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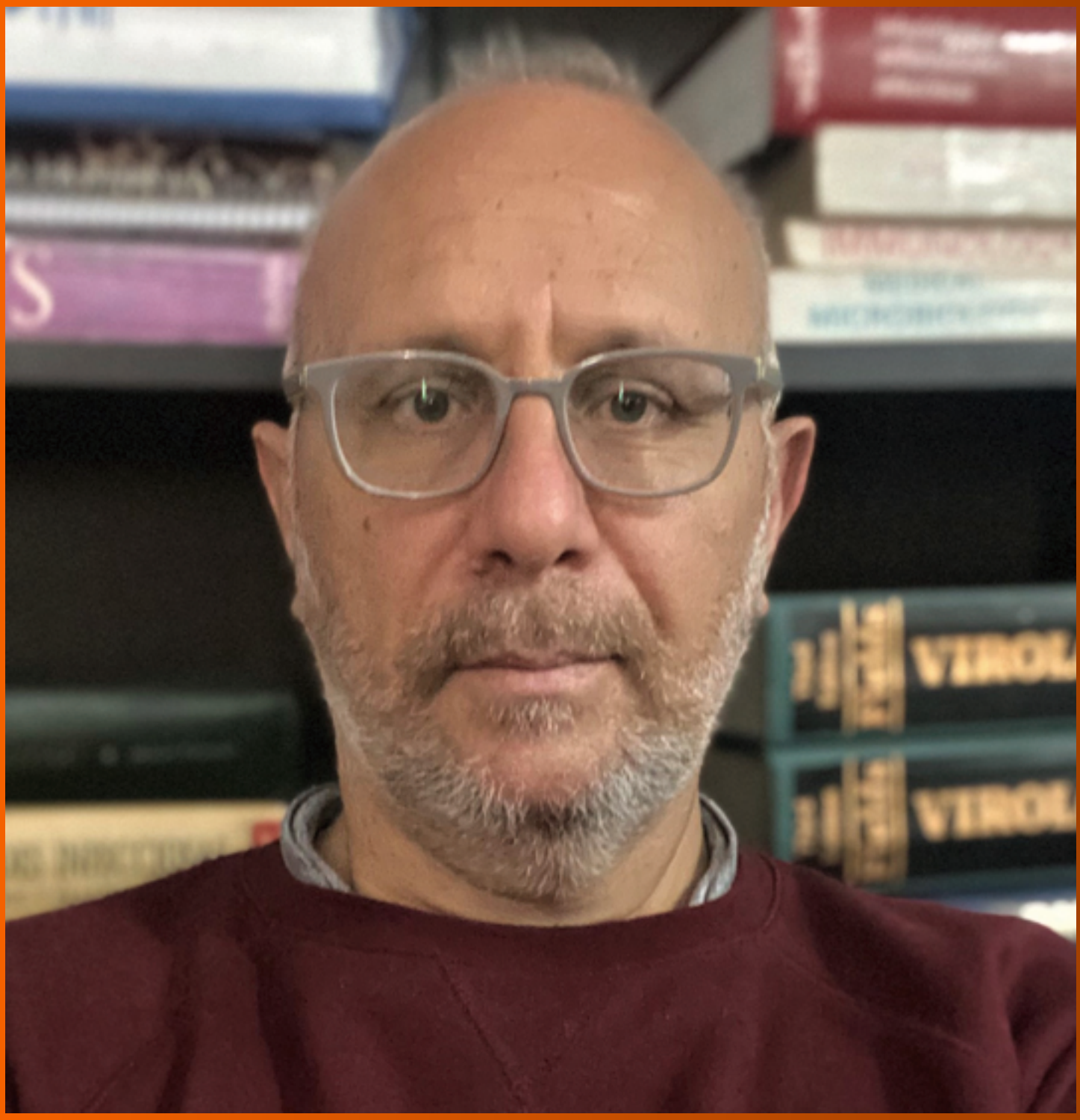

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The primary aim of *World Journal of Hepatology* (*WJH*, *World J Hepatol*) is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research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

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

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EDITORIAL

Current state of medical tourism involving liver transplantation-the risk of infections and potential complications

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Abstract

Liver transplant has been shown to significantly improve mortality and quality of life in various liver diseases such as acute liver failure, end-stage liver disease, and liver cancer. While the organ transplant demand is continuing to rise, the organ donation supply remains unmatched. The organ shortage, high cost, and long waiting lists have stimulated a desire for routes that may be unethical. This process which is named transplant tourism is the term used to describe traveling to another country to purchase an organ for transplant. Liver transplant tourism has been associated with post-transplant complications and higher mortality compared to a domestic liver transplant. Improper pre-and post-transplant infectious screening, inadequate opportunistic infection prophylaxis, and loss to follow-up were noted in patients who travel abroad for a liver transplant. It is crucial to understand the risk of transplant tourism to prevent morbidity and mortality.

Key Words: Commercial transplant; Liver transplant; Organ tourism; Transplant tourism

Core Tip: Liver transplant tourism can be associated with higher post-operative

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infections, biliary complications, and mortality compared to a domestic liver transplant. Pre-transplant education about the risk of liver transplant tourism and post-transplant management is essential to improve the patients' outcomes.

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INTRODUCTION

Liver disease and the role of transplantation

Acute liver failure, a rare and rapid deterioration of liver function in patients without pre-existing liver disease, is commonly caused by drug-related hepatotoxicity and viral hepatitis[1,2]. Without the transplant, mortality ranges from 26.7%-80%[3,4]. Chronic liver disease is frequently caused by non-alcoholic steatohepatitis, alcoholic and viral hepatitis, leading to cirrhosis and impaired function[5]. The immense morbidity and mortality of end-stage liver disease place a significant healthcare burden causing the liver transplant–its only 'cure'–the second most common transplanted organ globally[6-9].

Liver transplant has been shown to improve mortality and quality of life in various liver diseases such as acute liver failure, end-stage liver disease, liver cancer, liver disease with hepatopulmonary syndrome, and Porto-pulmonary hypertension[10,11]. Moreover, patients with metabolic disorders such as alpha 1-antitrypsin deficiency, familial amyloidosis, glycogen storage disease, hemochromatosis, and Wilson disease are also considered liver transplant candidates[11].

According to the United States Department of Health and Human Services, about 180000 liver transplantations were performed until 2020. While the organ transplant demand is continuing to rise, the supply remains unmatched. In 2018, the number of new registrants for the liver transplant waitlist in the United States was 11844, while 8250 liver transplants were performed[12]. The European Union has also stated a similar predicament with a severe donor shortage. This problem has been a constant stimulus for alternative–not so legal–pathways to obtain organ transplants.

TRANSPLANT TOURISM

According to World Health Organization, transplant tourism is the term used to describe traveling to another country to purchase an organ for transplant[13]. Travel for transplantation was defined by the 2018 edition of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism as the movement of the person across jurisdictional borders for transplant purposes and considered transplant tourism if it is related to trafficking humans for organ removal intention or trafficking in human organs, or if the resources dedicated to providing transplants to non-resident patients undermine the country's ability to supply transplant for people in its own country [14]. Transplant tourism can be divided into four models (Figure 1). First, the donor and recipient who are from the same country travel to another country for transplantation. Second, the donor travels to the country where the recipient resides. Third, the recipient travels to the country that the donor resides. Forth, the donor and recipient from different countries travel to the third country for transplantation[15]. Transplant tourism is a rampant phenomenon that needs more undivided attention. It accounts for approximately 10%-20.6% of global transplantation[16,17].

According to a national United States survey, many foreign transplants included young and male gender Asians with non-resident alien status[18]. Most of the countries that patients traveled to for transplant tourism were China, the Philippines, or India[18]. An interesting study from Syria pointed out the effects of a law passed in 2003 which legalized the use of organs from deceased donors, benefited patients, and increased commercialization as the poor used it as a means for monetary gain[19]. The

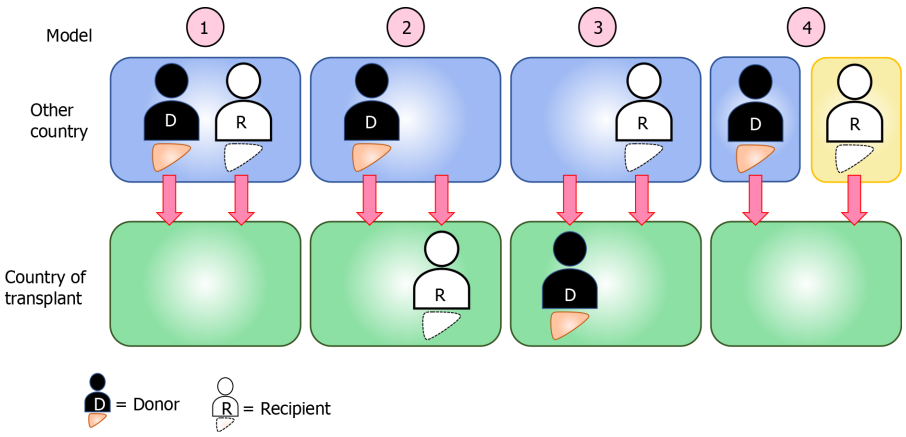

Figure 1 Transplant tourism models. 1: Donor and recipient from the same country travel to another country for transplantation; 2: Donor travels to the country where the recipient resides; 3: Recipient travels to the country that the donor resides; 4: Donor and recipient from different countries travel to the third country for transplantation.

formulation of law cannot be completed without enacting the regulation. The exploitation of the poorer population who give up organs for monetary benefit cannot be ignored. Although reports on tourism related to transplant have continued to decrease after great interest in the initial decade at the start of the 21st century, the lack of data is obvious as there is zero probability of anything remotely illegal to be documented. There is a great paucity of data involving liver transplantation pursued through illegal means and international travel for medical tourism for organ procurement. Most of the current data available is on renal transplantation. There has been a report of end-stage liver disease patients who traveled from Saudi Arabia and Egypt to China for liver transplantation due to lower associated financial burden and shorter waiting time[20]. From 2000 to 2016, a total of 1229 Korean patients traveled overseas for liver transplants based on the Korean Network for Organ Sharing. Of these, 98% of the patients underwent liver transplants in China[21]. In Taiwan, 5%-24.5% of patients who underwent liver transplants came from abroad[22,23].

From 2013 to 2016, 2806 patients who were non-United States citizens/non-United States residents registered for an organ transplant in the United States[24]. Of these patients, 1149 patients were foreigners who traveled to the United States for transplantation purposes. Deceased donor liver transplants were conducted in more than 5% of non-United States citizen/non-United States resident patients[24]. Liver transplant tourism is not limited to adult patients and can also be found in the pediatric population. In a study from Taiwan, pediatric cases comprised 79% of all foreign living donor liver transplant cases[22]. Liver transplant tourism can be costly. The price of liver transplants ranges from $40000 to $300000 which is higher than kidney transplants[17].

OUTCOME

The transmission of infectious diseases is one of the problems related to liver transplant tourism (Table 1) that can occur due to the lack of proper evaluation and management before and after the transplant for both donor and recipient[25]. Donor risks have been studied in detail and associated morbidity and mortality have been established. The people who remain vulnerable to trafficking, putting themselves at increased risks of surgical complications, infections, and increased mortality with 'less intensive' and 'poorly regulated' protocols need to be protected. Most of the time, this certain group of people appears vulnerable due to the existing inequities in health care. The financial drain resulting from this is bound to impact subsequent health care post-transplant, which carries significant importance. There have been reports of a lack of screening for even general pathogens like hepatitis-causing viruses. Thus, it compromises the general principles and practices which are crucial for such a sensitive procedure.

According to questionnaires from severe United Kingdom liver transplant centers, the top destinations for patients who traveled abroad for liver transplant were China, Egypt, India, followed by South Africa, France, and the United States[26]. This report

Table 1 Problems related to liver transplant tourism compared to domestic transplant[20,25,26]

	Previous reported problems related to liver transplant tourism
1	Higher surgical procedure complications
2	Inadequate pre-operative infection screening, prophylaxis documentation and higher post-operative infections rate
3	Higher mortality

showed that patients underwent liver transplants without or with unknown screening for hepatitis B and C viruses in some places. Unknown screening is also noted for carbapenemase-producing Enterobacteriaceae, cytomegalovirus, varicella-zoster virus, and Epstein-Barr virus. The majority of intraoperative and post-transplant prophylaxis in these patients is even unknown[26]. Indigenous infections such as malaria, Zika, rabies may be able to transmit through commercial transplant. Compared to domestic transplants in Saudi Arabia, overseas transplants in China showed a higher rate of sepsis (9.5% *vs* 0.83%) and acquired hepatitis B infection (5.4% *vs* 0%) following transplantation[20]. Surgical procedure complications can be difficult to manage by the new surgeon who did not perform the transplant for the patient in the first place. Compared to domestic transplantation, patients who received transplants abroad in China had significantly higher biliary complications (32.4% *vs* 11.7%) and significantly higher post-transplant interventions[20].

An eleven-year retrospective study from Taiwan demonstrated significant discrepancies between domestic and foreign liver transplants and their outcomes, with the latter faring worse mainly attributed to malignancy and liver disease. Survival rates within the 1st, 5th, and 10th year of the Taiwanese patients who received liver transplants domestically *vs* abroad were 89.2%, 79.5%, 75.2% *vs* 79.8%, 62.3%, and 49.9%, respectively[23]. An unfavorable outcome of transplant tourism was also noted in China. One- and three-year survival rates of liver transplants were 83% and 62% for Saudi and Egyptian patients who received a liver transplant in China while 92% and 84% were reported for domestic transplants in Saudi Arabia[20]. In the United States, post-liver transplant outcomes of non-United States citizen/non-United States resident were comparable to those of a United States citizen/United States resident, except the former group which had an increased risk of being lost to follow-up[27]. The significant influx of Taiwanese people to China appeared to decrease after the Human Organ Transplant Act was passed in 2007. This followed suit by Taiwan in 2015 when they passed amendments to the act by punishing organ brokers, and those patients received illegal transplants[23].

CLINICAL IMPLICATIONS

This article provided an overview of liver transplant tourism and outcomes.

CONCLUSION

Liver transplant has been shown to improve mortality in various advanced liver diseases. However, due to the shortage of organ donations, patients may seek liver transplant tourism. To prevent liver transplant tourism and its ongoing complications, it is crucial to educate patients regarding the risks of transplant tourism, the importance of proper screening, transplant center follows ups and liver transplant tourism morbidity and mortality. While efforts have been made at innumerable national and international platforms, more aggressive implementations to raise the awareness of organ donations are needed to overcome the rise in liver transplant tourism.

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OPINION REVIEW

Hepatitis E virus in professionally exposed: A reason for concern?

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Abstract

The zoonotic risk of hepatitis E virus (HEV) is well established. The HEV seroprevalence rates vary according to geographical region, assays used, and study cohorts. HEV infection is still underdiagnosed, implying the need to evaluate the disease's burden in the general population and specific risk groups, such as professionally exposed. Close contact with various animal reservoirs such as pigs, rabbits, sheep, dogs, wild boars, and deer has been associated with higher anti-HEV seroprevalence as a part of occupational exposure. While exact transmission routes remain to be determined, some general preventive measures such as proper hand hygiene, the usage of personal protective equipment, and the thermal processing of food before consumption should be followed. A "One-Health" multisectoral approach should be implemented to achieve optimal health and well-being outcomes, recognizing the interconnections between humans, animals, plants, and their shared environment, in which a vaccine against the zoonotic genotypes 3 and 4 and swine vaccination should be considered as a possible public health measure. This opinion review comprehensively addresses the HEV burden of professional exposure for butchers, slaughterhouse workers, veterinarians, farmers, hunters, and forestry workers delineates the current limits of protective work measures, and tackles future directions.

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Key Words: Hepatitis E virus; Zoonotic infection; Occupational disease; Veterinarians; Farmers; Butchers; Slaughterhouse workers; Forestry workers; Hunters

Core Tip: The zoonotic risk of hepatitis E virus (HEV) is well established. Close contact with various animal reservoirs such as pigs, rabbits, sheep, dogs, wild boars, and deer has been associated with higher anti-HEV seroprevalence as a part of occupational exposure. However, precise HEV transmission routes yet need to be determined. This opinion review addresses the HEV burden of professional exposure, delineates the current limits of protective work measures, and tackles future directions.

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INTRODUCTION

The global burden of hepatitis E virus (HEV) is high, with an estimated 20 million new HEV infection events yearly, 3.3 million symptomatic cases, and 44,000 deaths[1]. HEV RNA genotypes 1 and 2, found only in humans, primarily cause waterborne epidemics in resource-poor regions. Infections are usually self-limiting and not associated with progression to chronic disease. In high-income countries, zoonotic HEV genotypes 3, 4, and 7 circulate in various animal species, and human infections are usually asymptomatic, cause sporadic, or clustered cases of hepatitis[2,3]. In immunocompromised individuals, chronic HEV infection can progress to cirrhosis[3,4].

Besides contaminated water, transmission routes include consuming insufficiently cooked meat and meat products from infected animals (*e.g.*, pork liver), transfusions of infected blood derivatives, solid-organ transplants, and vertical transmission[1,3].

In the last two decades, there has been an increase in autochthonous infections related to the transmission of zoonotic genotypes HEV-3 and HEV-4[5]. Seroprevalence rates in the general population of industrialized countries vary from < 5% to > 50%. Higher rates are observed in the southwest region of France, Poland, and Netherlands, moderate seroprevalence rates from 10% to 30% in the United States, United Kingdom, Belgium, and Germany, and the lowest in Canada, Ireland, Australia, and New Zealand[3,6].

In 1995, the first HEV animal strain was found in sera and stool of swine in Nepal's Kathmandu Valley[7]. Since then, different reservoirs (infected pigs, rabbits, wild boars, and deer) and various zoonotic transmission routes[5] have been associated with professional exposures of those in close contact with the reported HEV reservoirs. Detected HEV sequences in pigs, rabbits, and humans are tightly related[8]; however, it is still unclear whether HEV strains from other animals can cross the species barrier and infect humans. Recently described HEV-7, distributed in dromedary camels from the Middle East[9,10], has been detected in a transplant recipient who consumed camel milk and meat[4]. In addition, a Chinese study showed that viral RNA of HEV-4 could be excreted by cow milk[11], implicating possible HEV transmission through milk or milk products.

Accordingly, professionally exposed workers such as butchers, slaughterhouse workers, veterinarians, farmers, hunters, and forestry workers are considered a risk group for HEV infections. This article addresses the burden of professional exposure to HEV, determines the current situation, delineates the limits, and tackles the future directions.

HEV IN VETERINARIANS AND FARMERS

Among domestic animals, pigs are considered the main reservoir of zoonotic HEV-3 and -4 in industrialized countries. High seroprevalence of HEV IgG antibodies was

detected in pigs in many countries, which implicate a high risk of zoonotic transmission to professionally exposed workers, such as veterinarians and farmers. Indeed, the occupational risk is well known and confirmed by numerous studies and several meta-analyses (Table 1) that investigated the association between direct contact with animals and HEV seroprevalence.

However, when interpreting serological studies, it is important to bear in mind that there are considerable variations in sensitivity and/or specificity between different HEV antibody assays. Thus, it is difficult to compare prevalence estimates using different assays[12], and the lack of a gold standard hampers the interpretability of serological studies[13].

The United States data confirmed that swine veterinarians were 1.51 times more likely to be anti-HEV positive than blood donors[14]. Similarly, studies from Norway and Austria show that swine veterinarians are twice as likely to be HEV seropositive than other veterinarians[15,16]. Other studies from France[17], Germany[18], and Israel[19] support high HEV professional exposure in pig farm workers. In Portugal, in addition to pig farmers, higher HEV seroprevalence was also found in sheep farmers and cheesemakers (29.3%) compared to the general population (16.1%)[20]. In east Africa, Rwandan farmers have higher HEV seroprevalence compared to other professions, with the highest being in high-density pig breeding regions[21].

Studies from China demonstrate high IgG seropositivity in veterinarians (26.7%-43.7%)[22-24] and farmers (34.8%-53.0%)[22-24]. In high-density, pig-farming areas in central China, HEV IgG seroprevalence in swine farm workers rises to 48.35% and increases with age and working years, with all the isolates belong to HEV-4d[25]. Except in swine and sheep farmers, higher seroprevalence was observed in deer (40.2%) and mink farmers (31.8%)[22].

However, despite high HEV seroprevalence rates and zoonotic potential, the awareness of HEV is still inadequate in farmers and veterinarians, who report the lack of knowledge and low perception of the HEV's importance for implementing on-farm risk mitigation strategies[26].

Recent studies additionally highlight risk in small animal practitioners due to high HEV seroprevalence in pet animals. Seroprevalence in dogs in the Netherlands and Germany was 18.52% and 56.6%, respectively[27,28]. The same Dutch study showed that 14.89% of cats had HEV antibodies. Nevertheless, the results of a German study show that pet ownership is inversely associated with infection[29]. On the on the hand, American data indicate that having a pet in the home increases odds of HEV seropositivity [odds ratio (OR), 1.19 (95% Confidence interval (CI), 1.01–1.40)][30]. These results are in line with the observation that veterinarians and farm staff exposed to dogs in the southwest of China have significantly higher seroprevalence than the general population[23]. In Finland, veterinarians have almost two times higher HEV seroprevalence (10.2%) than non-veterinarians (5.8%), and surprisingly, among veterinarians, the highest HEV seroprevalence (17.8%) was detected among small animal practitioners[31]. Similar results were confirmed in Estonia, where all antibody-positive veterinarians were small animal practitioners[32]. A high HEV seroprevalence in pet animals highlighted that in addition to generally known occupational exposure in pig farm workers (farmers and veterinarians), small animal practitioners could also be professionally exposed to HEV. High HEV seroprevalence in pet animals raises the question of their role in the HEV epidemiology as a potential risk of HEV transmission from pets to their owners, which needs to be further investigated.

HEV IN BUTCHERS AND SLAUGHTERHOUSE WORKERS

In geographically distinct locations, studies on swine related occupational exposure report a higher HEV seroprevalence in butchers and slaughterhouse workers compared to the general population; for Germany (41.7% *vs* 15.5%)[18], Portugal (29.7% *vs* 19.9%)[33], Republic of Moldova (14.3% *vs* 0)[34], India (75% *vs* 10.71%)[35], and Burkina Faso (76% *vs* 47.8%)[36]. However, the general population in these studies should be interpreted with caution, *e.g.*, a control group of freshman students who drank only filtered water may be misleading[35].

The results of several meta-analyses substantiate higher HEV risk in swine-related professions. A meta-analysis on 28 studies from mainland China showed that those professionally exposed (swine farmers, slaughters, swine vendors, and veterinarians) have a 2.63-fold higher risk for HEV IgG seropositivity than the general population [24]. Additionally, a recent meta-analysis on 32 studies on swine-related occupations (swine farmers, butchers, meat processors, port retailers, and veterinarians) from 16

Table 1 Occupation-related key points from meta-analyses on hepatitis E virus infection

Meta-analysis: Region/Period/No of studies	HEV IgG seroprevalence: occupational/general population	Occupation-related key points
16 countries; 1999-2018; 32 studies[37]	32.85%/21.70%	The anti-HEV IgG PR for all swine workers was 1.52 (95%CI: 1.38-1.76); butchers 1.75 (95%CI: 1.31–2.35), swine farmers 1.51 (95%CI: 1.32–1.74), meat processors 1.46 (95%CI: 1.13–1.89), veterinarians 1.36 (95%CI: 1.15–1.61) and pork retailers 1.19 (95%CI: 1.09–1.29) compared to the general population; The anti-HEV IgG PR for swine workers in Asia was 1.49 (95%CI: 1.35-1.64) and in Europe 1.93 (95%CI: 1.49-2.50)
Mainland China; 2004-2018; 28 studies[24]	47.4%/27.3%	Anti-HEV IgG positivity: Swine vendors (77.0%), producers (56.0%), swine farmers (53.0%), slaughters (51.7%) and veterinarians (43.7%); The OR for HEV IgG seropositivity in swine occupational population was 2.63 (95%CI: 1.87–3.70) compared to the general population
Europe; 2003-2015; 73 studies[51]	17%/28% using WT	Seroprevalence rates depend on the serologic assays used; increased with age, were unrelated to gender, varied within countries; Individuals in contact with swine/wild animals had higher seroprevalence rates than the general population, irrespective of assay used ($P < 0.0001$)
Global, non-endemic HEV countries; 1994-2018; 163 studies[52]	Not calculated	The OR for HEV seropositivity for occupational contact with pigs was 1.95 and for the employment in forestry population 2.49 compared to the general population; Recreational hunting was a non-significant predictor for HEV seropositivity; Contact with pigs (not categorized as occupational), cats or horses was non-significantly associated, contact with dogs was significantly associated with increased odds of HEV IgG seropositivity; The consumption of meat (uncooked liver sausage, rabbit and game meat, liver or organ meats, bacon or ham, and pork) was a significant predictor of HEV IgG seropositivity (median OR = 1.44, range (1.12-2.77)

CI: Confidence interval; HEV: Hepatitis E virus; OR: Odds ratio; PR: Prevalence ratio; WT: Wantai test.

different countries demonstrated that swine workers are 1.52-fold more likely to be HEV IgG seropositive than the general population. Interestingly, the association with the HEV exposure, the prevalence ratio (PR) is higher in Europe (PR = 1.93, 95%CI 1.49-2.50) than in Asia (PR = 1.49, 95%CI 1.35-1.64)[37] (Table 1).

Furthermore, the data show that rabbit slaughterhouse workers have a 6.9-fold increased risk for HEV compared to the general population and that their seropositivity also increases with working years[38].

The precise HEV transmission route among occupationally exposed workers remains to be determined. However, it is possible that increased risk of infection during slaughtering results from manipulation of raw HEV-rich organs and tissues (*i.e.* liver and bile) without direct consumption[18]. In addition, well-known risk factors for anti-HEV IgG seropositivity are the frequency and duration of contact with animals [33,39].

Over the past decades, it has become clear that a collaborative and multisectoral approach across boundaries of animal, human, and environmental health (a One-Health approach) is needed to develop control and achieve optimal health outcomes in a setting of zoonotic diseases. The use of protective equipment and vaccination (when possible) should be an integral part of the prevention of zoonotic infections. The HEV studies on protective equipment in butchers and slaughterhouse workers are scarce with conflicting results. An Indian study showed that slaughterhouse workers are routinely in contact with swine without adequate protective equipment[35]. A South Korean study demonstrated that anti-HEV IgG positive slaughterhouse workers use protective equipment (vinyl gloves, aprons, boots, and disposable protective suits) more often than anti-HEV IgG negative workers, suggesting that the equipment does not prevent the HEV infection or that the equipment is not appropriately used[40]. Although the clinical course of HEV infection in most cases is subclinical, in middle-aged and older men workers with underlying liver disease, the risk of HEV infection should be especially minimalized given the frequency of complications in this population group[41]. The authors propose that for workers at continued risk of exposure, strict hygiene measures, personal protective equipment, and a vaccine against the zoonotic genotypes 3 and 4 and swine vaccination should be considered. However, the first and only HEV vaccine produced and licensed in China is not approved for widespread use, even though it shows a good tolerance and the efficacy of 86.8% on the extended follow-up[42,43]. Despite these results, the efficacy in different genotypes of the virus and safety in chronic liver disease and other vulnerable populations remains unclear[43].

HEV IN FORESTRY WORKERS AND HUNTERS

In Europe, hunting and forestry work, particularly woodcutting, are associated with increased HEV seropositivity[17,44-47]. It is a well-known fact that the HEV seroprevalence increases with age, duration, and animal-related activity frequency. This general trend is also confirmed for the forestry workers and hunters[47,48].

However, some studies do not support previous data. Studies from central Germany and Northern Italy showed no differences in anti-HEV IgG antibodies in hunters[49] and forestry workers compared to the general population[50].

A meta-analysis on HEV seroprevalence in Europe conducted on 73 studies shows that individuals in contact with swine/wild animals have significantly higher seroprevalence rates than the general population. It is important to notice that they vary according to geographical region, assays employed, and study cohorts[51].

As wild boars and deer represent important HEV reservoirs, HEV transmission route in hunters may occur during skinning and disemboweling of an infected animal or through contact with its blood or feces[49]. Studies show that hand hygiene immediately after disembowelment reduces the HEV infection risk[48] and that the regular use of protective gloves is associated with an 88% lower HEV seroprevalence [49]. Additionally, a study from Southern France found that wearing work boots by forestry workers is associated with significantly lower HEV seroprevalence (46% without *vs* 28% with boots). Interestingly, no differences were detected for wearing gloves (39% without *vs* 34% with gloves)[17]. Despite conflicting evidence, the authors believe the use of personal protection minimizes the risk of infection.

In conclusion, most of the published studies showed that the risk of HEV infection is higher in forestry workers and hunters than in the general population. However, some studies did not identify hunting activity as an important risk factor for the HEV seropositivity. Close and frequent contact with HEV-infected animals, especially wild boars, represents important risk factors, where the use of personal protection minimizes the risk of infection.

CONCLUSION

Given the high seroprevalence rates observed in swine workers, veterinarians, farmers and hunters, contacts with infected animal reservoirs (mainly pigs, wild boars, deer) have been recognized as risk factors for the transmission of HEV. The list of new animal reservoirs is ever-expanding as new HEV strains are continuously being found in a broad range of hosts. Although the precise HEV transmission route in occupationally exposed workers remains to be determined, occupational exposure plays a significant role.

HEV infection is still an underdiagnosed disease due to the lack of routine diagnosis and surveillance protocols, limiting the knowledge of the data about the HEV burden. Thus, there is a need for a realistic evaluation of HEV disease's burden in humans in general and in specific risk groups, such as professionally exposed.

A better understanding of HEV transmission routes from the infected animals to workers might help develop more specific preventive measures for specific occupational groups that have shown to be associated with the higher risk of acquiring HEV. Until other evidence is found, several protective measures to decrease the risk in occupationally exposed groups should be respected: the proper hand hygiene following contact with animals known to be HEV reservoir, the usage of recommended personal protective equipment, and the proper thermal processing of food before consumption. Although HEV infection is not an economically important pig disease, developing a vaccine against the zoonotic genotypes 3 and 4 and swine vaccination should be considered a possible public health measure. Epidemiologically important pet animals should also be further investigated as a potential additional risk factor for small animal practice veterinarians and pet animal owners.

Further testing of different populations including the general population and professionally exposed persons as well as animals are needed to better understand the epidemiology of hepatitis E.

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REVIEW

Clinical algorithms for the prevention of variceal bleeding and rebleeding in patients with liver cirrhosis

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Abstract

Portal hypertension (PH), a common complication of liver cirrhosis, results in development of esophageal varices. When esophageal varices rupture, they cause significant upper gastrointestinal bleeding with mortality rates up to 20% despite state-of-the-art treatment. Thus, prophylactic measures are of utmost importance to improve outcomes of patients with PH. Several high-quality studies have demonstrated that non-selective beta blockers (NSBBs) or endoscopic band ligation (EBL) are effective for primary prophylaxis of variceal bleeding. In secondary prophylaxis, a combination of NSBB + EBL should be routinely used. Once esophageal varices develop and variceal bleeding occurs, standardized treatment algorithms should be followed to minimize bleeding-associated mortality. Special attention should be paid to avoidance of overtransfusion, early initiation of vasoconstrictive therapy, prophylactic antibiotics and early endoscopic therapy. Pre-emptive transjugular intrahepatic portosystemic shunt should be used in all Child C10-C13 patients experiencing variceal bleeding, and potentially in Child B patients with active bleeding at endoscopy. The use of

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carvedilol, safety of NSBBs in advanced cirrhosis (*i.e.* with refractory ascites) and assessment of hepatic venous pressure gradient response to NSBB is discussed. In the present review, we give an overview on the rationale behind the latest guidelines and summarize key papers that have led to significant advances in the field.

Key Words: Portal hypertension; Endoscopy; Non-selective betablockers; Transjugular intrahepatic portosystemic shunt

Core Tip: Variceal bleeding is a severe, and often deadly, complication of portal hypertension. Screening for varices, effective bleeding prophylaxis and standardized management of bleeding is critical to improve clinical outcomes. While carvedilol seems to be the treatment of choice for primary prophylaxis in compensated cirrhosis, the use of hepatic venous pressure gradient measurements and safety of non-selective betablockers in advanced cirrhosis with refractory ascites is controversial. The pre-emptive use of transjugular intrahepatic portosystemic shunt within 72 h after variceal bleeding prevents rebleeding and mortality in Child C10-C13 patients.

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INTRODUCTION

Chronic liver diseases cause recurrent liver damage and can result in the development of liver fibrosis and, ultimately, liver cirrhosis[1]. Fibrosis and cirrhosis lead to gradually increased intrahepatic vascular resistance, splanchnic vasodilatation and increased portal blood flow, which subsequently results in increased portal pressure and the development of collaterals[2]. To allow risk stratification, evidence-based guidelines have been developed to grade portal hypertension severity, and the term clinically significant portal hypertension (CSPH) has been defined to indicate a high risk of complications[3]. CSPH is defined as a hepatic venous pressure gradient (HVPG), an invasive surrogate parameter of portal venous pressure, of ≥ 10 mmHg[4]. This definition is based on studies demonstrating that esophageal varices (EV) develop above the 10 mmHg HVPG threshold, subsequently increasing the risk of bleeding[5]. In cross sectional studies, between 40%-60% of patients with liver cirrhosis show EV, highlighting the clinical importance of this condition[6,7]. Variceal bleeding is, next to liver failure, hepatocellular carcinoma, infections and the hepatorenal syndrome, one of the main causes of mortality in patients with CSPH and adequate diagnosis as well as treatment is of utmost importance, given that variceal bleeding episodes are still associated with a high mortality rate of up to 20%[8-12]. Thus, to avoid unnecessary fatal outcomes, variceal bleeding and re-bleeding must be prevented, ideally by (primary or secondary) prophylactic treatment of portal hypertension *per se*. Therefore, this review focusses on clinical algorithms and summarizes the available evidence on prevention and treatment of variceal bleeding.

PREVENTION OF ESOPHAGEAL VARICEAL BLEEDING

Screening for gastroesophageal varices in patients with portal hypertension

In patients with cirrhosis but without EVs at baseline, the incidence of developing EV rises from 5% after one year to 28% after three years, independently of liver function or compensated/decompensated liver cirrhosis[13]. In a cross-sectional study of 494 patients of which 48% had decompensated liver cirrhosis, 38% of patients had EV at the time of screening[14]. Thus, EV are common in patients with advanced chronic

liver disease, and it was shown that patients with EV suffer from significantly higher mortality rates and decompensating events than patients without[14]. Of note, however, bleeding risk is correlated with HVPG values, and patients with a HVPG of ≥ 12 mmHg are at significantly higher bleeding risk than patients with < 12 mmHg, despite the diagnostic CSPH cutoff value of 10 mmHg[15,16]. Although HVPG is considered the gold standard, measurement requires specific expertise and equipment, comes at relatively high cost and is invasive. Thus, it is not considered as standard of care and not available to most centers[17]. As an alternative, transient elastography (TE) has been established as a well-validated cheap, non-invasive tool to measure liver stiffness, as fibrosis/cirrhosis severity and portal pressure directly correlate[18,19]. TE allows to classify patients with liver cirrhosis, defined as a liver stiffness measurement value > 15 kPa and can be used as screening tool[3,20]. Efforts to establish clear cutoff values have been made[21], and evidence indicates that patients with TE values < 20 kPa and platelet count > 150 G/L are unlikely to have varices (< 5%)[22]. These values can be used to avoid screening gastroscopies for EV, and the next TE screening for EV can be postponed for another year[22]. Screening gastroscopy is, however, required in patients with diagnosed liver cirrhosis who do not meet these mentioned criteria[3,17,22] and allows to identify "high risk" varices, which are referred to as "varices needing treatment" (VNT) in recent guidelines[22]. VNT are varices of large size (> 5 mm diameter) or small varices (< 5 mm diameter) with red spot signs/red wale markings, as both of them are at high risk of bleeding[22]. When VNT are detected, treatment with non-selective betablockers (NSBB) or endoscopic band ligation (EBL) should be initiated for primary prophylaxis of variceal bleeding[3,17,22].

While evidence is clear on these VNTs, current guidelines are less validated whether endoscopic screening is indicated for small varices[23]. Augustin *et al*[24] found that following the current Baveno VI criteria spared more screening endoscopies with a minimal risk of missing VNT, but when guidelines are followed strictly, small varices would be missed in a significant number of patients. Thus, treatment decisions in these cases should be made on a case-to-case basis until further evidence is available.

Preprimary and primary prophylaxis for patients with small esophageal varices

When patients with high risk EV are identified, treatment should aim to prevent variceal bleeding as primary prophylaxis. Current guidelines recommend either NSBB or EBL for prevention of first EV bleeding in patients with medium to large varices, while they do not specifically recommend treatment for small varices due to above mentioned lack of decisive studies[3,17].

While available evidence uniformly demonstrated that NSBB therapy effectively prevents first, as well as recurrent, EV bleeding and reduces mortality when EV are diagnosed[25,26], it is under debate whether NSBB should be prescribed without signs of EV. One large randomized multicenter study assigned patients with CSPH without EV to timolol or placebo and found that although HVPG was lower in timolol-treated patients, the subsequent development of EV or variceal bleeding rate did not differ between timolol or placebo treated patients[27]. Although the HVPG-response to NSBB differs in patients with or without CSPH, the results were relatively unexpected [27].

Little high-quality evidence is available regarding treatment of patients with small and low risk varices in primary prophylaxis[22,28]. It seems as if some trials were underpowered to see sufficient effects of NSBB on the incidence of first variceal bleeding in patients with small varices[23] while others demonstrated that NSBB effectively prevented the progression from small to large varices, especially in patients assigned to carvedilol[29,30]. The recently published PREDESCI trial showed that NSBB were associated with a decreased risk of decompensation [hazard ratio: 0.51 (95%CI: 0.26-0.97), P = 0.041] in patients with CSPH and low risk varices, potentially resulting in longer decompensation-free survival[31]. Taken together, the conflicting evidence led the authors of the current international guidelines to not recommend NSBB treatment for patients with no EV or for prevention of varix progression. However, some experts still recommend using NSBB in patients with cirrhosis as soon as CSPH is evident (*e.g.* by HVPG ≥ 10 mmHg or by any size of varices) to prevent clinical decompensation.

Beta blocker therapy for primary prophylaxis in patients with medium and large esophageal varices

Prescribing NSBB for primary prophylaxis is less expensive, has no procedural risk, does not require repetition of esophageal gastroscopy after initiation of NSBB for prevention of variceal bleeding and saves time for gastroenterologists[3,17]. Therefore,

NSBB are sometimes favorable compared to EBL, with dosing intensities summarized in Table 1. Beside the positive effect of NSBB on variceal bleeding (absolute risk reduction of up to -16%, NNT = 6), several studies have also demonstrated benefits that are likely mediated by their additional non-hemodynamic effects[32-35]. With regards to beta blocker selection, some trials showed a better or comparable efficacy in primary prophylaxis of carvedilol in comparison to other NSBBs, probably as carvedilol has additional anti-α-1-adrenergic activity and does therefore result in a more potent decrease of portal pressure[36-38]. Thus, carvedilol is recommended as first line therapy in some national guidelines[3,39-41]. However, carvedilol for the sole indication of portal hypertension should not be prescribed in doses above 12.5 mg per day, as higher doses (> 12.5 mg/d) do not lead to further reductions of portal pressure [36,37]. Importantly, carvedilol may be prescribed when NSBB have already failed, as our group could show that in 58% of patients who did not respond to propranolol, carvedilol still resulted in a significant HVPG response (defined as reduction of HVPG of more than 20% or reduction to a HVPG value < 12 mmHg)[36].

Despite the easy handling of NSBB or carvedilol, up to 15% of patients require a dose reduction or discontinuation due to common and severe side effects such as hypotension, shortness of breath and/or fatigue[42], and 15% to 25% of patients have absolute or relative contraindications for NSBB initiation[35,42]. In addition, there is a great abundance of studies comparing NSBB to EBL in primary prophylaxis, and there is no clear outcome benefit for one or the other. In a Cochrane analysis from 2012, patients who underwent EBL as primary prophylaxis showed reduced variceal bleeding rates compared to patients using NSBB alone, while bleeding did not impact on mortality[43]. Another meta-analysis demonstrated that there was no difference in bleeding rates when high-quality studies were assessed[44]. In contrast to these meta-analyses, one large multicenter study showed better efficacy of carvedilol for primary prophylaxis compared to EBL alone[41], and another meta-analysis of 32 randomized controlled trials and a total number of 3362 patients with large varices in primary prophylaxis found that NSBB monotherapy was associated with a decrease of all-cause mortality, decrease risk of first variceal bleeding and a better safety profile compare to patients treated with EBL[45]. Overall, bleeding rates in primary prophylaxis greatly vary between studies and no reproducible differences between the overall effectiveness, especially the overall- or bleeding-related mortality, could be established so far[46-49]. To address certain limitations of previous studies, another large randomized controlled open-label multicenter study, CALIBRE, is currently recruiting patients with liver cirrhosis and medium to large EV, and will investigate the effect of carvedilol or EBL on the incidence of variceal bleeding within 1 year of treatment initiation[50], potentially impacting on treatment regimes in the future.

NSBB in patients with complicated ascites and/or spontaneous bacterial peritonitis

Due to vasodilating effects, sympathetic activation, increased left ventricle systolic function and, therefore, impairment of renal perfusion, several studies questioned the safety of NSBB and carvedilol in patients with decompensated cirrhosis[51-59]. This is in line with evidence that NSBBs were associated with higher mortality in patients with refractory ascites[51,60,61]. However, these findings were not uniformly confirmed and some studies report no impact on outcome[62-64]. As a result of this conflicting evidence, current guidelines suggest to monitor blood pressure, serum sodium levels and kidney function in patients with decompensated cirrhosis[3,17,22], but do not state that NSBB are contraindicated[17,22]. Nevertheless, high doses of NSBB (*e.g.* propranolol > 160 mg/day) should be avoided as they seem to be associated with worse outcome[65]. In addition, there is limited evidence supporting a switching strategy from carvedilol to propranolol in patients with ascites and/or renal impairment[56]. Thus, carvedilol should not be used in patient with severe ascites[3].

Similar conflicting results were reported for NSBB use in patients with spontaneous bacterial peritonitis (SBP) and/or acute kidney injury[56]. In one retrospective study, NSBB use was associated with a higher risk for the development of a hepatorenal syndrome in patients with newly diagnosed SBP, resulting in impaired survival[59]. However, a more recent study suggests that NSBB maintenance during an SBP-episode is not associated with increased mortality as long as there is no severe arterial hypotension, highlighting the importance of the guideline's recommendations to monitor blood pressure[66].

EBL for patients in primary prophylaxis with medium or large esophageal varices

EBL has a very low procedural risk and is the most effective endoscopic choice for EV [3,17,22,67,68]. When EBL is chosen for primary prophylaxis, it should be repeated every two to four weeks until varices are completely eradicated (small "remnant"

Table 1 Recommended use of non-selective betablockers in patients with primary and secondary prophylaxis [adapted from the Austrian (Billroth III), European (Baveno VI) and American (Guidance by the AASLD 2017) guidelines][3,17,22]

Beta blocker	Initial dose	Goal	Treatment duration	Further guidance
Propranolol	20–40 mg twice daily	Maximum dosage of 160 mg/day; Or until the resting heart rate of 55–60 beats/min; Maximum dosage of 80 mg/day in patients with ascites	Indefinite	Adapt every 2-3 d until optimal dose is reached; Discontinue during spontaneous bacterial peritonitis, hyponatremia (Na < 125 mmol/L) or acute kidney injury; Systolic blood pressure should not decrease below 90 mmHg; EGD for further variceal screening is not needed
Carvedilol	6.25 mg once daily	Maximum dosage of 12.5 mg/day	Indefinite	Adapt dose after 3 d and increase to 6.25 mg twice daily; Discontinue during spontaneous bacterial peritonitis, hyponatremia (Na < 125mmol/L) or acute kidney injury; Systolic blood pressure should not decrease below 90 mmHg; EGD for further variceal screening is not needed; Potential switch from carvedilol to propranolol in case of new onset of ascites
Nadolol	20-40 mg once daily	Maximum dosage of 160 mg/day; Or until the resting heart rate of 55–60 beats/min; Maximum dosage of 80 mg/day in patients with ascites	Indefinite	Adapt every 2-3 d until optimal dose is reached; Discontinue during spontaneous bacterial peritonitis, hyponatremia (Na < 125mmol/L) or acute kidney injury; Systolic blood pressure should not decrease below 90 mmHg; EGD for further variceal screening is not needed

EGD: Esophagogastroduodenoscopy

varices can be tolerated) and endoscopy should subsequently be repeated after six and twelve months[3]. If EV reappear, the treatment algorithm has to be restarted in the same intervals[3]. Compared to NSBB, EBL for primary prophylaxis has a lower overall rate of adverse events, but if adverse events occur they are more severe and life-threatening (*e.g.* EBL-related ulcer bleeding)[47,49,69]. Procedure related bleeding as a potential complication after EBL has been described to occur in 2%-6% of interventions[68,70-72]. In addition to potential esophageal injuries, EBL induces/accelerates the development of gastric collaterals[73] as it does not affect the underlying cause of increased portal pressure and thus has no disease-modifying effects. In summary, however, both treatments, namely NSBB or EBL, are effective and physicians should choose individually which primary prophylaxis is used, based on patients' concomitant risk factors and local availability. As a brief overview, we have summarized the recommended clinical algorithms in Figure 1.

ACUTE ESOPHAGEAL VARICEAL BLEEDING

Management of acute variceal bleeding

When EV are not detected in time, or if primary prophylaxis fails and acute variceal bleeding cannot be prevented, a determined and rapid treatment initiation as well as intensive care are required to optimize outcome. Despite improved mortality rates in the past decades, bleeding-related mortality remains as high as 15%-20%[9,10,12,74]. Patients presenting with acute variceal bleeding are classified as "decompensated cirrhosis", irrespective of fibrosis severity[5,17]. Despite this classification, 5 year mortality rates are affected by the underlying fibrosis severity as complications such as ascites and/or hepatic encephalopathy also impact on overall survival[14]. Fluid resuscitation, pharmacological treatment and endoscopy/EBL are the three main pillars for acute variceal bleeding treatment (see Figure 2)[3,17,22].

Initial fluid resuscitation to counteract hemorrhagic shock is the first important step in patients with acute variceal bleeding, and packed red blood cell (PRBC) transfusions are indicated when hemoglobin levels are below 7 to 8 g/dL, as too liberal administration of PRBCs has been shown to impair outcome[3,75]. In the randomized controlled study by Villanueva *et al*[75], patients with "liberal" use of PRBC transfusion showed significantly increased mortality rates compared to patients in which PRBCs were only transfused at a threshold of 7 g/dL, maintaining hemoglobin levels of 7-9 g/dL. Thus, the threshold of 7 g/dL is still recommended by current guidelines [3,17,22].

In contrast to PRBCs, transfusion of platelets, the use of fresh frozen plasma or administration of recombinant factor VIIa to correct platelet count or international normalized ratio (INR), respectively, did not demonstrate a clear benefit and is therefore not recommended[3,17,22,76,77].

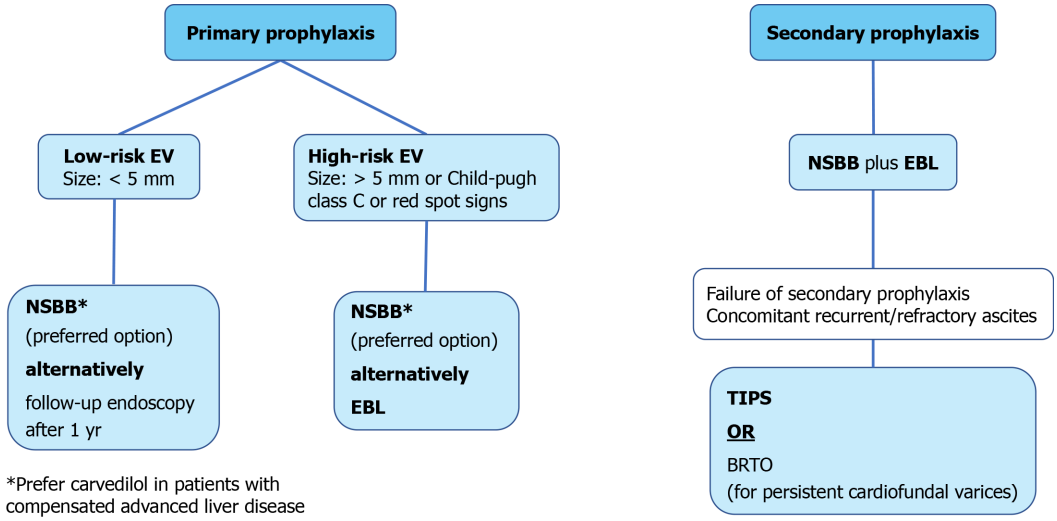

Figure 1 Clinical algorithms recommended for cirrhotic patients in primary prophylaxis and secondary prophylaxis (adapted from the Austrian Billroth-III guidelines)[3]. EV: Esophageal varices; NSBB: Non-selective betablocker; EBL: Endoscopic band ligation; TIPS: Transjugular intrahepatic portosystemic shunt; BRTO: Balloon occluded retrograde transvenous variceal obliteration.

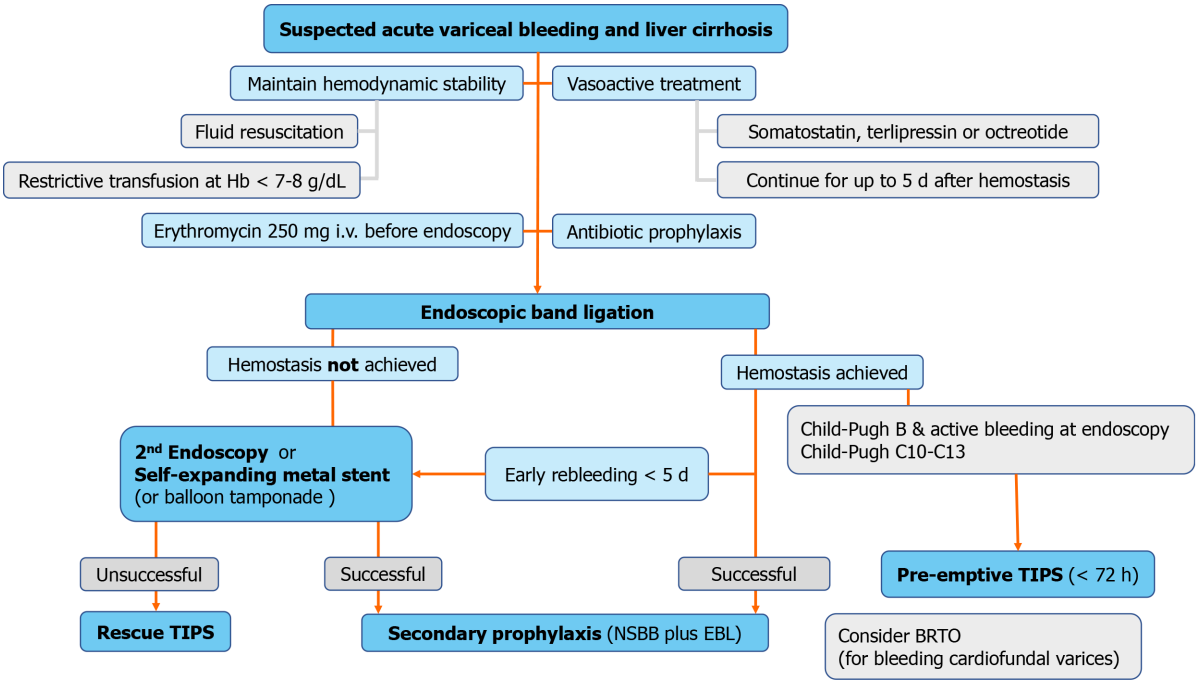

Figure 2 Clinical algorithm for treatment of patients with acute variceal bleeding (adapted from the Austrian Billroth-III guidelines)[3]. TIPS: Transjugular portosystemic shunt; i.v: Intravenous; NSBB: Non selective betablocker; EBL: Endoscopic band ligation; BRTO: Balloon occluded retrograde transvenous variceal obliteration.

To counteract active bleeding, vasoactive drugs (vasopressin, terlipressin, somatostatin or octreotide, dosing regimens summarized in Table 2) have been shown to reduce portal pressure by reducing portal systemic collateral blood flow, portal blood flow and intravariceal pressure *via* systemic and splanchnic vasoconstriction[17,78,79]. Thus, they are recommended for use in patients with acute variceal bleeding, while none of the vasoactive treatments has been shown to be superior to the others in terms of bleeding control and impact on mortality[3,17,22,80,81]. Of note, however, terlipressin has been associated with hyponatremia, especially in patients with preserved liver function and sodium levels should therefore be monitored, although these systemic sodium alterations did not translate to any outcome difference[80].

In addition to fluid resuscitation and administration of vasoactive drugs, antibiotic treatment is indicated as patients with acute variceal bleeding suffer from a significant risk of infection[82]. Thus, intravenous broad spectrum antibiotics (*e.g.* ceftriaxone at a

Table 2 Recommended vasoactive agents for management of acute variceal bleeding [adapted from the Austrian (Billroth III), European (Baveno VI) and American (Guidance by the AASLD 2017) guidelines][3,17,22]

Regimen	Dosing	Duration of regimen	Further guidance
Somatostatin	Bolus of 500 μg, followed by 500 μg/h *via* continuous infusion (6 mg/50 mL, infusion rate of 4.2 mL/h)	2-5 d	Bolus can be repeated in case of uncontrolled bleeding
Terlipressin	Bolus of 2mg every 4 h for the first 24-48 h, followed by giving bolus of 1mg every 4 h; Or continuous infusion 2 mg/d; maximum 12 mg/d	2-5 d	Be caution in patients with coronary artery disease, peripheral arterial occlusive disease hyponatremia (< 125 mmol/L), cardiac arrhythmia and severe asthma or chronic occlusive pulmonary disease
Octreotide (somatostatin analogue)	Bolus of 50 μg, followed by 50 μg *via* continuous infusion	2-5 d	Bolus can be repeated in case of uncontrolled bleeding

dose of 1g every 24 h with a duration for 7 d or less) should be administered before endoscopic therapy is initiated[3,17,22]. In addition, erythromycin should be administered ideally 30-120 min before endoscopy to improve sight during the procedure *via* facilitation of gastric emptying[3,17,22,83].

Finally, EBL is the gold standard of endoscopic treatment after hemodynamic stabilization and should ideally be performed within the first six to twelve hours of admission when EV bleeding is suspected or detected[3,17,22,84,85]. Performing endoscopists should be adequately trained, and EBL has been proven to be the best available treatment in terms of rebleeding, further development of esophageal strictures, and associated mortality[86].

Recently, however, data suggests that instead of vasoactive drugs and endoscopic therapy, preemptive implantation of a transjugular intrahepatic portosystemic shunt (TIPS) to lower portal pressure can be effective. An international multicenter observational study compared pre-emptive TIPS to endoscopy plus vasoactive drugs in patients with Child-Pugh C or Child Pugh B cirrhosis with active bleeding at the time of endoscopy[87]. The authors found that pre-emptive TIPS implantation, compared to standard of care with medication and endoscopic treatment, significantly reduced treatment failure and rebleeding in Child-Pugh C, and Child-Pugh B patients with active bleeding. This translated into a significantly lower mortality rate in Child-Pugh C patients, while mortality in Child-Pugh B patients with active bleeding were low in both, EBL/medication and TIPS, groups and did not improve by pre-emptive TIPS implantation[87]. Thus, pre-emptive TIPS implantation emerges as a valid option in patients with high risk of rebleeding, especially in Child-Pugh C patients.

Therapy-refractory variceal bleeding

These favorable results are in line with findings in patients with therapy refractory acute variceal bleeding in which rescue-TIPS implantation is the best choice when standard treatment fails[3,17,22]. Rescue-TIPS, *e.g.* TIPS implantation after EBL failure to control bleeding, achieves bleeding control in 90%-100% and results in very low rebleeding rates of approximately 15%[88]. However, despite the available encouraging results, use of TIPS in acute settings is limited by technical challenges and availability[89,90]. Therefore, balloon tamponade (Sengstaken tube and Linton-Nachlas tube) is the most commonly used treatment for uncontrolled bleeding in real-world settings. By compressing bleeding varices, it controls EV bleeding in up to 90%, but half of the patients suffer from rebleeding events after deflation of balloon tamponade[91-95]. Furthermore, it is associated with often life-threatening complications in 60% of patients, such as perforation, esophageal ulceration and aspiration pneumonia[91-94,96,97]. Additionally, balloon tamponade can only be left *in situ* for 24-48 h due to the high risk of pressure-induced necrosis[98].

As these high complication rates are considered unacceptable in modern medicine, a self-expanding metal stent (SEMS), SX-ELLA Stent Danis, has been developed to improve procedure related complication rates. It can easily be deployed without endoscopic guidance and can be left *in situ* for up to seven days. Several studies showed successful bleeding control in 70%-100% of patients[99-101] with lower complication rates than balloon tamponade, although this did not improve mortality rates[102,103]. Current guidelines nevertheless recommend the use of SEMS because of its better safety profile[3,17,22].

On the basis of these poor outcome data, balloon tamponade and SEMS are usually only used as a bridging to further definitive therapy, such as TIPS implantation. Despite this large body of favorable evidence, however, we recently reported a lack of systematic use of TIPS implantation after SEMS in acute variceal bleeding[101]. This is in line with recently published real-life data from France which showed that approximately 1/3 of patients with variceal bleeding fulfilled the criteria for early TIPS, but only 7% underwent subsequent early TIPS implantation[90]. This knowledge gap on TIPS indication criteria was also evident in our recently published survey in which only 20% of the respondents could report TIPS criteria correctly[104]. Therefore, knowledge on early TIPS implantation must be improved among all specialists.

Furthermore, in case of additional cardiofundal variceal bleeding and/or ongoing variceal bleeding after TIPS implantation, balloon occluded retrograde transvenous variceal obliteration (BRTO) should be considered[3,105-107]. A recently published meta-analysis showed improved outcome in terms of rebleeding, mortality and hepatic encephalopathy in patients who also underwent BRTO as compared to patients who only underwent TIPS implantation[106].

PREVENTION OF ESOPHAGEAL VARICEAL REBLEEDING

Secondary prophylaxis of EV bleeding

Secondary prophylaxis is defined as the prevention of recurrent variceal bleeding. Patients who survive and recover from an episode of acute variceal bleeding are at high risk of rebleeding and death, which is 60% and 33% in the first year, respectively [17,108]. Older studies found that HVPG measurement at the time of the first bleeding episode can predict rebleeding risk, and a HVPG of ≥ 20 mmHg was associated with a significantly increased risk for rebleeding and death[109]. Despite several non-invasive scores (APRI, FIB-4, AST/ALT, King´s score) are available, their role as non-invasive predictors for the presence of esophageal varices in patients with cirrhosis is not established. Kraja *et al*[110] showed that the FIB-4 is a powerful predictor of EV (cut off value: 3.23; AUC: 0.66, 95%CI: 0.54-0.78) but a poor predictor for EV bleeding (AUC: 0.42, 95%CI: 0.28-0.56) and that all other non-invasive biomarkers were not useful. This is in line with several other available studies that showed great variation in accuracy in different populations and etiologies of liver cirrhosis[111-113]. Recently, Drolz *et al*[68] reported high bilirubin and larger size varices as risk factors for rebleeding within 30 d of prophylactic EBL, while reduced platelet counts, elevated INR, and decreased fibrinogen levels were associated with procedure-related bleeding in other studies [113-115]. Another study showed an adequate prediction value for predicting in-hospital rebleeding using Child-Turcotte-Pugh score (cut off > 7) and Clinical Rockall score (cut off > 2)[116], while the well-established MELD and MELD-Na scores showed good results for predicting in-hospital mortality[116]. Thus, non-invasive prognostic scoring systems cannot be recommended to predict risk for recurrent variceal bleeding but are useful tools to estimate overall mortality rates[116-118].

In terms of secondary prophylaxis to avoid rebleeding, monotherapy of NSBB or EBL are associated with higher mortality in secondary prophylaxis than combined NSBB + EBL therapy, which is in contrast to studies in the primary prophylaxis setting [35]. Thus, current guidelines recommend the combination of EBL + NSBBs[3,17,22, 119,120], while the combined treatment of NSBB and low-dose isosorbide mononitrate, a combination used in the past, is no longer recommended due to high rates of adverse events[3,17,22].

With regard to NSBB choice, propranolol is recommended at a daily dosage of 80–160 mg/day in most guidelines, with a maximum dosing of 80 mg/day in patients with ascites[3]. Similar to primary prophylaxis, some guidelines also recommend carvedilol, while others do not (yet) recommend its general use[17,22]. Guidelines that do recommend carvedilol suggest to use it at a concentration of 6.25–12.5 mg/day and only in patients without ascites[3]. Finally, with regards to EBL for secondary prophylaxis, endoscopy and banding intervals are equal to the intervals in primary prophylaxis (complete eradication, re-endoscopy after 6 and 12 mo).

Similarly, when first-line therapy with EBL + NSBB to prevent rebleeding fails, TIPS implantation is the best choice for further treatment[3,17,22], as it decreases portal pressure and therefore targets the underlying cause of EV bleeding. Of note, however, no significant benefit on survival rates was found despite the better outcomes in terms of rebleeding rates[15,126,122]. In patients with gastric varices and contraindications for TIPS implantation such as spontaneous episodes of hepatic encephalopathy, BRTO can be considered as treatment option in selected patients, as it may even decrease

portosystemic shunting through the collaterals that are scheduled for occlusion[3]. Furthermore, surgical shunts, devascularization, splenectomy or (partial) splenic embolization may be considered if first-line treatments fail[3].

CONCLUSION

The continuous efforts of hepatologists and gastroenterologists around the world, as well as initiatives of international collaborations to generate high-quality evidence has translated to improved survival in patients with EV bleeding in the last decades. Thus, we have summarized recent advances and highlighted the rationale for specific treatments now recommended by several national and international guidelines.

In primary prophylaxis, NSBB or EBL are equal in outcomes and are therefore both recommended as monotherapies to prevent a first variceal bleeding event[3,17,22]. However, carvedilol – due to its higher potency to lower portal pressure[36] resulting in higher proportions of HVPG responders – may be the treatment of choice for primary prophylaxis in compensated cirrhosis. No clear recommendation for the use of betablockers can be made for patients with small varices (even with additional risk factors), as their efficacy in this setting remains unclear. Importantly, due to non-hemodynamic effects of NSBBs on intestinal permeability[34], systemic inflammation [124] and considering the results of the recent PREDESCI trial[31] showing reduced risk of decompensation and mortality, NSBB may already be recommended for small varices.

To monitor NSBB treatment response, invasive HVPG measurement is still considered as gold standard, but other non-invasive surrogates to monitor NSBB response to prevent variceal bleeding such as ultrasound-based elastography or transient elastography assessment of the spleen are currently under consideration as HVPG measurement is not widely available[125,126].

When acute variceal bleeding occurs, standardized treatment algorithms recommend conservative transfusion strategies, early initiation of vasoactive drugs, prophylactic antibiotics, and EBL[3,17,22]. More recently, the pre-emptive use of TIPS implantation in selected high-risk patients with variceal bleeding has been demonstrated to not only decrease rebleeding rates but also mortality[3,17,22,127,128].

Due to logistic challenges with the "time-critical" use of pre-emptive TIPS implantation, specialist should be familiar with this concept and infrastructure and networks need to be developed in order to improve the outcomes of patients with variceal bleeding.

In secondary prophylaxis, the combination of NSBB and EBL has proven to be superior to either monotherapy[3,17,22].

In conclusion, NSBBs remain the cornerstone of medical therapy of portal hypertension and are still used for pharmacological bleeding prophylaxis. EBL may also be used for primary prophylaxis, but its main role is in effective control of acute variceal bleeding and variceal eradication in secondary prophylaxis. Standardized concepts and the infrastructure for the general use of pre-emptive TIPS in selected patients with high-risk variceal bleeding need to be developed. This review should have provided clinicians with valuable concepts for the management of PH, including variceal screening, primary bleeding prophylaxis, management of acute variceal bleeding and finally effective secondary prevention of variceal rebleeding.

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REVIEW

Liver injury associated with drug intake during pregnancy

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Abstract

Drug use during pregnancy is not common. Drug-induced liver injury (DILI) is a potential complication that is rare but can adversely affect both the mother and the fetus. Although many drugs can directly cause hepatotoxicity, idiosyncratic liver injury is common in pregnancy. Underreporting of adverse drug reactions, lack of adequate literature regarding drug safety in pregnancy, and the inherent difficulty in diagnosing DILI during pregnancy make the management of this condition challenging. This review attempts to describe the existing literature regarding DILI in pregnancy, which is mainly in the form of case reports; several studies have looked at the safety of antithyroid drugs, antiretroviral drugs, and paracetamol, which have an indication for use in pregnancy; the relevant data from these studies with regard to DILI has been presented. In addition, the review describes the diagnosis of DILI, grading the disease severity, assessment of causality linking the drug to the adverse event, regulatory guidelines for evaluating the potential of drugs to cause liver injury, efforts to ensure better participation of women in clinical trials and studies in pregnant women population in particular, and the challenges involved in generating adequate research evidence. The establishment of DILI registries in various countries is an encouraging development; however, there is a need for promoting active, spontaneous reporting of adverse events during pregnancy to ensure rapid generation of evidence regarding the safety of a drug in pregnant women.

Key Words: Drug induced liver injury; Pregnant women; Liver failure; Adverse effects; Pregnancy outcome; Registries

Core Tip: Drug-induced liver injury is a rare but potentially life-threatening consequence of drug administration. Few drugs are indicated for use in pregnant

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women based on their lack of teratogenic risk; however, these can be hepatotoxic. This review collates information from case reports and other research studies to present the current knowledge regarding the hepatotoxic potential of drugs used in pregnancy. The challenges in diagnosis and methods for causality assessment are described. Attempts to generate evidence by formulating guidelines enabling the conduct of inclusive clinical trials involving women as well as reinforcing the pharmacovigilance activities by developing adverse event registries are described.

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INTRODUCTION

Liver injury is an uncommon but potentially life-threatening adverse consequence of drug administration. Although the marketing of a new drug entails substantial effort in ensuring drug safety, both in the pre- and post-marketing phase, the limited size of the population that can be formally monitored in a controlled setting of a clinical study makes detection of uncommon adverse events a challenging task. Drug-induced liver injury (DILI) remains one of the common post-marketing events leading to drug withdrawal or significant labelling changes[1]. An incidence of up to 24 cases per 100000 population has been reported; the exact incidence reported varies widely and is probably not a true reflection of the magnitude of the problem[2-4]. Moreover, the inter-drug risk is highly variable, with the risk of hepatotoxicity with azathioprine being 1 in 133[3] and for chlorpromazine being approximately 1 in 800 users compared with less than 10 per 100000 users for many other drugs[5]. Traditional and complementary medicines also contribute significantly to DILI burden to varying extent in different countries[6,7]. It is to be noted that drugs generally considered safe and used in pregnancy, such as cephalosporins, amoxicillin-clavulanate, ibuprofen, *etc*., are commonly implicated inciting drugs[8].

DILI is one of the least studied aspects of pregnancy. Although accurate estimates of liver disease incidence and prevalence during pregnancy are not available, a study conducted using a nationwide inpatient sample in the United States showed that the rate of liver disease among hospitalized pregnant women ranged from 0.3% for chronic and alcohol-related liver disease to 7.18% for liver disorders of pregnancy[9]; apart from the adverse health impact on the mother, cases of fetal liver injury and mortality have also been reported. In general, liver disease during pregnancy can be categorized into three types. First, liver diseases that are specific to pregnant women and tend to occur at a specific trimester. Second, diseases such as viral hepatitis which occur irrespective of the pregnancy status; third, pre-existing liver disease in a pregnant woman.

Most of the available literature regarding DILI in pregnancy is in the form of case reports. Though DILI has become the leading cause of acute liver failure in the United States and Europe[10] and acute liver injury is more likely to progress to acute liver failure in women[11], only a few studies concerning pregnant women are found. A study in the United Kingdom found that drugs accounted for 2.8% of the abnormal liver function tests in pregnant women[12]. Similarly, a study in Singapore reported that 2.1% pregnant women with abnormal LFT overall, and 3.4% women presenting in the third trimester, had DILI[13]. However, not all studies have been able to identify similar rates of DILI in pregnancy[14]. Difficulty in diagnosis or underreporting is likely to account for a significant number of such cases[12]; subclinical cases due to the use of over-the-counter and herbal medications are also likely to be missed, especially since spontaneous resolution occurs following the withdrawal of the inciting drug. Furthermore, under-reporting is all the more likely since the clinical presentation of liver injury may occur weeks to months following drug exposure.

DILI IN PREGNANCY

Drug intake during pregnancy, although requires careful discretion on the part of the physician as well as the expectant mother, is common[15,16]. Antimicrobials, antiemetics, and analgesics are the common categories of drugs used. The use of herbal medicines and dietary supplements, either inadvertently or based on personal and cultural beliefs of benefit, is common.

Liver injury due to drugs may be direct, idiosyncratic, or indirect[17]. The direct form is the commonest and has become the leading cause of acute liver failure in western countries[10]; it is related to the pharmacological properties of the drug, is dose-dependent, and can affect any individual. The idiosyncratic form is not predictable, is rare, has variable features, and affects susceptible individuals[18]. The indirect form occurs due to a drug exacerbating a pre-existing liver disease or inducing clinical manifestation of subclinical liver disease.

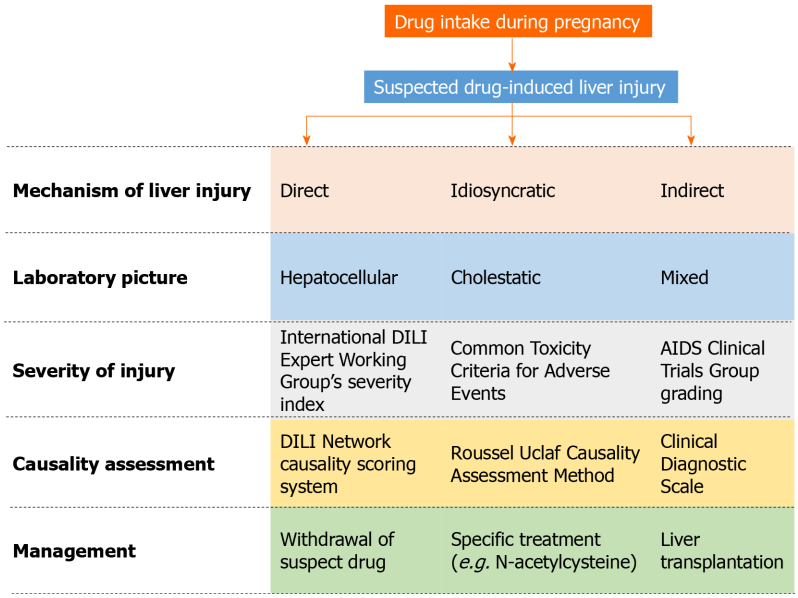
Drugs considered safe for use in pregnancy are known to cause idiosyncratic DILI. Co-morbidities like malnutrition, obesity, diabetes, and pre-existing liver disease may further intensify the risk of DILI during pregnancy[19]. Drug factors like the pharmacological class, dosage, and polypharmacy could also contribute[20]. Other factors that have a potential role in contributing to DILI causation include the circadian rhythm, presence of infection, intestinal microbiome, alcohol consumption, smoking status, environmental pollutants, and socioeconomic conditions[21]. The common medications reported in literature associated with DILI in pregnancy, such as paracetamol, alpha methyldopa, nevirapine, and propylthiouracil, are known for their safety and efficacy. Hence, an index of suspicion is important for the early detection of DILI in pregnancy.

Besides the above-mentioned factors, physiological changes that occur during pregnancy are also known to affect the pharmacokinetics of drugs. In particular, changes in the hepatic blood flow, microsomal enzyme activity levels, body fluid distribution, and serum albumin levels are important. There is a significant increase in the hepatic blood flow, mainly due to increased venous return[22]; this influences the metabolism of drugs with high hepatic extraction. Similarly, fall in serum albumin levels due to hemodilution can alter the pharmacokinetics of highly protein bound drugs, such as efavirenz[23]. An important change during pregnancy is in the hormonal milieu; this has significant effect on the hepatic metabolizing enzymes[24]. While the activity of a large number of cytochrome enzymes is increased, a decrease in activity is seen for CYP1A2 and CYP2C19[25]. The potential effect of such changes on the hepatotoxic potential of a drug would depend on whether it is the parent drug or its metabolite that causes the liver damage. In studies where specific drug use has a higher risk of hepatotoxicity in pregnant women compared with non-pregnant women, the mechanisms underlying the increased risk is unclear; for example, severe hepatotoxicity and temporary drug withdrawal during antitubercular therapy has been shown to be more frequent in pregnant women[26]. Similarly, nevirapine-induced hepatotoxicity is more frequent in pregnant women[27]. It is to be noted that in both the above examples, it is pregnancy, rather than the drug, which is a risk factor for hepatotoxicity, suggesting that the changes that occur during the pregnant state influence the likelihood of a drug to cause hepatic damage. However, it is to be noted that while there are several studies of changes in drug pharmacokinetics in pregnancy and several pharmacokinetic models have been developed to predict these[28], the actual clinical significance of these changes has not been adequately studied[29].

The management of DILI in pregnancy is similar to that in the non-pregnant population, in that the suspect drug is discontinued based on the clinical feasibility and risk-benefit assessment[30]. Although glucocorticoids have been used in severe cases, there is no adequate evidence to support their use; moreover, their use in pregnancy is associated with a higher risk of inducing diabetes[31]. Liver transplantation is also an option to be considered in severe cases.

DILI ASSESSMENT

Various algorithms, scales, and decision pathways have been proposed for the diagnosis, causality assessment, and grading of severity of DILI (Figure 1). The initial step is to suspect DILI; although an obvious case of liver injury may present with symptoms of hepatitis prompting an enquiry into the possible causes, a number of cases may go unaware initially unless alerted by an abnormal liver chemistry result. The challenge further is to determine whether liver injury is drug-induced, partic-



Mechanism of liver injury	Direct	Idiosyncratic	Indirect
Laboratory picture	Hepatocellular	Cholestatic	Mixed
Severity of injury	International DILI Expert Working Group's severity index	Common Toxicity Criteria for Adverse Events	AIDS Clinical Trials Group grading
Causality assessment	DILI Network causality scoring system	Roussel Uclaf Causality Assessment Method	Clinical Diagnostic Scale
Management	Withdrawal of suspect drug	Specific treatment (*e.g.* N-acetylcysteine)	Liver transplantation

Figure 1 Overview of drug-induced liver injury management including various grading scales and assessment methods. AIDS: Acquired immunodeficiency syndrome; DILI: Drug-induced liver injury.

ularly in the presence of pre-existing or new-onset liver disease. Although a correlation is not always present, DILI can be classified as hepatocellular, cholestatic, or mixed based on the initial liver enzyme levels at the time of clinical presentation [32]. The ratio of alanine aminotransferase (ALT) to alkaline phosphatase (ALP) normalized to the upper limit of normal indicates the biochemical nature of the liver insult; a ratio ≥ 5 suggests hepatocellular injury, ≤ 2 suggests cholestatic injury, and 3-4 suggests a mixed pattern of injury. Aspartate aminotransferase values can be used to determine the liver injury pattern in the absence of availability of ALT data; gamma-glutamyl transferase is considered less reliable as an ALP substitute[33]. The biochemical tests may be supplemented with imaging and biopsy to determine the liver histology and rule out alternative causes of liver injury. Each hepatotoxic drug is more likely to be associated with a specific pattern of liver injury[34]; this may help in narrowing down the suspected medications or confirming DILI.

DILI rank is a database that consists of 1036 Food and Drug Administration-approved drugs that are divided into four classes based on their potential for causing DILI; most-DILI-concern drug, less-, no-, and ambiguous-DILI-concern drug[35]. Screening of this database will further help in associating a drug with an event. In terms of causality assessment, general assessment scales, such as the World Health Organization-Uppsala Monitoring Centre scale and Naranjo scale, lack validity and reproducibility; assessments based on expert opinion, such as the DILI Network (DILIN) Causality Scoring System, are limited by lack of availability of such expertise in usual clinical care[36]. A widely used tool specific for DILI is the Roussel Uclaf Causality Assessment Method (RUCAM). This scale by the Council for International Organizations of Medical Sciences, consisting of seven domains, includes weighted scoring of an event according to "the temporal relationship between exposure to a particular drug and the liver injury (both its onset and course), exclusion of alternative non-drug-related etiologies, exposure to other medications that could explain DILI, risk factors for the adverse hepatic reaction, evidence in the literature regarding DILI from the drug in question and response to re-exposure to the medication"[33]. However, it is relatively complex and involves workup to collect all the relevant data before arriving at a conclusion. Modifications have been done to the RUCAM scale to overcome some of its limitations; these include the Clinical Diagnostic Scale and Digestive Disease Week Japan 2004 Scale[37]; however, their performance is not significantly better than RUCAM which remains a useful tool, both in the context of clinical trials and routine assessment, to be used in DILI cases[38].

Determining the severity of DILI helps in provisioning appropriate care and prognostication. Severe DILI is one of the factors associated with mortality and chronic liver injury, although a majority of the cases will resolve completely[39]. Various DILI severity categorization schemes have been developed that take into consideration a combination of factors such as liver enzyme levels, bilirubin level, presence of

comorbid liver diseases, hospitalization, literature evidence, *etc.* For example, the DILIN prospective study proposed a five-point system for grading severity based on ALT, ALP, total bilirubin levels, need for hospitalization, signs of hepatic failure, and death or need for liver transplantation[39]. The International DILI Expert Working Group's severity index consisting of four severity classes is in principle similar to the DILIN scale but does not take into consideration hospitalization[32]. The Common Toxicity Criteria for Adverse Events, developed by the Cancer Therapy Evaluation Program of the National Cancer Institute of the National Institutes of Health, is a commonly used grading scale for adverse drug events. The scoring is based on the levels of liver enzymes and total bilirubin. However, this general purpose grading scale has not been shown to correlate with the clinical outcomes; it categorizes liver enzyme/bilirubin levels but does not evaluate DILI per se[40]. A similar grading that uses slightly different lab value limits is that developed by the Acquired Immune Deficiency Syndrome Clinical Trials Group[41].

DRUGS CAUSING DILI

The case reports describing DILI in pregnancy have been summarized in Table 1. Literature evidence in the form of prospective/retrospective, mostly observational, studies has been summarized in Table 2. Some of the commonly implicated drugs for liver injury in pregnancy are described below.

Paracetamol

Paracetamol is one of the most commonly used agents for fever/pain and is used in pregnancy as well. However, it has been known from previous studies that it can cross the placenta and, in higher than recommended doses, may even harm the fetal and maternal liver cells[42]. There are case reports of liver failure warranting the need for liver transplantation during or immediately after pregnancy[43-45]. The presenting symptoms have been severe abdominal pain, vomiting and signs of hepatotoxicity. The reasons for consumption of paracetamol have been for pain, self-medication, and in a couple of cases, even intentional poisoning has been reported[46,47]. Histology has shown acute fatty liver of pregnancy and toxin-induced injury consistent with paracetamoluse[43].

Fetal hepatocytes breakdown paracetamol into a variety of metabolites, some with a toxic activity that can directly damage the fetal hepatocytes. The antidote N-acetylcysteine has been seen to cross the placenta to combine with these metabolites [48]. Though the available data is sparse, it has been suggested that if *N*-acetylcysteine therapy, which is safe in pregnancy, is initiated early (within 16 h of paracetamol intake), the morbidity from paracetamol overdose can be significantly reduced[42]. Cases of intentional poisoning by ingestion of paracetamol have been reported. In both cases the fetal outcome was favorable, and both the patients recovered without sequelae[46,47] (Table 3).

Antithyroid drugs

Hyperthyroidism is a common endocrine disorder affecting 2% of females and 0.5% of males worldwide. Most of the times, anti-thyroid drugs are the mainstay of treatment. However, these drugs are also known to cause several side effects. Liver failure is a rare yet life-threatening adverse effect of these drugs[49]. In the case of the latter, post-mortem histology showed submassive necrosis[50]. Though hepatotoxicity is common, otherwise uneventful pregnancies with successful outcomes have been reported widely. In many such cases, propylthiouracil was changed to carbimazole leading to the resolution of the liver injury[51,52]. However, few severe cases of fulminant hepatitis that needed liver transplantation have also been reported[53-55]. Though fetal outcomes have been largely favorable, cases with adverse outcomes such as fetal growth restriction, oligohydramnios, frequent episodes of focal seizures, delayed developmental milestones, have been reported[53]. Transient thyrotoxicosis and signs of acute hepatic injury have also been reported[56,57].

Antiretroviral drugs

The role of nevirapine in causing hepatic damage more frequently in pregnancy is known, although conflicting results regarding the same have been reported[27,58,59]. The treatment duration is likely to play a significant role in the causation of hepatotoxicity. A shorter course of nevirapine for human immunodeficiency virus (HIV) prophylaxis is seen to be linked with fewer hepatotoxic reactions for non-HIV-infected

Table 1 Data available from case reports regarding drug-induced liver injury in pregnant women

Suspect drug	Pathological finding(s)	Outcome in mother	Outcome in child
Azithromycin[78]	Intrahepatic cholestasis	Recovery without sequelae	Birth by caesarean section
Chlorpromazine	Severe reduction in the number of bile ducts; marked cholestasis and pseudoxanthomatous transformation of ductular epithelia and hepatocytes in the region of the limiting plate; progressed to cirrhosis[85]; Ductopenia, long-standing cholestasis with pseudoxanthomatous transformation of hepatocytes and ductular epithelia[84]	Prolonged liver disease culminating in vanishing bile duct syndrome and cirrhosis [85]; Gradual resolution with non-active periportal and septal fibrosis[84]	Premature birth by cesarean section [84,85]
Combination antiretroviral therapy	Fulminant hepatitis[105]	Recovery without sequelae [70,105]; death[105]	Nonreassuring fetal testing; improved following drug withdrawal; normal delivery[70]
Human chorionic gonadotropin and follicle stimulating hormone for *in vitro* fertilization[87]	Cholestasis	Recovery without sequelae	Premature birth by cesarean section
Methyldopa	Cytolytic hepatitis and cholestasis, toxic hepatitis [106]; hepatitis[73,74,107,108]	Improved following drug withdrawal[72-74]	-
Nitrofurantoin[109]	Toxic liver damage	Recovery without sequelae	Normal
Paracetamol	Acute fatty liver of pregnancy and toxin-induced injury[43]; fulminant hepatitis[45]	Liver transplantation[43,45]	Fetal death[43]; intrauterine fetal demise with extensive pericerebral and intraventricular hemorrhage with extensive periventricular leukomalacia[45]; intracranial hemorrhage, fetal hepatotoxicity[110]; preterm birth[111]
Propylthiouracil	Liver necrosis[50,53,54,112]; widened portal triads, and lymphoplasmocytic infiltrate[50]; hepatitis[52]; portal hepatitis[112]; acute liver failure[55]	Liver transplantation[53,55]; recovered[52,54]; death[50]	Miscarriage[50,54]; Antenatal ischemic encephalopathy, delayed developmental milestones[53]; normal [52,55]; caesarian delivery[112]
Tetracycline[83]	Fatty liver	Death	-

individuals or pregnant HIV-infected women and the fetus. However, intake of nevirapine for ≥ 2 wk for prophylaxis has a higher risk of hepatotoxicity among non-HIV-infected individuals and HIV-infected pregnant women[60]. Various studies have also been conducted to study the relation between CD4 counts and the occurrence of nevirapine toxicity. It has been noted that initiating nevirapine-based antiretroviral regimens during pregnancy at higher pre-treatment counts (CD4 ≥ 250 cells/μL) increases toxicity risk and should be avoided. The severity of hepatotoxicity was also more[61-63]. However, there are conflicting reports regarding this aspect as well, as no correlation was observed between high CD4 counts and adverse events in some studies[64-67].

Hepatitis C coinfection has been implicated as a risk factor for hepatotoxicity in pregnant women on antiretroviral therapy as a higher risk of liver toxicity to combination antiretroviral therapy has been observed[68].

Overall, it has been largely observed that there is no direct association between antiretroviral therapy in pregnancy and harmful effects on the fetal liver or the hepatic parameters at birth. However, a detailed and regular follow-up would be recommended before ruling out the harmful effects of maternal ARV treatment[69]. Antiretroviral-induced hepatotoxicity presenting as non-reassuring fetal testing has been known, wherein a detailed assessment later revealed maternal metabolic acidosis and transaminitis[70].

Alpha methyldopa

Alpha methyldopa is one of the first-line drugs for hypertension during pregnancy due to its long-known safety profile. However, there have been reports of methyldopa-induced hepatitis cases in pregnancy[71-73], with a temporal relationship between drug exposure and serum liver enzyme elevations. Also, a rapid decrease of liver enzymes on withdrawal of the drug further supports this observation[72,74]. Postpartum methyldopa-induced hepatotoxicity, up to two months after delivery, has also been reported; despite a full recovery from the acute phase, a residual underlying hepatic fibrosis was reported[71].

Table 2 Studies other than case reports describing effect of drugs on maternal/fetal/neonatal liver function

Ref.	Study design	Study population	Suspected medication (s)	Study outcome
Snijdewind *et al*[68]	Retrospective, comparative	Pregnant women	Antiretroviral therapy and hepatitis C virus co-infection	Nevirapine use related to hepatotoxicity in pregnant as well as non-pregnant women; the risk is significantly associated with hepatitis C coinfection during pregnancy
Beck-Friis *et al*[26]	Retrospective, comparative	Pregnant *vs* non-pregnant	Antitubercular drug	Severe hepatotoxicity and temporary drug withdrawal more frequent in pregnant women compared to non-pregnant women
Mandelbrot *et al*[113]	Retrospective, comparative	Pregnant women	Atazanavir	Three women had abnormal liver enzyme levels; grade 3 bilirubin elevations in 5 patients; jaundice in 5 neonates requiring phototherapy.
Heaton *et al*[82]	Retrospective, case-control	General population including pregnant women	Doxycycline, tetracycline	Doxycycline potentially less hepatotoxic than tetracycline
McCormack *et al*[114]	Prospective, placebo-controlled	Pregnant women	Erythromycin estolate, clindamycin hydrochloride, placebo	Erythromycin estolate resulted in raised liver enzymes; use not advised in pregnancy
Tempelman *et al*[115]	Retrospective, comparative	Pregnant women	Highly active antiretroviral therapy	Nelfinavir or nevirapine containing regimens are safe and effective in pregnant women with HIV
Franks *et al*[77]	Retrospective	Women with isoniazid hepatitis	Isoniazid	A 2.5-fold increased risk of isoniazid hepatitis and 4-fold higher mortality rate in the prenatal clinic group compared to non-pregnant women.
Gupta *et al*[116]	Multicenter, double-blind, placebo-controlled, noninferiority trial	Women with HIV (efavirenz-based antiretroviral therapy) receiving isoniazid preventive therapy either during pregnancy or after delivery	Isoniazid	Risk of composite adverse pregnancy outcome was greater in those who initiated isoniazid preventive therapy during pregnancy than those during postpartum period; majority of liver enzyme elevations and symptomatic hepatitis occurred in postpartum period.
Sato *et al*[117]	Single-cohort interventional	Pregnant women with choriocarcinoma and high-risk gestational trophoblastic neoplasia	Methotrexate, etoposide, actinomycin D	Of the 23 patients who received methotrexate, etoposide and actinomycin D, treatment changed to etoposide and actinomycin D in 14 patients due to leukocytopenia, hepatotoxicity, and stomatitis.
Fang *et al*[118]	Single-cohort, prospective, interventional	Pregnant women	Nelfinavir	Of the 16 women studied, one developed serious adverse event of elevated AST; the drug was well tolerated in general.
Timmermans *et al*[59]	Retrospective, comparative	Pregnant and non-pregnant women	Nelfinavir, nevirapine	Nevirapine related hepatotoxicity more frequent in pregnant than in non-pregnant women.
Joy *et al*[119]	Single-cohort, retrospective, observational	Pregnancy women in third trimester	Nevirapine	Incidence of adverse events lower; study in larger cohorts recommended to determine the relationship between nevirapine hepatotoxicity and trimester use.
Natarajan *et al*[58]	Retrospective, comparative	Pregnant women	Nevirapine	Risk of nevirapine-associated toxicity not higher in pregnancy; CD4 counts not predictive of toxicity.
Kondo *et al*[65]	Retrospective, comparative study	Pregnant women	Nevirapine	Hepatotoxicity occurred in those with pre-treatment CD4 counts ≥ 250 cells/μL; no correlation between high CD4 counts and adverse events.
Phanuphak *et al*[66]	Retrospective, comparative	General population including pregnant women	Nevirapine	Pregnant women with high CD4 counts have higher rate of symptomatic hepatotoxicity.
Kondo *et al*[67]	Single-cohort, retrospective, observational	Pregnant women	Nevirapine	No correlation between high CD4 counts and adverse events; hepatotoxicity occurred only in pregnant women with CD4 counts > 250 cells/μL
Ouyang *et al*[120]	Prospective, comparative	Pregnant women	Nevirapine	No significant association between nevirapine use and liver enzyme elevation regardless of pregnancy status; pregnancy associated with increased hepatotoxicity.
Ouyang *et al*[27]	Retrospective, comparative	Pregnant women	Nevirapine	No increased risk of hepatotoxicity among HIV-infected pregnant women on nevirapine *versus* other drugs, including in those treatment naïve.
Peters *et al*[64]	Prospective,	Pregnant women	Nevirapine	Severe hepatotoxicity and rash higher with

	comparative			nevirapine than with nelfinavir; no association with CD4 counts.
Lyons *et al*[62]	Single-cohort, retrospective, observational	Pregnant women	Combination antiretroviral therapy	Women with more severe hepatotoxicity had higher pretreatment CD4 counts.
Jamisse *et al*[63]	Single-cohort, prospective, observational	Pregnant women	Nevirapine-containing combination antiretroviral therapy	Severe hepatotoxicity more common at higher CD4 counts in pregnancy.
Sheng *et al*[121]	Prospective, comparative	Pregnant women with high viral loads of hepatitis B virus	Nucleos(t)ide analogues	Telbivudine therapy was safe in pregnant women.
Zhang *et al*[122]	Disproportionality analysis	Pregnant women	Omeprazole, lansoprazole, amoxicillin	The risk of cholestasis associated with these drugs higher in pregnant women; re-assessment of safety recommended.
Cecchi *et al*[88]	Single-cohort, prospective, observational	Pregnant women	Organophosphate pesticides	Subclinical hepatotoxicity during the second trimester in spraying period.
Trakulsrichaia *et al*[123]	Single-cohort, retrospective, observational	Pregnant women	Paraquat poisoning	Hepatotoxicity more common in patients who died.
Andersen *et al*[57]	Single-cohort, observational	General population including pregnant women	Antithyroid drugs	Antithyroid drug-associated liver failure observed less frequently in pregnant women than in the general population.
Brunet *et al*[124]	Single-cohort, prospective, observational	Pregnant women	Saquinavir/ritonavir	Among the 58 women who received the drug, one developed severe grade 3 hepatotoxicity; in general, the drug was effective and safe.
Jharap *et al*[125]	Single-cohort, prospective, observational	Pregnant women	6-Thioguanine nucleotide, 6-methylmercaptopurine	Fetal exposure to 6-thioguanine but not to 6-methylmercaptopurine; 60% had anemia at birth; no major congenital abnormalities.

HIV: Human immunodeficiency virus.

Table 3 Case reports of drug poisoning/abuse and alternative medicine use resulting in liver injury during pregnancy

Suspect drug	Clinical finding(s)	Maternal outcome	Fetal outcome
Cocaine[126]	Hepatic rupture	Prolonged hospital stay	Emergency caesarian delivery
Paracetamol	Raised liver enzymes[46,47]; coagulopathy[46]	Recovery without sequelae[46,47]	Normal[47]; prematurity, respiratory distress, metabolic acidosis, full recovery[46]
Mushroom (Amanita species)[127]	Low prothrombin activity	Recovery without sequelae	Normal
Mountain germander (*Teucrium polium*)[128]	Raised liver enzymes	Recovery without sequelae	Normal

Antitubercular drugs

Studies in the past have reported that the risk of hepatotoxicity to antitubercular drugs is significantly higher in pregnancy. Temporary drug withdrawals due to elevated transaminase levels were more frequent for pregnant than non-pregnant women, and cases of fatal hepatotoxicity have also been reported. The reason for the increase however has not been elucidated[26].

Administering isoniazid to prevent tuberculosis constitutes isoniazid preventive therapy (IPT); the benefit of treating active tuberculosis in pregnancy plus providing isoniazid preventive therapy to minimize the risk of developing active tuberculosis in persons with HIV, has been seen. However, data regarding the benefit of IPT in pregnant women who are on antiretroviral therapy is sparse, owing to the fact that pregnant women have usually not been included in various trials of isoniazid preventive therapy[75,76].

Studies have reported increased isoniazid toxicity among pregnant women as well [77]. From the limited data on IPT available so far, a higher incidence of unfavorable pregnancy outcomes, such as stillbirth or spontaneous abortion, has been reported. Also, the risks associated with initiating IPT during the postpartum period were seen to be lower than that associated with initiating it during the course of pregnancy[75].

Antibiotics

Azithromycin-induced liver injury has been rarely reported in the general population. There is a report of azithromycin-induced intrahepatic cholestasis in a pregnant woman; on withdrawal of azithromycin, the liver enzyme levels returned to normal within 4 wk without any symptoms after treatment with silymarin and bifendate, which help reduce ALT level and protect the liver from further injury[78].

A unique case of drug-induced mononucleosis-like hepatic injury in a patient with systemic lupus erythematosus has been reported following the administration of multiple antibiotics. An allergic reaction to the administered drugs was implicated based on a positive lymphocyte stimulation test[79].

Tetracycline is another antibiotic that has been known since decades for its potential to cause hepatic adverse events[80]. Tetracycline-induced liver injury typically causes fatty infiltration of the liver. The presence of kidney dysfunction and pregnancy are some of the risk factors for hepatotoxicity to tetracycline[81,82]. Fatal hepatotoxicity to tetracycline, when given in pregnancy, has also been reported, and post mortem examination has shown major histological changes in the liver along with fatty degeneration of the renal tubular epithelial cells[83].

Miscellaneous drugs

Individual case reports implicating other drugs, herbal medicines, and dietary components (Table 3) have also been described. Cholestatic liver disease in a pregnant woman in the 33rd week of pregnancy who received chlorpromazine and chlorprothixene has been reported; no signs of liver damage were present in the newborn[84]. A case of a primary biliary cirrhosis-like syndrome that developed after 2 wk of chlorpromazine therapy has also been reported[85]. A case of intrahepatic cholestasis of pregnancy, worsening after dexamethasone administration has also been reported [86]; however, the authors concluded that it was more likely due to the progression of the primary disease rather than drug-induced. Cholestasis developing following *in vitro* fertilization and ovarian hyperstimulation syndrome is also known[87].

Reports of the effect of environmental xenobiotics on pregnancy have also been reported. A prospective study conducted in a rural area where organophosphates were intensively applied, found that the liver enzymes were raised in the spraying period, which could be indicative of subclinical hepatotoxicity. Though the offspring at birth were normal, a follow up would be required to assess the delayed effects of raised maternal cortisol during pregnancy[88].

REGULATORY GUIDELINES FOR CLINICAL EVALUATION OF DRUGS FOR DILI IN PREGNANCY

Clinical trials seldom study drug effects in pregnant women due to ethical and safety concerns, unless the drug is to be specifically used in pregnant women. In fact, even in the case of non-pregnant females, the inclusion of females in eligible clinical trials is significantly less than men despite the regulatory intent of ensuring adequate participation opportunities[89]. The findings of drug studies in the general population regarding the effect of hepatic function on the drug kinetics and dynamics, including the possible toxic effects of drugs on liver, are generally applicable to pregnant women; however, the physiological changes that occur during pregnancy need to be considered in determining how the drug effects are likely to be affected.

DILI is often rare; although good, the relative rarity of the event also makes its detection during the clinical trial phase difficult. For example, most known drug-hepatotoxicity events occur with an incidence of < 1 in 10000; hence, such events are seldom detected during a clinical trial. Keeping this issue in mind, regulatory guidelines emphasize the need to detect lesser grades of liver injury, which may not necessarily manifest clinically/symptomatologically, but are potential markers for occurrence of serious liver injury if used in the wider population[90]. Accordingly, drugs which not only cause elevation of liver enzymes but also impair bilirubin metabolism or affect clotting factor synthesis are likely to cause severe liver injury. In general, considering the occurrence of mild elevations in liver enzyme levels even in placebo/control groups, an isolated 3-fold elevation is considered the minimum threshold for concern[90].

The above-mentioned aspects are also applicable to drug use in pregnancy. Although drug use is to be discouraged during pregnancy to the extent possible, studies show that a large number of women do receive drugs for various reasons[91-

93]. Regulatory guidelines encourage that drugs to be used specifically in pregnancy or includes an indication for use in pregnant women for a general indication should be studied in the pregnant population[94-96]. These may be studies conducted exclusively among pregnant women or in the general population that does not exclude subjects who are pregnant. Such studies provide useful data regarding the potential safety of the drug in relation to liver function, although the limited sample size of such studies precludes arriving at definite conclusions. The safety update reports from drug manufacturers, based on drug use in the general population as well as the pregnancy exposure registries, may provide information regarding the hepatotoxic potential of a drug; the latter are not regulatory in nature but do provide vital information in this population. The increasing emphasis on pharmacovigilance activities in various countries is also expected to contribute to earlier identification of DILI in pregnancy. However, the reporting of adverse drug events in pregnant women has so far been low[97,98]; underreporting is the norm, and much needs to be done to improve reporting. Most of the DILI cases have been identified through published case reports, with some of these forming the basis for specific clinical studies in pregnant women, particularly for antiretroviral drug-associated hepatotoxicity. The regulatory mandated section of drug effects in pregnancy in the drug labels is a good source of information regarding drug safety specifically in pregnancy for prescribers[99].

CHALLENGES FOR EVIDENCE GENERATION

Besides the lack of adequate representation of females in clinical trials, assessment of the hepatotoxic potential of a drug in pregnant women has two important challenges. The first is a general challenge, not limited to pregnant women, of differentiating liver injury incited by drugs in contrast to that by liver disease; the challenge arises due to lack of any specific clinical or biochemical marker for drug-induced injury. Hence, clinical and medication intake history and knowledge regarding the pharmacology of the suspected medication to a large extent dictates the identification of the cause of injury. Large adverse event databases, which contain spontaneously reported adverse events from consumers and healthcare professionals, are excellent sources for determining a signal[100]; however, the lack of adequate recording of history/sequence of events in these spontaneous reports often precludes any definitive conclusions to be made. The second challenge is to differentiate DILI from intrahepatic cholestasis of pregnancy, which is not uncommon[101,102]. These challenges are compounded by the infrequent identification and reporting of such cases. Given the hurdles, spontaneous active reporting by health professionals and patients seems to be the most appropriate way for evidence generation, supplemented by the safety data from pre- and post-market approval clinical studies. Recognizing the inability to identify potential hepatotoxic drugs during clinical trials and the immediate post-marketing period, a number of regions/countries have started DILI registries to gather data regarding cases of potential DILI so that the data can be collectively evaluated to identify signals[103-105].

CONCLUSION

DILI is a real concern in pregnancy, although most of the cases have a favourable outcome and require only withdrawal of the drug. Advances in diagnostic modalities and access to liver transplantation have further improved the outcomes. Most of the DILI cases during pregnancy go unreported; there is a need to capture these incidents efficiently to ensure an informed decision can be made regarding drug use in pregnancy. The establishment of DILI registries in various countries is encouraging and will add significantly to this effort.

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MINIREVIEWS

Racial differences in prevalence and severity of non-alcoholic fatty liver disease

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Abstract

The aim of this review is to assess the evidence regarding racial differences in the prevalence and severity of nonalcoholic fatty liver disease (NAFLD). We reviewed the published literature that reported prevalence, severity, and genetic associations of NAFLD in different ethnic groups. The metabolic syndrome (MetS) has been associated with NAFLD, but each component of the MetS is present in various races in different percentages and their effect on NAFLD appears to be dissimilar. An elevated triglyceride (TG) level seems to have the strongest association with NAFLD. The latter is more prevalent in Hispanic patients; Blacks have lower TG levels and a lower NAFLD prevalence, compared to Caucasians or Hispanics. The severity of liver fibrosis is lower in some, but not all biopsy-based studies of Black patients. No study has evaluated the severity of liver disease controlling for the individual components of MetS, especially TG. Important racial differences in the prevalence of selected genetic polymorphisms, particularly PNPLA-3 and MBOAT7 have been documented, together with their effects on the prevalence of liver steatosis and fibrosis. Data on overall and liver mortality have found no significant differences according to race/ethnicity, with the possible exception of one paper reporting lower cirrhosis mortality in Black patients. We conclude that NAFLD is more prevalent in Hispanics and less in Blacks. This is supported by differences in key genetic polymorphisms associated with hepatic

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fat storage. However, there is presently insufficient evidence to firmly conclude that race, per se, plays a role in the development of liver fibrosis and its complications. Further studies, appropriately controlled for diet, exercise, and individual MetS parameters are needed.

Key Words: Race; Ethnicity; Nonalcoholic fatty liver disease; Fatty liver disease; Metabolic syndrome

Core Tip: Nonalcoholic fatty liver disease is one of the most common diagnoses made in a Gastroenterology practice. The prevalence and severity of nonalcoholic fatty liver disease in different ethnic groups need to be evaluated by controlling for the individual variables of the metabolic syndrome. This is because these variables are different in various ethnicities.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common diagnoses made in a gastroenterology practice. Several articles suggested differences in the prevalence and severity of liver disease according to patient race/ethnicity. If such differences were proven, this would have an important impact on resource allocation to decrease health disparities. Thus, it is imperative that the available literature be critically reviewed and existing knowledge gaps, if any, identified.

RACE AND NAFLD

Definitions

NAFLD is a condition marked by excess fat storage accounting for > 5% of the liver's volume in the absence of known alcohol abuse[1]. The latter is usually defined as the use of > 20 g alcohol/day for women and > 30 g/d for men[2], although lower limits have been used[3]. No study addressing race differences has verified absence of alcohol by testing hair for alcohol or using blood phosphatidylethanol levels[4,5]. The diagnosis is usually inferred by imaging studies, typically an ultrasound showing increased hepatic echogenicity[6,7]. Elevated alanine aminotransferase (ALT) in the absence of known competing causes has also been accepted as "suspected NAFLD" [7]. It is also crucial to differentiate primary *vs* secondary causes (medications, genetic or nutritional disorders); however only approximately 12% of studies excluded the latter[8].

We accepted the authors' race classification, which was typically based upon self-reporting. We recognize that race and ethnicity are "constructs that have no clearcut definition"[9]. It is important to keep in mind that Hispanics and Asians include significantly heterogeneous sub-populations[3,7,9].

Since Asians are underrepresented in most United States studies, this review will focus on Blacks (or African-Americans), Hispanics (or Latinos) and Whites (or Caucasians).

For the purpose of this paper, we will accept that the alcohol history is accurate, that a compatible ultrasound and/or elevated transaminases in the appropriate clinical setting are reasonable diagnostic tools, and that all reported cases are primary NAFLD.

Specific aim

To assess the strength of evidence suggesting that race-ethnicity in adults is associated with not only prevalence, but also with severity and prognosis of NAFLD.

Methods

We queried the PubMed English language database using the following keywords in the title or abstract: "fatty liver", "nonalcoholic fatty liver disease", "NAFLD", "liver or hepatic steatosis", "steatohepatitis" AND "race" or "ethnicity". We eliminated articles including alcoholic liver disease or HIV infected patients. We restricted this narrative review to adult populations.

Prevalence of NAFLD by race/ethnicity

The prevalence of NAFLD is reported to be highest in the Middle East (32%) and South America (31%), followed by Asia (27%), and Europe (23%)[10,11]. In Africa and India, the prevalence of NAFLD is approximately 9% of the population[12,13].

The most recent estimate places the United States prevalence of NAFLD at about 32%[14]. The United States is unique due to its mix of various races and ethnicities, while maintaining relative homogeneity in terms of geography and alimentary patterns. Therefore, it seems like an optimal population to study to uncover potential racial differences in disease.

A recent meta-analysis[8] shows that in population-based cohorts (*i.e.*, not high-risk patient groups such as diabetics) 23% of Hispanics have NAFLD, *vs* 14% of Caucasians, and 13% of African Americans. These percentages translate into a higher relative risk (RR) for Hispanics being diagnosed with NAFLD (RR = 1.5), and lower for African/Americans (RR = 0.7) compared to Whites[8]. If one focuses on patient subgroups that are at high risk for NAFLD, these differences become smaller (Hispanics RR = 1.2 and African-American RR = 0.8) but remain significant[8]. Interestingly, a NHANES based study[6], not included in the above meta-analysis, also found that Hispanics have a RR for NAFLD of 1.7 and African-American a RR of 0.8 compared to Whites: however when restricted to 'never drinkers', those differences are no longer significant, implying that small amounts of alcohol may have different effects on different races[6]. Thus, despite higher rates of HTN and insulin resistance, African-Americans have a lower prevalence of NAFLD[1,6,15-18].

There is relatively little written about Asian patients other that the prevalence may be about 25% in Asia[19], but may be lower in US-residing Asians, where NAFLD is noted in 20%[14]. A summary of the estimated prevalence of NAFLD in the United States is shown in Table 1.

Prevalence of the metabolic syndrome by race/ethnicity

Contributors to the rising worldwide prevalence of NAFLD include non-modifiable factors like genetics, but also modifiable variables such as diet and lifestyle choices[7,21,22]. Identifying, quantifying and controlling for these factors will be useful to establish whether some groups may be at higher risk, and therefore help allocate resources to mitigate those differences[23].

Diet and exercise has been found to be different in different ethnic groups. Asians have better diets (measured with an adapted healthy eating index) than Caucasians who in turn have better diet scores than Latinos and Blacks[21]. In Hawaii, however, intake of fruits and vegetables was lowest in Japanese-Americans compared to Filipinos or Native Hawaiians[22]. Yet, it is not clear whether a better diet score necessarily translates into a lower NAFLD risk[21,22]; and if so, by how much.

Similarly, exercise habits appear to be different, highest in Caucasians and lowest in Asians[9,22]. This is important because exercise decreases intrahepatic fat by MRI, even in the absence of weight loss[24]. Unfortunately, in articles focusing on NAFLD, these potentially important variables have not been adjusted for.

Metabolic syndrome (MetS) is accepted as the major association with NAFLD. MetS is defined by the presence of 3 or more out of 5 criteria: Increased fasting glucose, central obesity/waist circumference, low high-density lipoprotein (HDL), elevated triglycerides (TG), and elevated arterial pressure. Meeting this definition is associated with future development of diabetes type 2 (DM) and cardiovascular disease (CVD)[23]. There are differences in the prevalence of MetS according to race ethnicity, in non-institutionalized adult individuals living in the United States. A recent assessment shows that the prevalence of MetS was 35% in Whites, 30% in Blacks, followed by Hispanics (termed "Mexican Americans") (29%)[15]. No increased prevalence was noted in the Latino population surveyed[15]. A United States military study looked at the incidence of MetS (by ICD-10 codes), and found the highest was in Pacific-

Table 1 Estimated prevalence of nonalcoholic fatty liver disease in the general United States population (three main Race-ethnicities)

Ref.	Whites			Blacks			Hispanics		
	No.	Denom	Percentage	No.	Denom	Percentage	No.	Denom	Percentage
Rich *et al*[8]	24454	200510	0.12	3625	54790	0.07	5125	40591	0.13
Kallwitz *et al*[7]							1691	9342	0.18
Zou *et al*[14]	2229	4341	0.51	538	2833	0.19	1686	3886	0.43
Lim *et al*[20]	82	400	0.2	49	297	0.16	180	377	0.48
Foster *et al*[16]	189	1244	0.15	106	992	0.10	208	775	0.27
Total	26954	206495	0.13	4318	58912	0.07	8890	54971	0.16

Islanders, and the lowest in White personnel[25].

However, there are 3 important problems with MetS as a dichotomous variable. First, individual components of the MetS have a different distribution among races, elevated TG being more common in Latinos and White males and abnormal waist circumference in Blacks and White females[23]. In fact, the low TG levels in Blacks have been called "the TG paradox"[26]. Thus, African American patients have a higher body mass index (BMI) and similar prevalence of DM, yet they display a better lipid profile and therefore are less likely to have MetS compared to Hispanics (Table 2)[17]. The prevalence of DM is lowest in Whites (12%) and similar in Asians (19%), Blacks (20%) and Hispanics (22%)[18]. The latter group showed major heterogeneity, South American patients having less DM (12%) compared to other Latino groups[18].

Second, a diagnosis of MetS predicts the development of DM or CV disease differently in different races. For example, in patients with MetS, rates of incident DM are highest in Black males and females (17%) and lowest in white women (8%); whereas the rate of development of CVD is highest in White men (25%) and lowest in Black women (6%)[23]. Third, the association between individual MetS variables and NAFLD is not the same. In a recent study from China (Asian patients), NAFLD patients had higher levels of each of the 5 MetS parameters *vs* controls. However, when a multivariable analysis was run, adjusted for age and sex, the strongest association was with an elevated TG; the prevalence of NAFLD in the highest and lowest TG quartile was 50% *vs* 5 %[27]. Therefore a *z*-score, where the MetS is measured on a continuous scale (from -1 to +4) has been developed and shown to predict the development of diabetes and CVD better than the binary MetS[23]. When controlled for the z-score, Black individuals have double the rate of DM and higher rates of hypertension *vs* whites[16,23]. There are no data assessing the prevalence and severity of NAFLD, in patients matched by the *z*-score.

Fat distribution/obesity

Lean NAFLD (*i.e.*, with normal BMI) is found in as many as 5% of those with NAFLD in the United States[14] and this subgroup has a 65% chance of being metabolically abnormal, *i.e.*, fulfilling criteria for MetS[28]. On the other hand, overweight and obese NAFLD patients have a correspondingly higher chance of having MetS, 92% and 95%, respectively. Lean NAFLD seems more common in Asians *vs* other ethnic groups[14,20]. Elevated TG appears to be the commonality in patients with NAFLD, independent from BMI[17,27,28].

Patterns of visceral and liver fat depositions show ethnic differences and may contribute to the prevalence and severity of NAFLD. Total adiposity, measured by DEXA and MRI to account for visceral, liver and truncal fat was found to be highest in Japanese Americans and lowest in African Americans[17]. Interestingly, women had lower visceral fat area than men, except in the Japanese American group[20]. African-American adolescents have less visceral fat than either Hispanics or Whites[29].

A study using transient elastography and controlled attenuation parameter estimated hepatic steatosis and fibrosis in 2000 Korean patients. Obese (*i.e.*, BMI 25 or greater) but metabolically healthy (no MetS) individuals had greater liver steatosis and fibrosis than non-obese patients[30]. However, in the non-obese group, those with MetS, had higher steatosis estimates but similar fibrosis to those without MetS. BMI rather than MetS was the variable independently associated ($P < 0.001$) with both steatosis and fibrosis[30]. The Dallas heart study quantified visceral fat percentage by MRI in the general population: unfortunately, 3% to 8% of the individuals reported alcohol intake levels exceeding those used to define NAFLD[1]. The findings were that

Table 2 Prevalence of metabolic syndrome and its components in African Americans *vs* Hispanics[17]

	AA	H	*P* value
Percentage MetS	19	33	< 0.0001
% Diabetes	17	17	NS
Mean HDL	53	47	< 0.0001
Mean TG	107	160	< 0.0001
Mean BMI	31	29	0.008

AA: African Americans; H: Hispanics; MetS: Metabolic syndrome; HDL: High-density lipoprotein (mg%); TG: Triglyceride (mg%); BMI: Body mass index.

male Hispanic and White individuals had similar risk (42% to 45%) of having hepatic steatosis greater than 5.5 g TG per 100 g of liver tissue, much higher compared to Black males (23%). Women, both White and Black, had lower rates of abnormal hepatic steatosis (24%) compared to Latinas (45%). The fact that Blacks had higher HTN and Insulin resistance rates, but lower circulating TG levels, suggests racial and genetic differences in intrahepatic TG storage[1,16,20,31].

Genetics

Pathways of lipolysis or lipogenesis (MBOAT7, PNPLA3, TM6SF2,) are some of the genetic polymorphisms that have been linked to NAFLD prevalence and its severity [16,32-34].

In individuals of European descent, a T mutation in the MBOAT7 gene (rs641738) has been associated with severity of NAFLD in those with TT homozygosity[34]. Even the presence of one T polymorphism was associated with a small [odds ratio (OR) = 1.3] but significant risk of biopsy-proven F2, F3 or F4 fibrosis[34]. However, the association between the PNLPA3 G allele and F2-F4 was stronger (OR = 1.6)[34].

The PNPLA3 gene controls hepatic VLDL excretion, likely leading to hepatic TG accumulation; it may also sensitize the liver to environmental stressors, thus contributing to elevated transaminase levels in the presence of obesity[2]. The G allele mutation (rs738409), termed I148M (*vs* CC wild type) is a single-nucleotide polymorphism (SNP), which increases the risk of fat accumulation in the liver and thus NAFLD four-fold[17,32,33]. The G allele was found to be more frequent in Hispanics (40%) compared to Africans and Europeans (both 15%). In those with GG alleles, the risk of having NAFLD was similar in Asians and Caucasians (3-fold) and Hispanics (4-fold) but was much higher in Black patients (9-fold) compared to those with wild type genotype[35].

Within the United States population, the PNPLA3-G allele had a significant association with a non-invasive estimate of liver fibrosis, the FIB-4 score[7], but in one study this association was not clear (Table 3)[3]. The GG homozygosity has also been associated with a 5-fold increase in HCC risk[33]. A recent study from Sicily confirmed that the G allele (either heterozygous or homozygous) was associated with more advanced liver fibrosis[36]. In patients with stage 3 and 4 fibrosis, the G allele was associated with more liver decompensation, HCC and liver related death, despite a relatively small total number of patients followed (n = 471)[36]. Interestingly, 2/3 patients had the G allele and almost a quarter was homozygous GG[36].

In Hispanics with American ancestry (Mexican-, Central-, and South American), the frequency of PNPLA3-G is higher than in those of European or Afro-Caribbean background[3]. A small study in Hmong patients suggests that some Asian subpopulations have high rates of the G SNP and thus may have increased risk for NAFLD[37]. These findings underscore the existence of distinct and potentially relevant subpopulations within a traditional race/ethnicity group.

A minor allele (rs58542926) in transmembrane 6 superfamily member 2 (TM6SF2) was associated with hepatic TG content measured by magnetic resonance spectroscopy, in the Dallas Heart Study[1]. The C to T polymorphism decreases VLDL excretion, thus increasing TG concentration in the liver[33]. In addition, this TM6SF2 polymorphism was noted to increase the risk for hepatic fibrosis independent of age, obesity, diabetes, and PNPLA3 genotype[38]. On the other hand, the TM6SF2-T allele mutation E167K had similar low frequencies between Hispanics[3] and those from European ancestry and had a strong association with ALT levels[15].

Table 3 Percentage of patients with the *PNPLA3* G allele polymorphism and FIB-4 > 2.67[3]

	% PNPLA3-G allele	% Suspected NAFLD	% FIB-4 > 2.67
Mexican American	52	21	0.4
South American	51	20	0.3
Central American	48	23	0.9
Puerto-Rican	35	16	2.0
Cuban	28	16	1.8
Dominican	22	13	0.5

NAFLD: Nonalcoholic fatty liver disease.

NAFLD, liver fibrosis and liver complications

Several studies assessed metabolic factors associated with varying histopathological severity of NAFLD. There is agreement that the degree of steatosis is proportional to the number of elements of the MetS[7,16,20,32,39,40]. Additionally, one study showed that the MetS was associated with significantly greater risk of liver fibrosis stage 3 or 4 (33% *vs* 15% in those without MetS) and necroinflammation (61% *vs* 44%)[39]. The same study showed that in patients with NASH, 88% had MetS compared to 67% of those with simple fatty liver[39].

NAFLD is more prevalent in Hispanics[6,15,18,31], but the significance of this finding is debatable, as fibrosis is the only histological variable consistently associated with liver mortality[41]. While mortality in NAFLD patients is chiefly associated with cardiovascular events[42,43], it would be useful to tease out whether race independently affects the development of cirrhosis, and therefore liver mortality.

The fact that there is a relationship between elements of MetS and liver steatosis, inflammation and fibrosis[23,39] means that studies comparing liver disease severity between races must be controlled for the 5 MetS variables, keeping in mind that each may be more predictive in specific races.

The multi-ethnic cohort[44] looked at a United States population enriched with Asian minorities. The results showed that NAFLD was the most common cause of chronic liver disease in Japanese Americans (64% of those with liver disease) followed by Hawaiians (58%), Latinos (46%), Whites (41%) and Blacks (39%). When looking at the percentage of patients who had NAFLD-related cirrhosis (by ICD-9 codes) by race, the percentages were 4% (Japanese), 3.1% (Latinos), 1.7% (Whites) and 1.5% (Blacks)[44].

Dulai *et al*[42] reviewed 5 studies that assessed baseline liver fibrosis (mostly by biopsy) in patients with NAFLD or NASH. At baseline these 5 studies showed that most (67%) of patients had stage 0/1 fibrosis; 14% had F2; 12% F3 and 7% cirrhosis. Mortality was mainly cardiovascular related (about 40%) followed by cancer (20%) and liver disease (10%)[43]. There were no details comparing races within each study. In fact, one study had only Asians[45] and another 88% Whites[34]. A Canadian study did not mention race or ethnicity[46]. While baseline advanced fibrosis stage (F3/4) varied from 27% in Asians[45] to 12% in Whites[43], the percentages of MetS was also different (63% *vs* 33% respectively).

Within NAFLD, however, NASH on liver biopsy is less common in African-Americans (57%), but not significantly, *vs* Caucasians (73%)[47].

A recent meta-analysis[8] noted that 11 studies assessed stage of fibrosis (mostly by biopsy) in NAFLD and had data on race. The pooled proportion of patients with NAFLD and significant fibrosis (stages 3 and 4) was 19.5% [95% confidence interval (CI): 18.1-20.9]. The percentages were numerically highest in Whites (22.3%) and Hispanics (19.6%) and lowest among Blacks (13.1%). However, differences were not statistically significant for Whites *vs* Hispanics (RR 1.02, 95%CI: 0.94-1.11), and borderline significant for Whites *vs* Blacks (RR = 1.10, 95%CI: 1.00-1.22)[8]. A later paper showed that morbidly obese Black patients (mean BMI > 45) had lower % of NASH (4%) and lower % of fibrosis stages 3 and 4 (1.4%) *vs* Whites (17% and 9% respectively). The 2 groups had similar percentages of DM and hypertension[48]. A retrospective but well detailed study based on liver biopsy found advanced fibrosis (F3/F4) in 16% Caucasians *vs* 2.6% Blacks, despite the fact that the latter had greater BMI and higher DM rates. However, their lipid profile was healthier than Caucasians[49].

The most recent NHANES (1999-2016) evaluation[14] used the US Fatty Liver Index to define NAFLD and two noninvasive marker (FIB-4 and NAFLD Fibrosis Score) to assess advanced liver fibrosis (*i.e.*, stages 3 and 4). The results show that Latinos and Whites had higher likelihood of NAFLD (43% and 33%, respectively), *vs* Asians (20%) and African Americans (19%). Overall, mortality was associated with DM2 and FIB-4 but not race, and was higher in lean or overweight patients *vs* obese[14].

Interestingly, a work by Lomonaco *et al*[50] found that, when metabolic factors are controlled for, hepatic steatosis, inflammation and fibrosis scores (all by histology) were similar between Caucasians and Hispanics. A study assessing biopsy-confirmed NASH and comparing Latinos and Whites reported that the former were younger, had increased carbohydrate intake, and had a lower prevalence of hypertension[31]. However, while there were numerical different rates of F3/F4 (Whites and Blacks 30%, Asians 28% and Latinos 23%) these were not significant. Multivariable analysis identified only age, female gender, HTN and abnormal HOMA-IR as significantly associated with advanced fibrosis, but not race[31].

The preponderance of evidence shows that while Latinos have more NAFLD, they don't have significantly higher rates of advanced fibrosis. Studies based on liver biopsy, except one[31] have shown Black patients to have less fibrosis[8,49]. However, adequate controlling for the variables of the MetS has not been done.

NAFLD is associated with the development of hepatocellular carcinoma (HCC). One report demonstrated that patients with NAFLD have a 10-fold higher chance of developing HCC compared to controls[51]. The overall risk of HCC in NAFLD was low (estimated 0.02/100 patient-years), and it was higher in older (> 65 years) Hispanics and lower in Blacks: these subgroups were not matched by MetS risk[51].

Finding racial differences in mortality (especially liver mortality) in patients with fatty liver requires evaluation of a very large database. A NHANES analysis (1988-1994), looked at (mostly) NAFLD patients and found a correlation between high estimated liver fibrosis (by non invasive tests such as FIB-4) with mortality (both all cause and liver-related) up to 2006[52]. Unfortunately, liver mortality represented only 3% of the total mortality, so there were too few endpoints to make inferences about racial associations[52]. A review of total United States mortality captured in the latest National Vital Statistics (NVSS) database, showed that Hispanics with a diagnosis of NAFLD have lower mortality than Caucasians, although in both groups the trend is towards increased mortality the past 10 years: there was no attempt to adjust the data for underlying metabolic disease[53]. In 2016, the NIAAA issued a report on liver cirrhosis mortality. The age-adjusted mortality rates for cirrhosis "without mention of alcohol" were 50% lower in Blacks *vs* whites, but NAFLD codes were not specifically reported[54]. However, a paper looking at hospital charges, length of stay and mortality in non-Federal Community hospitals across the United States, showed that mortality was not statistically different across races in patients admitted with a NAFLD diagnosis[55].

More data on race-specific cirrhosis, HCC and mortality rate in patients with NAFLD are needed.

Response to therapy

There is considerably less data on racial responses to therapy for NAFLD. To date, this includes mainly weight loss strategies, including bariatric surgery.

Vilar-Gomez *et al*[56] published a small but well-designed study enrolling Cuban patients. They histologically documented decreased liver fibrosis (45% of patients) and resolution of NASH (90% of patients) when a 10% or greater weight loss was achieved [56]. The latter endpoint was noted in 10% of patients, all of them Cuban. However, diet and exercise may be beneficial to decrease liver steatosis in the absence of weight loss[24].

Behavioral therapy resulted in a maximum weight loss of 5 kg in Black patients, significantly less than 13 kg in Whites[57]. Metformin for one year significantly increased HDL-cholesterol (by 1-2 mg/dL) in White and Black patients: In Hispanics the HDL declined by approximately 1 mg/dL[58]. Lorcaserin lead to a placebo-adjusted weight loss of 3.2 kg, 2.7 kg and 1.4 kg in Whites, Blacks and Hispanics respectively[59]. Semaglutide as an injection for DM control showed minor changes in weight in different races[60].

A study in 3268 patients (1561 Hispanics, 660 Blacks, and 1047 Whites) examined the percentage of excess weight loss (EWL) after Roux-en-Y gastric bypass or adjustable gastric band placement[61]. EWL differed by ethnicity (-53% in Hispanics, -50% in Whites and -43% in Blacks), at 6 months post-operatively. These differences persisted at 1 and 2 years after surgery (-69%, -69% and -58%, respectively)[61]. A prior meta-analysis, looking at the percentage of EWL (between 12 and 24 mo post-

operatively) confirmed an average of 8% lower weight loss in Blacks compared to Whites[62].

In the future, large phase 3 studies using new NASH medications may uncover possible racial differences in baseline histology, and clinical liver outcomes. Those studies will have prospectively collected metabolic data, permitting investigators to assess risk by race, controlled for variables of the MetS[23].

CONCLUSION

In conclusion, there is convincing evidence that the prevalence of NAFLD depends on genetics and the prevalence of the MetS. Its individual components impact fatty liver differently in different populations. Socio-economic, dietary and lifestyle differences may also explain reported racial differences but have not been thoroughly studied in the NAFLD arena. In the United States, NAFLD and NASH seem more prevalent in Hispanics, however most studies have not been controlled for the individual variables of MetS, and this may have overestimated racial differences. African Americans have a lower prevalence of hypertriglyceridemia and this contributes to their lower prevalence of NAFLD despite higher rates of hypertension and DM. Fibrosis scores seem similar in Whites and Latinos: In most biopsy studies, Blacks have shown lower hepatic inflammation and fibrosis levels. There is no evidence that NAFLD mortality is higher in Latinos, and it may be lower in Blacks. We believe that there is presently insufficient evidence to confidently conclude that race, per se, plays a role in the development of the complications of NAFLD. Further studies, appropriately controlled for diet, exercise, and MetS parameters are needed.

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MINIREVIEWS

Torsion of spleen and portal hypertension: Pathophysiology and clinical implications

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Abstract

The displacement of spleen from its normal location to other places is known as wandering spleen (WS) and is a rare disease. The repeated torsion of WS is due to the presence of long pedicle and absence/laxity of anchoring ligaments. A WS is an extremely rare cause of left-sided portal hypertension (PHT) and severe gastric variceal bleeding. Left-sided PHT usually occurs as a result of splenic vein occlusion caused by splenic torsion, extrinsic compression of the splenic pedicle by enlarged spleen, and splenic vein thrombosis. There is a paucity of data on WS-related PHT, and these data are mostly in the form of case reports. In this review, we have analyzed the data of 20 reported cases of WS-related PHT. The mechanisms of pathogenesis, clinico-demographic profile, and clinical implications are described in this article. The majority of patients were diagnosed in the second to third decade of life (mean age: 26 years), with a strong female preponderance (M:F = 1:9). Eleven of the 20 WS patients with left-sided PHT presented with abdominal pain and mass. In 6 of the 11 patients, varices were detected incidentally on preoperative imaging studies or discovered intraoperatively. Therefore, pre-operative search for varices is required in patients with splenic torsion.

Key Words: Wandering spleen; Splenic torsion; Left-sided portal hypertension; Gastric variceal bleeding; Splenectomy

Core Tip: Wandering spleen (WS) is a rare disease. The repeated torsion of WS is due

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to the presence of long pedicle and absence/laxity of anchoring ligaments.WS is an extremely rare cause of left-sided portal hypertension and severe gastric variceal bleeding. This review comprehensively describes the pathophysiological mechanisms, clinico-demographic profile, and clinical implications of torsion of the spleen. In patients with splenic torsion, varices can be detected incidentally on preoperative imaging studies or intraoperatively. Therefore, pre-operative search for varices is required in patients with splenic torsion.

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INTRODUCTION

The displacement of spleen from its normal location to other places is known as wandering spleen (WS). It is a rare clinical entity in which the spleen is attached by a long vascular pedicle. It was first described by Van Horne in 1667[1]. WS-also known as splenoptosis or ectopic spleen or floating spleen or aberrant spleen-most commonly located in the pelvic cavity.

The spleen is anchored to its normal position by splenorenal and gastrosplenic ligaments. Due to absence or laxity of these ligaments, the spleen is displaced from the left hypochondrium to other places in the abdominal cavity. The laxity or absence of splenorenal and gastrosplenic ligaments can be caused by congenital or acquired pathology. Congenital causes of WS include an incomplete fusion of the dorsal mesogastrium and the parietal peritoneum, resulting in the absence of anchoring ligament formation[2,3]. While acquiring causes include pregnancy due to hormonal effects, lax abdominal wall in multiparous women or obese persons and splenomegaly. More than one risk factor can be involved in the pathogenesis of WS

The true incidence of WS is unknown. The incidence of WS was 0.2% in splenectomies performed in 1003 patients. The patient is usually asymptomatic and remains undiagnosed for long periods. A WS is usually diagnosed in childhood and the third and fourth decades of life, with a strong female preponderance. In a study, Viana *et al* [4] reviewed the data of 266 cases of WS and found that the average age at the time of diagnosis was 25.2 years, with a male-female ratio of 3.3:1.

More than half of the patients present with recurrent abdominal pain due to repeated torsion. Abdominal mass is the most common finding on examination[5-8]. In a systematic review, 197 (M:F = 1.5:1) pediatric patients with WS were analyzed, and abdominal pain was found to be the most frequent (43%) symptom[7]. Another systematic review was performed in 376 surgically treated patients of WS. Abdominal pain and abdominal mass were the most frequent clinical features. More importantly, nearly half of the patients presented with acute clinical onset[8]. The diagnosis of a complicated WS needs a high index of suspicion. Delay in diagnosis can lead to emergency surgeries. It can be avoided by reducing time-consuming repeated imaging studies[9].

WANDERING SPLEEN AND SPLENIC TORSION: AN OVERVIEW

WS can be complicated with splenic torsion, splenic infarction, hypersplenism and left-sided portal hypertension (PHT). Acute abdomen, splenic abscess, acute pancreatitis, pancreatic necrosis, gastric volvulus, pancreatic volvulus, intestinal obstruction, and gastric outlet obstruction are the other rare complications of WS[5,10-15].

Splenic torsion is the most common complication of WS. In a systematic review, splenic torsion was diagnosed in 56% of pediatric patients with WS[7]. The repeated torsion of WS is due to the presence of long pedicle and absence/Laxity of anchoring ligaments. Torsion usually occurs clockwise. Torsion of pedicle leads to increased back pressure in splenic vein (SV), resulting in parenchymal congestion, splenomegaly, and hypersplenism. Extreme torsion can lead to the arterial supply being compromised, causing infarction and necrosis. The enlargement of the spleen further aggravates

splenic torsion. Torsion can be precipitated by movements of the body, changes in intra-abdominal pressure, peristalsis, or distension of adjacent organs[16,17].

WS is diagnosed using abdominal ultrasound (US), computed tomography (CT), and magnetic resonance imaging. US demonstrates the absence of spleen from its normal position and its location elsewhere in the abdominal cavity. US examination is limited by the presence of gas, suboptimal assessment of adjacent viscera and difficulty in identifying twisted pedicle and the infarcted spleen. CT scan is the preferred modality of investigation for the diagnosis of WS. CT scans delineate the exact location of the spleen and demonstrates the twisting of the splenic pedicle known as whirl sign-alternating radiolucent and radio dense bands formed due to splenic vessels and adjacent fat. The whorled appearance of splenic vessels and surrounding fat is diagnostic of splenic torsion. CT scans also demonstrate other associated findings, such as ascites and entrapment of the adjoining viscera secondary to torsion. Scintigraphy and angiography can also diagnose WS but are rarely used due to their high costs and invasive nature[18-21].

Splenopexy is the first-line treatment of WS and is indicated even in asymptomatic patients (except elderly and high-risk surgical candidates) because of the potential risk of serious complications. Detorsion and splenopexy are preferred in patients with torsion, whose spleen parenchyma is shown to be viable and without signs of hypersplenism. Splenectomy is considered in cases of splenic infarction, splenic vessel thrombosis (SVT), portal vein thrombosis (PVT), hypersplenism, PHT, and suspicion of cancer[5,22]. In recent years, there has been a growing trend toward more conservative and minimally invasive approaches, such as splenopexy or laparoscopic techniques[4,7,8,23,24]. Viana *et al*[4] reviewed the data of 266 cases of WS and found that splenectomy and splenopexy were performed in 70% and 29% of patients, respectively. The majority of patients had open surgery (79%), while about one-fifth of patients were treated using laparoscopic surgery. A very recent systematic review by Ganarin *et al*[7] showed that splenectomy and splenopexy were performed in 55% and 39% of surgically treated patients (n = 197), respectively. About half of the splenopexies were performed using minimally invasive surgery. Frequently used techniques were the placement of a mesh (46%) or the construction of a retroperitoneal pouch (31%). Overall, splenopexy was effective in 95% of cases.

SPLENIC TORSION AND PORTAL HYPERTENSION: PATHOPHYSIOLOGICAL MECHANISMS

Left-sided PHT, also known as segmental or sinistral PHT, is a rare cause of gastric variceal bleeding. It usually occurs as a result of SV occlusion caused by splenic torsion, extrinsic compression of splenic pedicle and SVT. Left-sided PHT should be suspected in those who have gastric and/or splenic varices in the absence of esophageal varices and deranged liver function test. WS is an extremely rare cause of left-sided PHT[16].

The torsion of WS occurs mainly due to absence/laxity of anchoring ligaments, long pedicle and splenomegaly. Splenic torsion can also be predisposed by other causes of splenomegaly, including chronic liver disease (CLD), malaria, myeloproliferative disease, lymphoproliferative disorders, infectious mononucleosis, and splenic haemorrhagic cyst[5]. The torsion of the splenic pedicle leads to increased back pressure in the SV, resulting in splenic parenchymal congestion and splenomegaly. The occlusion of the SV can be caused by the chronic torsion of the splenic pedicle, SVT, and direct mechanical compression by an enlarged spleen. SV occlusion leads to impaired venous return and retrograde filling of the short gastric and left gastroepiploic veins. Decompression of splenic venous outflow occurs through the short gastric veins, coronary vein, and left gastroepiploic veins, producing gastric varices[16]. A few cases of mesenteric varices have been described in WS patients without PVT. The mechanical occlusion of the portal vein at the level of superior mesenteric and SV confluence due to splenic torsion can explain the mechanism of formation of mesenteric varices[25-28]. The coexisting gastric volvulus can further obstruct the venous drainage of the proximal stomach, leading to the development of PHT[12]. The pathophysiologic mechanisms of PHT in WS patients are shown in Figure 1.

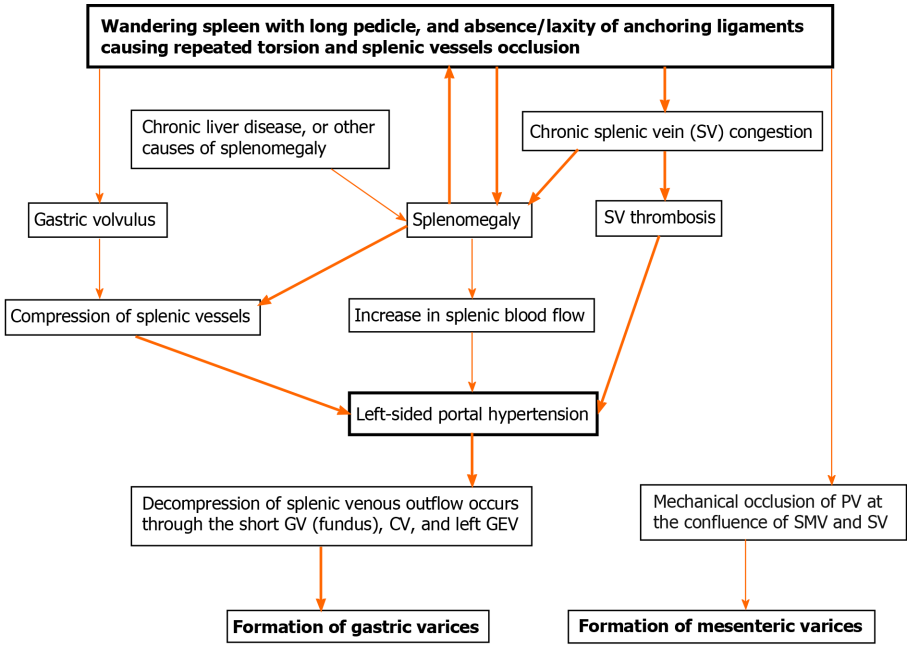

Figure 1 Schematic diagram of the mechanisms of varices formation in wandering spleen with splenic torsion. SV: Splenic vein; GV: Gastric varices; CV: Collateral vein; GEV: Gastroepiploeic vein; SMV: Superior mesenteric vein; PV: Portal vein. Please note that thick arrow denotes more frequent mechanism and thin arrow denotes less frequent mechanism.

SPLENIC TORSION AND PORTAL HYPERTENSION: CLINICAL IMPLICATIONS

Left-sided PHT is a rare manifestation of WS with torsion. Approximately 20 cases of WS with left-sided PHT have been described in English medical literature[5,11-13,25-39]. The clinico-demographic profile of the reported cases of patients with WS and PHT are summarized in Table 1. The majority of patients were diagnosed in the second or third decade of life (mean age: 26 years), with a strong female preponderance (M:F = 1:9). WS patients with PHT present earlier than WS patients without PHT. Upper gastrointestinal bleeding was the most common presenting complaint, followed by abdominal pain. The majority of the patients had gastric varices without esophageal varices, which is suggestive of left-sided PHT. Mesenteric varices and splenic varices were identified in about 25% of patients. In 14 patients, gastric varices were diagnosed in endoscopy or gastrointestinal series. In five patients, the presence of varices was only identified in imaging studies. One patient had intra-operative diagnosis of PHT. Splenectomy was performed on all patients, and the follow-up details of 14 patients revealed the disappearance of varices.

Esophageal varices are absent in WS patients with left-sided PHT. Coexisting CLD has been described in two-patients with WS[40,41]. Splenomegaly resulting from CLD can further aggravate the splenic torsion and PHT[40]. PVT has also been described in patients with WS[28,42,43]. Hence, the presence of esophageal varices in patients with WS warrants careful evaluation for coexisting CLD and PVT.

Splenectomy eliminates PHT, provides symptomatic relief, and prevents the relapse of varices (Table 1). However, splenectomy in patients with undiagnosed collaterals can be tricky due to increased blood loss. Splenectomy in these patients can necessitate additional transfusions of blood and blood products. Eleven patients of WS with undiagnosed PHT were presented with abdominal pain and mass. In six patients, varices were detected incidentally on preoperative imaging studies or discovered intraoperatively. Therefore, pre-operative search for varices with endoscopy and a good quality CT-scan are useful in patients with splenic torsion. These patients also require intra-operative inspection for small collaterals and careful dissection.

CONCLUSION

The repeated torsion of WS can lead to splenomegaly, SVT, hypersplenism, and, rarely left-sided PHT. The patients with WS and PHT usually present with gastric variceal

Table 1 Summary of reported cases of wandering spleen with portal hypertension, *n* (%)

Clinico-demographic features	Remarks	Frequency (%)
Reported cases (*n*)		20
Mean age (range)		26.15 (12-55) yr
Male:female ratio		1:9
Presenting complaints	Upper GI bleeding	9 (45)
	Abdominal pain	8 (40)
	Abdominal mass	2 (10)
	Acute pancreatitis	1 (5)
Type of varices	Gastric varices	18 (90)
	Mesentric varices	5 (25)
	Splenic varices	6 (30)
Diagnosis of varices	Endoscopy	12 (60)
	GI series	2 (10)
	Imaging only	5 (25)
	Intra-operative	1 (5%)
Venous thrombosis	Splenic vein	3 (15)
	Portal vein	1 (5)
Splenic infarction		4 (20)
Definitive treatment	Splenectomy	20 (100)
Post-operative variceal status	Documented (*n*)	14/20 (70)
	Resolved (*n*)	14/14 (100)

GI: Gastrointestinal.

bleeding. Nearly half of the WS patients with PHT can present without variceal bleeding. Splenectomy or splenopexy in patients with undiagnosed collaterals can be tricky due to increased blood loss. Therefore, pre-operative search for varices is required in patients with splenic torsion. They also require intra-operative inspection for small collaterals and careful dissection. Esophageal varices are absent in WS patients with left-sided PHT. Hence, the presence of esophageal varices in patients with WS warrants careful evaluation for coexisting CLD and PVT.

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MINIREVIEWS

Impact of coronavirus disease 2019 on prevention and elimination strategies for hepatitis B and hepatitis C

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has resulted in significant morbidity and mortality since its first case was discovered in December 2019. Since then, multiple countries have witnessed a healthcare system collapse due to the overwhelming demand for COVID-19 care. Drastic measures have been taken globally in order to curb the spread of the virus. However, those measures have led to the disruption of other aspects of healthcare, increasing the burden due to other medical conditions. We have also stepped back in achieving the ambitious goal set in place by World Health Organization to eliminate viral hepatitis as a public threat by 2030. Hepatitis B and C are chronic conditions with a significant worldwide burden, and COVID-19 has resulted in many hepatitis elimination programs slowing or stopping altogether. In this review, we elucidate the impact of the ongoing COVID-19 pandemic on the interventions targeted towards the elimination of hepatitis B virus and hepatitis C virus. Some of the salient features that we have covered in this review include hindrance to screening and diagnostic tests, neonatal vaccinations, the transmission dynamics affecting hepatitis B virus and hepatitis C virus, role of limited awareness, restrictions to treatment accessibility, and disparity in healthcare services. We have highlighted the major issues and provided recommendations in order to tackle those challenges.

Key Words: COVID-19; Chronic hepatitis; Review literature; Vaccine; World Health Organization; Pandemics

Core Tip: There has been a multi-fold impact of the pandemic on viral hepatitis elimination strategies. Due to supply chain disruptions, hepatitis B virus vaccination campaigns have been halted. Increased preference for home deliveries, poor antenatal

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care, and unavailability of at-birth hepatitis B virus vaccine has increased the risk of vertical transmission. With needle-sharing activities on the rise and closure of harm reduction centers, the spread of blood-borne infections including the hepatitis C virus has risen. Hospitals are either being avoided due to the fear of contracting severe acute respiratory syndrome coronavirus 2 or are being converted into coronavirus treatment wards, resulting in poor management of patients.

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INTRODUCTION

In December 2019 the first case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was isolated and identified in Wuhan, China[1]. The coronavirus disease 2019 (COVID-19) pandemic that ensued, has led to 2.47 million deaths as of February 21, 2021[2].

This medical emergency shed light upon our fragile healthcare system worldwide and its vulnerabilities including the immense vacuum questioning our preparedness for the next pandemic[3]. Although we were able to achieve making vaccines in record time[4], the impact on human life and our economies are yet to be quantified.

On the other hand, hepatitis B virus (HBV) and hepatitis C virus (HCV) have had their impact quantified and have been studied for decades. In 2016, the World Health Organization (WHO) estimated the prevalence of chronic hepatitis B to be 257 million worldwide[5], while it was 71 million for chronic hepatitis C. Chronic hepatitis has a worldwide burden that is mostly clinically silent, as it goes undiagnosed in most low to middle-income countries (LMICs)[6,7].

We evaluated the sustainable development goals (SDGs) set in place by the WHO for the task of eliminating hepatitis B and C as a public health threat by 2030[8]. The SDGs include goals such as coverage of three-dose HBV neonatal vaccine, prevention of mother-to-child transmission, and harm reduction services such as sterile syringe set distribution for people injecting drugs. The efforts done to achieve these sustainable goals have been severely compromised due to the current pandemic.

Although it is debatable that having chronic viral hepatitis influences the outcomes of having the COVID-19[9-12], worse outcomes with acute respiratory distress syndrome in COVID-19 can be expected due to impaired immunity[1,13].

This review elucidates the impact of the COVID-19 pandemic on chronic viral hepatitis B and C; since hepatitis A and E contribute relatively less significantly to morbidity, mortality, and long-term impact[8]. We evaluated SDGs and current existing data in light of them. Some of the salient features, as shown in Figure 1, can be identified as a hindrance to screening tests and neonatal vaccinations, the transmission dynamics affecting HBV and HCV, the role of limited awareness, restrictions to treatment availability, and disparity in healthcare services.

DISRUPTED HEPATITIS B VACCINATION CAMPAIGNS

The COVID-19 pandemic brought in conditions and circumstances that were unusual for countries and the world as a whole with factors not previously anticipated. Although the rate of hepatitis B vaccinations has been steadily on the rise since the 1990s, we have learned that geopolitical factors, financial priorities, the image of the government, and the health sector have played a huge role in their success or failure [3]. A recent example within an epidemic can be found in the Ebola outbreak in 2013 in West Africa. Due to disrupted vaccination services, limited availability, and allocation of funds, a sharp rise in the incidence of measles was reported during the epidemic and in the months that followed[14].

The Institute for Health Metrics and Evaluation at the University of Washington showcased an overall drop in global vaccination coverage in 2020 to levels as low as those seen in the 1990s with words depicting its severity as "… we have been set back

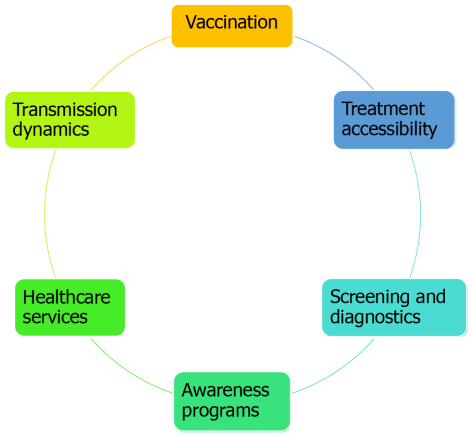

Figure 1 This wheel represents, in no particular order or flow, the focal points that give an insight to the impact of coronavirus disease 2019 on viral hepatitis.

25 years in 25 wk"[15]. High-income countries like the United States had a drop in pediatric vaccinations being ordered and administered after an emergency was declared on March 13, 2020[16]. Between February and April of 2020, the United Kingdom also saw a drop of almost 20% in the administration of measles, mumps, and rubella vaccines, as compared to 2019[17].

Reduced availability and provision of HBV vaccines during this COVID-19 pandemic will have detrimental effects on the incidence of HBV during infancy, childhood, and in later years, thus increasing the chances of chronicity in the generation to come. This severely impedes our progress to the 2030 elimination goals set in place by WHO[8].

Vaccination rates are not in line with the target goals set in SDGs in the LMICs[18], and poor screening in the case of viral hepatitis might pose a greater threat in the long run compared to the pandemic.

Despite being a high-value investment, vaccines are the most cost-effective way of avoiding disease[19]. The decline in measles, mumps, polio, and yellow fever can be credited to this. Nothing can truly represent the effectiveness of vaccines other than the global eradication of the smallpox virus. This disfiguring disease that had infected over 11 million people from 1920 to 1977, was eradicated in 1978 following a world-wide vaccination campaign.

Although the HBV vaccine is an effective modality, this modality does not exist for HCV. Progress has been made on HCV over the past few decades with the year 2020 being its limelight when Drs. Michael Houghton, Harvey Alter, and Charles Rice were awarded the Nobel Prize in Physiology/Medicine for their discovery of the HCV[20]. This raises hopes for a cure and even so a vaccine that will be beneficial for the years to come. Eliminating HCV as a global threat should be a priority as the disease is present actively in 71 million people and accounts for 500000 deaths annually[21,22].

Abbas *et al*[23] conducted a benefit-risk analysis study in Sub-Saharan Africa during the pandemic. The study compared the SARS-CoV-2 pandemic and its impact on routine childhood vaccination programs, encompassing several preventable diseases including hepatitis B as well as others such as diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, rotavirus, measles, meningitis A, rubella, and yellow fever. The model found that in a high-impact scenario, for every one excess COVID-19 death attributable to SARS-CoV-2 infections acquired during routine vaccination clinic visits, 84 deaths in children could be prevented by sustaining routine childhood immunization in Africa[23].

HBV vaccination campaigns have also been halted due to disruptions in the supply chain. LMICs regions like Pakistan and Sub-Saharan Africa were faced with a shortage of HBV vaccines during the pandemic[24,25]. The latter had breakdowns in the cold chain and limited financial support from the government[25]. Despite healthcare services being ramped up, changes in healthcare-seeking behavior led to a change in attitude resulting in reluctance for availing vaccinations[25]. The acceptance and readiness of vaccinations are closely linked to the fear of the linked disease and the trust placed in the government and its practices[26-28]. Due to heightened misinformation on media outlets and a general chaotic atmosphere worldwide, people had an anti-science sentiment and heightened distrust in most places[3]. Furthermore, the pandemic resulted in increased home-births, which hindered access to vaccines,

limiting dosages being given at birth[29].

INTERRUPTION IN THE TRANSMISSION DYNAMICS OF HEPATITIS B AND C

The actual numbers to quantify the effects on transmission dynamics in viral hepatitis spread are limited[3]. Even though as a result of movement restrictions and worldwide lockdowns, the physical spread is expected to decrease, such limiting behaviors give rise to risky attitudes on the part of undiagnosed and stable hepatitis. Alcohol consumption and unprotected sexual intercourse have increased. Drug abuse has been on the rise during the pandemic[30]. Disruption of needle exchange programs and harm-reducing services are already scarce in LMICs and with lockdowns in place and financial constraints, such limitations would result in cross-contamination of blood-borne viruses *via* needles especially HCV[31]. Stowe *et al*[32] reported the closing down of numerous harm reduction service centers in South Africa leading to rising in overdose cases in street-based heroin-using individuals. In general, the incidence of viral hepatitis will increase by the closing of harm reduction centers [33].

In Sub-Saharan Africa, liver diseases are highly prevalent although extremely underdiagnosed[25]. Being unaware of their viral hepatitis status creates ground for increased transmission dynamics in the population that already has limited funding for screening, vaccinations, and treatment as a whole. Government efforts will need a clear pragmatic strategy as the pandemic progresses to counter such transmission dynamics.

The chances of vertical transmission have also increased as the preference for home deliveries has surged during the pandemic[29]. There is an increased likelihood of missing out on routine HBV and HCV antenatal screening tests. The initial dose of HBV vaccine usually administered at birth could either be delayed or skipped. The intrapartum administration of hepatitis B immunoglobulin to decrease the vertical transmission has also been affected due to home deliveries. These above-mentioned limitations all increase the chances of vertical transmission, which will affect a generation that is to come, making them highly susceptible to chronic hepatitis due to early exposure.

LACK OF AWARENESS PROGRAMS FOR HEPATITIS DURING COVID-19 PANDEMIC

Lack of awareness is an issue faced by multiple LMICs. Increasing the awareness amongst the general population about modes of transmission of viral hepatitis, symptoms, screening and diagnosis, management, and follow-up plays an important role in elimination programs[34]. Measures taken during the pandemic have led to the closure of community-based education and screening programs and in-person events. A decrease in voluntary activities such as the NoHep program seems to have decreased the diagnosis rate[35].

A lack of information dispersal has been noticed during the pandemic in regards to people suffering from viral hepatitis. According to a study conducted by the World Hepatitis Alliance, 99 countries were sent a survey to access viral hepatitis services during the pandemic. Only 39 (30%) of 131 analyzable responses indicated adequate information on COVID-19 had been provided to people living with viral hepatitis in their country. One participant from Ukraine said that no specific information had been provided for people living with viral hepatitis, although information had been provided for people living with human immunodeficiency virus[36].

In low-income countries like Pakistan, new and known cases of HBV and HCV patients were compared between January to June of 2020 to the corresponding months in 2019. These 23 centers were mostly government-run with free of cost hepatitis treatment being provided. All the centers remained open, with no shortage of staff. Despite this, the centers still recognized a lesser number of new people coming in for treatment; for example, in January 2020 a mean number of 45 new patients registered in these centers when there were no cases, while in June 2020, the number has fallen by 84%[37]. This highlights the lack of awareness amongst individuals regarding the seriousness of viral hepatitis.

IMPAIRMENT IN SCREENING AND DIAGNOSTIC FACILITIES

One of the most important steps in eliminating viral hepatitis is to screen and diagnose in a timely fashion in order to start treatment and prevent transmission. Underdiagnosis is a key hurdle in eliminating viral hepatitis, as it can have a long-term impact on transmission dynamics.

In 2017, it was estimated that 91% of patients with chronic HBV and 80% of patients with chronic HCV had not been diagnosed. In a World Health Alliance survey conducted across 32 LMICs, only 36% of the respondents reported that testing services were accessible to people. The key issues identified in the survey were either the closures or avoidance of testing services[31]. A study revealed that within Sub-Saharan Africa, there was a reduction of 71%, 95%, and 83% in the number of patients in the hepatitis clinics of Burkina Faso, Tanzania, and the Gambia, respectively, from January to April 2020[38]. The primary reason for such a striking decline in the use of outpatient services was attributed to the fear of contracting the severe acute respiratory syndrome coronavirus 2. Similarly, a decline of 84% in HBV and 74% in HCV positive patients coming for a follow-up visit in district hepatitis clinics were recorded in Pakistan from January to June 2020[37].

In order to control the pandemic, multiple aggressive measures have been taken worldwide, leading to financial disruption of hospitals and healthcare services, often resulting in their closures[39]. There have also been shortages in the testing reagents of HBV and HCV due to global supply chain disruption. In Italy, a law was enacted in February 2020 to conduct graduated birth cohort screening for hepatitis, however, it had not been put into action as of May 2020. In Egypt, all the ongoing screening programs were also suspended in March 2020, as reported by Blach *et al*[40] to reserve polymerase chain reaction tests for COVID-19; all polymerase chain reaction testing for viral hepatitis was halted in Pakistan[37].

REDUCED ACCESS TO TREATMENT FACILITIES FOR CHRONIC HEPATITIS

In most countries, travel bans have been enforced, making access to critical care difficult. In multiple high-income countries, continuity of care is being maintained by utilizing telemedicine services. This has made it convenient for patients to have access to remote healthcare. However, in LMICs including Sub-Saharan Africa, telemedicine is impractical due to a lack of resources including cell-phones, internet services, and modes of payment[25]. The task of generating dedicated phone numbers for gastroenterology and hepatology services and spreading awareness regarding telemedicine amongst the population is not easily established in communities with a low literacy rate. Furthermore, it is difficult for the patients to understand or perform the investigations that the doctor asks them to do.

Even though all the LMICs are not facing or responding to the pandemic in the same way, there has been a global negative impact on access to treatment and care. For instance, even though a strict lockdown was not imposed in Egypt, HCV management centers had a 50% reduction in new patients and follow-ups[40]. A study conducted across three clinical sites in the United States, Japan, and Singapore reported a significantly decreasing trend in the number of patients who visited liver clinics across the three clinical sites during February, March, and April in 2018, 2019, and 2020[41]. Although most Spanish harm reduction centers continued to operate during the pandemic, there was a reduction in the number of clients using them, which resulted in decreased testing and increased discontinuation of ongoing hepatitis C treatment [42]. A web-based survey conducted in Italy revealed that initiation of HBV and HCV treatment was deferred in 23% of the centers, and even in patients considered at high risk for serious complications, treatment had been started in only 20%-28% of the cases [43].

In many countries including Egypt, medications are not manufactured and are imported from other countries. Interruption of the supply chain and necessary reallocation of healthcare resources has resulted in a remarkable shortage of medications for viral hepatitis, as reported by studies conducted in Egypt[44], Sub-Saharan Africa[38], and Pakistan[37]. In Italy, 26% of the hepatology wards had been converted to COVID wards, and 33% had bed reductions[43].

As a result of interrupted and substandard treatment of viral hepatitis, there is an increased risk of disease flares that could promote transmission and also increase resistance to viral drugs. Routine monitoring of laboratory investigations including

liver function tests and complete blood counts were also significantly reduced because of increased priority given to COVID tests, as reported by Mustafa *et al*[37]. This is likely going to result in higher rates of severe worse outcomes such as decompensated liver disease and hepatocellular carcinoma. Certain reports have suggested that medications such as tocilizumab and corticosteroids, which are commonly being used to treat COVID-19 infections, can result in the reactivation of dormant HBV infection [45,46]. This may be an important cause of increased morbidity and mortality in patients with a prior HBV infection as a rapid rise in alanine aminotransferase levels following viral reactivation can in some cases lead to a fulminant hepatic failure. Hence, antiviral prophylaxis against HBV reactivation should be considered[47]. Furthermore, it is also recommended that liver tests should be performed routinely in all COVID-19 patients, particularly the ones receiving remdesivir and tocilizumab, regardless of their baseline values[48].

WIDENING DISPARITIES IN HEPATITIS-RELATED HEALTHCARE

The pandemic is causing health care and socioeconomic inequalities between regions and countries. The communities most underserved by the healthcare systems have an increased risk of contracting the SARS-COV-2 virus and are more likely to have non-communicable comorbidities, which further increases the chances of COVID associated complications[3,49].

The WHO survey reported that in LMICs, treatment access has been hampered due to movement restrictions and suspension of clinical services. Fifty-two percent of the frontline health workers from the 32 LMICs reported that treatment was not accessible by patients[31]. However, only 8% of the respondents from the United States reported an issue with access to treatment. This highlights the discrepancy between high-income countries and LMICs, the latter suffering from more severe consequences as a result of the pandemic[36].

National economies are crumbling and most giants in the varied sectors are downsizing to get through the pandemic. This increases the risk for people living in countries where universally accessible health care systems are not present, especially in rural areas of LMICs like India and Nigeria where daily wage earners are limited to healthcare by access and out-of-pocket expenditure for medical facilities[36]. Similar cases have also been accounted for in the United States, a high-income country where almost 6.2 million people have lost their jobs, thus losing the medical insurance linked to their jobs, during the pandemic[50]. Health disparity has affected almost everyone in one way or the other but the basic difference lies in access to basic medical help.

Primary care settings and general practitioners, which have an essential role in hepatitis elimination, are now focusing on the COVID-19 pandemic and this change can further reduce both diagnosis and treatment rates of hepatitis patients. Countries with a low number of doctors to population ratio will be affected more[51,52].

OVERCOMING THE CHALLENGES

A pulse survey conducted across 100 countries of five different WHO regions not only provided an insight on the extent of healthcare disruption but also listed a few strategies that have been adopted by those regions to mitigate the impact of COVID-19 on essential health services during the pandemic[53]. Based on the approaches that the responding countries had implemented to overcome the healthcare disruptions, we have come up with a list of recommendations that can be utilized by researchers and policymakers to prevent transmission, increase screening and diagnosis, and provide prompt management of patients with HBV and HCV, to counter the impact of COVID-19 pandemic (refer to Figure 2). We can use this crisis as an opportunity to develop a healthcare system that is sustainable and does not collapse in case of continued morbidity and mortality due to the pandemic.

CONCLUSION

There is no doubt that drastic measures needed to be taken in order to curb the pandemic, but as a result of those measures, we might be stepping backward in achieving the goal of eliminating viral hepatitis by 2030. There is a dire need to come

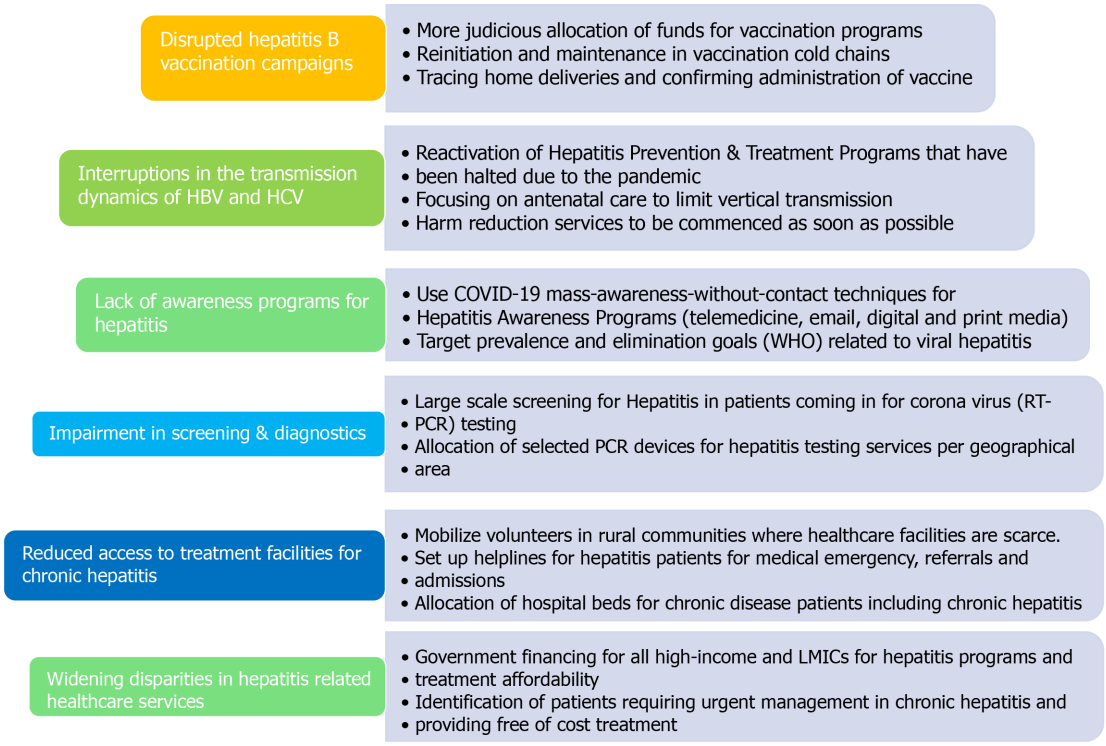

Figure 2 Overcoming the challenges. This figure addresses possible recommendations and solutions to the coronavirus disease 2019 pandemic crisis that has and is affecting our goal of achieving 2030 World Health Organization goal for elimination of chronic hepatitis B virus and hepatitis C virus. HBV: Hepatitis B virus; HCV: Hepatitis C virus; RT-PCT: Reverse transcriptase polymerase chain reaction.

up with guidelines that guarantee consistent care of patients with viral hepatitis, in case there is another wave of the pandemic. The impact of COVID-19 is going to extend beyond just the morbidity and mortality related to that disease. Hence, elimination efforts for viral hepatitis must be resumed as soon as possible.

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ORIGINAL ARTICLE

Retrospective Study

Prevalence and risk factors of steatosis and advanced fibrosis using transient elastography in the United States' adolescent population

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in children and adolescents.

AIM

To determine the prevalence and risk factors of steatosis and advanced fibrosis using transient elastography (TE) in the United States' adolescent population.

METHODS

Using the National Health and Nutrition Examination Survey 2017-2018, adolescent participants aged 13 to 17 years who underwent TE and controlled attenuation parameter (CAP) were included in this study. Forty-one factors associated with liver steatosis and fibrosis were collected. Univariate and multivariate linear regression analysis were used to identify statistically significant predictors.

RESULTS

Seven hundred and forty participants met inclusion criteria. Steatosis (S1-S3), based on CAP, and advanced fibrosis (F3-F4), based on TE, were present in 27% and 2.84% of the study population, respectively. Independent predictors of steatosis grade included log of alanine aminotransferase, insulin resistance, waist-to-height ratio, and body mass index. Independent predictors of fibrosis grade included steatosis grade, non-Hispanic black race, smoking history, and systolic blood pressure.

CONCLUSION

This study demonstrated a high prevalence of steatosis in the United States' adolescent population. Almost 3% of United States' adolescents had advanced fibrosis. These findings are concerning because a younger age of onset of NAFLD can lead to an earlier development of severe disease, including steatohepatitis, cirrhosis, and liver decompensation.

Key Words: Non-alcoholic fatty liver disease; Fatty liver; Metabolic syndrome; Cirrhosis, national health and nutrition examination survey; Pediatric; Adolescents

Core Tip: Adolescents in the United States were found to have a high prevalence of non-alcoholic fatty liver disease, which was estimated to be 27%. Nearly 3% were found to have advanced fibrosis diagnosed by transient elastography. The severity of steatosis was associated with alanine aminotransferase, insulin resistance, waist-to-height ratio, and body mass index. Risk factors of fibrosis included steatosis grade, non-Hispanic black race, smoking history, and systolic blood pressure.

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INTRODUCTION

With the rise of obesity and metabolic syndrome among younger populations, non-alcoholic fatty liver disease (NAFLD) is a growing concern in adolescents. NAFLD has become the most common cause of chronic liver disease in children and adolescents, with a prevalence previously estimated to be 3%-10% in the global pediatric population[1,2]. The prevalence of NAFLD in children with obesity is exceedingly high at 40%-70%[3]. Unsurprisingly, the rates of NAFLD have grown with the rise of childhood obesity over recent decades. Other established risk factors include insulin resistance, metabolic syndrome, and dyslipidemia. The development of NAFLD in childhood is clinically important because of the progressive nature of the disease. Earlier development of NAFLD increases the risk of earlier-onset fibrosis and frank cirrhosis[4].

Liver biopsy is the gold-standard diagnostic test for NAFLD. It not only confirms the diagnosis of NAFLD, but can also grade the level of inflammation and stage the liver fibrosis. However, this invasive procedure is ill-suited to serve as a general screening tool. Non-invasive alternatives which include a physical exam, biochemical tests, and serum biomarkers for fibrosis are not reliable predictors of fibrosis[5,6]. Because fibrosis is the single most important predictor of long-term mortality in NAFLD, transient elastography (TE) has emerged as a non-invasive, reproducible modality in the assessment of patients with NAFLD. Using ultrasound, TE measures the liver stiffness as a proxy for fibrosis stage. Its accuracy has been demonstrated in adult patients with fibrosis secondary to chronic hepatitis B and C, alcoholic and non-alcoholic liver disease, and biliary disease[7-9]. TE's accuracy however is reduced by active hepatitis, increased waist circumference, recent eating, and liver congestion. In adults with NAFLD, TE has an area under the receiver operating characteristic for detecting advanced fibrosis (bridging fibrosis or cirrhosis) of 0.88[10]. In children and adolescents, TE has been validated for chronic liver disease, including NAFLD with similar accuracy, but the data are limited[11-14]. Further research is needed to confirm the liver stiffness thresholds for fibrosis used in the pediatric population.

In addition to liver stiffness, modern TE is also able to calculate the controlled attenuation parameter (CAP). CAP is a quantitative measurement for steatosis. In adults, significant steatosis is defined by having more than 33% of the hepatocytes on a liver biopsy contain steatotic architecture. This correlates to CAP scores greater than 250 db/m[7]. Cut-offs for CAP of 248 db/m, 268 db/m, and 280 db/m were proposed

Statistics (NCHS). The survey collected multiple data sets, including demographic, interviews, physical examinations, and laboratory testing of biologic samples. It is available to the public.

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to correspond with steatosis ≥ 11%, ≥ 33%, and ≥ 66%, respectively[15]. CAP cut-offs in children are suspected to be similar[16,17], but require additional validation.

In the present study, we reported the prevalence of NAFLD characterized by TE and CAP in United States adolescents. Our study employed novel data from the unselected, general cohort of the 2017-2018 National Health and Nutrition Examination Survey (NHANES). We also assessed risk factors associated with NAFLD in this young demographic.

MATERIALS AND METHODS

Study population and study design

NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States, conducted by the National Center for Health Statistics (NCHS)[18]. The survey collected multiple data sets, including demographic, interviews, physical examinations, and laboratory testing of biologic samples. NHANES protocol was approved by the NCHS Research Ethics Review Board.

Currently, NHANES has been collecting data in a 2-year cycle. The liver ultrasound transient elastography examination was first introduced in NHANES 2017-2018, which has been released in March 2020 along with other data files. Out of 9254 participants in NHANES 2017-2018, there were 740 participants aged younger than 18 years that met inclusion criteria for this study. The exclusion criteria included: (1) Incomplete TE exam status; and (2) Hepatitis B, hepatitis C, or hepatitis E infection. It is worth noting that alcohol consumption data in participants younger than 18 years is not publicly accessible and has not been published by the time of writing this article.

We included 41 factors associated with liver steatosis and fibrosis in this study: demographic (*i.e.*, age , gender, race/ethnicity, and smoking), body measurement (*i.e.*, body mass index (BMI), waist-to-height ratio, and waist-to-hip ratio), physical activities (days of physical active, hours of TV/videos watching, and hours of computer usage), diet (*i.e.*, energy, protein, carbohydrate, sugars, dietary fiber, fat, saturated fatty acids, and cholesterol), blood pressure (*i.e.*, systolic and diastolic), laboratory tests [*i.e.*, triglycerides, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase, alkaline phosphatase, total bilirubin, total protein, albumin, iron, total iron binding capacity, transferrin saturation, ferritin, total cholesterol, direct HDL-Cholesterol, high-sensitivity C-reactive protein, platelet count, HbA1c, fasting glucose, and insulin)]. Additionally, we manually calculated LDL-cholesterol and homeostatic model assessment of insulin resistance (HOMA-IR) from the existing variables.

The above variables were chosen based on the availability of data in NHANES 2017-2018, the usage in clinical practice, and the supporting evidence that demonstrated an association with NAFLD. Additionally, we compared the predictive performance of liver fibrosis indices with the steatosis grade and fibrosis stage. Three liver fibrosis indices used in this study included (1) AST to platelet ratio index (APRI)[19]; (2) Fibrosis-4 (FIB-4) index[20]; and (3) Pediatric NAFLD fibrosis index (PNFI)[21].

Definitions

Assessment by liver ultrasound TE examination resulted in measurement of CAP. CAP is a standardized non-invasive measure for assessment of fibrosis and quantification of steatosis in NAFLD[22]. Cut-off values for median CAP score for different grades of steatosis (S0-S3) were derived from a meta-analysis on CAP technology. S0 was defined as a score of less than 248 dB/m (< 10% steatosis). S1 was defined as a score of 248 to less than 268 dB/m [10% to < 33% steatosis (mild)]. S2 was a defined as a score of 268 to less than 280 dB/m [33% to < 66% steatosis (moderate)]. S3 was defined as a score of 280 dB/m or more [≥ 66% steatosis (severe)][15]. Median CAP scores of 248 dB/m or greater (≥S1) were considered as suspected steatosis.

Participants were also categorized according to stage of hepatic fibrosis. The METAVIR scoring system was used for fibrosis staging (F0-F4)[23]. Stages of hepatic fibrosis ranged from no fibrosis (F0) through intermediate stages of hepatic fibrosis (F1-F3) to end-stage cirrhosis (F4)[24]. The degree of fibrosis was equivalent to the liver stiffness measured in kPa as calculated by liver ultrasound transient elastrography[25]. Stage F0-F1 were defined as a median stiffness < 7 kPa. Stage F2 was defined as a median stiffness of 7 to < 8.6 kPa. Stage F3 was defined as a median stiffness of 8.6 to < 11.5 kPa. Stage F4 was defined as a median stiffness ≥ 11.5 kPa. Participants with a median stiffness of 8.6 kPa or greater (≥F3) were considered to have

advanced fibrosis[26].

BMI was discretized into four classes (1) Underweight, BMI < 5th percentile; (2) Normal, 5th percentile ≤ BMI < 85th percentile; (3) Risk of overweight, 85th percentile ≤ BMI < 95th percentile; and (4) Overweight BMI ≥ 85th percentile[27]. Participants who smoked during the past 30 d or had ever smoked ≥ 100 cigarettes in their entire lives were classified as smokers in this study.

Statistical analysis

Statistical analyses were performed using STATA Release 16 (StataCorp LP, TX, United States). Categorical and ordinal factors were presented in frequency (%). Continuous factors were presented in median (interquartile range). All continuous factors were first tested for skewness; if the distributions were extremely skewed to the right (herein defined as skewness > 3), the factors were log transformed before using them as predictors in regression models. Since the response variables evaluated in this study are the steatosis grade (0 to 3) and the fibrosis score (0 to 4), linear regression model is an appropriate model for determining if predictors are significantly associated with each response variable. The significant factors in univariate level were included as predictors in stepwise regression to determine the significant predictors in multivariate level. The significance level was 0.05.

RESULTS

A total of 740 participants were included in the data analysis as shown in Figure 1. General characteristics of the study population are shown in Table 1. The median age was 15 years old with male comprising greater than 50% of the study population (n = 386, 52.16%). The largest race was Non-Hispanic White (n = 229, 30.39%), followed by Non-Hispanic Black (n = 171, 23.11%) and Mexican American (n = 130, 17.57%) respectively. The majority of the study population had a steatosis grade of S0 (n = 538, 72.8%) and fibrosis stages of F0 and F1 (n = 693, 93.65%). Steatosis (S1-S3) was present in 27% of the study population. Advanced fibrosis (F3-F4) was present in 2.84% of the study population. 53.33% (n = 392) of the study population had a normal BMI, while 28.71% (n = 211) were overweight and 0.54% (n = 4) were underweight.

Data concerning social history and physical activity were also analyzed. A smoking history was endorsed by 6 participants (0.84%). The percent of study participants who spent ≥ 5 h per day of watching TV in the past 30 d was 20.63% (n = 150). Similarly, 35.85% (n = 261) of study participants reported spending ≥ 5 h per day on the computer for the past 30 d.

Table 2 is a univariate analysis of participant characteristics stratified according to steatosis grade. Out of the 47 variables, there were 28 significant predictors. Statistically significant variables that were positively associated with steatosis grade in the multivariate analysis were log of ALT (P = 0.001), HOMA-IR (P = 0.006), waist-to-height ratio (P = 0.001), and BMI (P = 0.011) (Table 3).

Similarly, Table 4 is a univariate analysis of participant characteristics stratified according to fibrosis stage. Out of the 48 variables, there were only 9 significant predictors. In the multivariate analysis (Table 5), steatosis grade (P < 0.001), non-Hispanic black race (P = 0.002), a smoking history (P = 0.028), and systolic blood pressure (P = 0.035) were predictors of fibrosis stage that were statistically significant and positively associated with fibrosis stage.

The performance of liver fibrosis indices (APRI, FIB4, and PNFI) were summarized in Table 6. PNFI was the only significant predictor of steatosis grade. However, all liver fibrosis indices had very low positive predictive values (0%-3.26%) for predicting cirrhosis (F4).

DISCUSSION

This study reported the prevalence of steatosis and fibrosis in United States adolescents who participated in NHANES 2017-2018 as diagnosed by TE and CAP. We also identified predictors of steatosis grade and fibrosis stage in this study population. Although there was a recent study on a similar topic that utilized the same database from Ciardullo *et al*[28], the study designs were distinct as follows: (1) The maximum age in this study is 17 since the age 18 and above was used as a cut-off for many adult questionnaires in NHANES (*e.g.*, alcohol use, physical activity, and smoking); (2) We

Table 1 General characteristics of study population

	All participants (*n* = 740)
Age	15 (13-16)
Sex, *n* (%)	
Male	386 (52.16)
Female	354 (47.84)
Race, *n* (%)	
Mexican American	130 (17.57)
Other Hispanic	55 (7.43)
Non-Hispanic White	229 (30.95)
Non-Hispanic Black	171 (23.11)
Non-Hispanic Asian	83 (11.22)
Other race-including multi-racial	72 (9.73)
Smoking, *n* (%)	6 (0.84)
Steatosis grade, *n* (%)	
S0	538 (72.8)
S1	63 (8.53)
S2	39 (5.28)
S3	99 (13.4)
Fibrosis result, *n* (%)	
F0-F1	693 (93.65)
F2	26 (3.51)
F3	12 (1.62)
F4	9 (1.22)
Waist-to-height ratio	0.48 (0.43-0.55)
Waist-to-hip ratio	0.57 (0.53-0.63)
Body mass index, *n* (%)	
Underweight	4 (0.54)
Normal	392 (53.33)
Risk of overweight	128 (17.41)
Overweight	211 (28.71)
Days physically active at least 60 min	4 (2-5)
Hours/day watch TV or videos past 30 d, *n* (%)	
Less than 1 h	107 (14.72)
1 h	121 (16.64)
2 h	166 (22.83)
3 h	105 (14.44)
4 h	78 (10.73)
5 h or more	150 (20.63)
Hours/day use computer past 30 d, *n* (%)	
Less than 1 h	68 (9.34)
1 h	85 (11.68)
2 h	131 (17.99)

3 h	83 (11.4)
4 h	100 (13.74)
5 h or more	261 (35.85)

discretized the steatosis grades and fibrosis levels into 4 Levels each; (3) Advanced fibrosis was defined as ≥F3 (≥ 8.6 kPa) rather than ≥F2 (≥ 7.4 kPa); (4) We included more risk factors that were widely known to be associated with NAFLD (*e.g.*, smoking, physical activity, diet, and insulin resistance); and (5) Linear regression was used instead of logistic regression. For this reason, our results on prevalence and significant predictors are different from the previous study even though we used the same database.

We found that significant steatosis was present in over a fifth of the adolescents studied as indicated by a median CAP ≥ 248 dB/m and that advanced fibrosis (F3-F4) was present in 2.84% of the adolescents studied. Log of ALT, waist-to-height ratio, HOMA-IR, and BMI were significant predictors of steatosis in multivariate level. These four factors can be categorized into three groups that are commonly known as risk factors of NAFLD: liver chemistry (ALT), insulin resistance (HOMA-IR), and body fat (BMI and waist-to-height ratio). North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) guidelines suggested using ALT as a screening test for NAFLD with the cutoff levels of 22 mg/dL for girls and 26 mg/dL for boys[29]. BMI, waist-to-height ratio, and insulin resistance have been heavily documented as risk factors for hepatic steatosis in obese children[30,31]. In fact, insulin resistance plays a central role in the pathogenesis of non-alcoholic fatty liver disease[32].

Identifying predictors of fibrosis in adolescents is important because fibrosis has been shown to be a strong predictor of liver related complications and overall mortality[33]. Having sensitive and specific predictors of fibrosis allows us to effectively prevent and manage associated liver-related complications such as hepatocellular carcinoma and cirrhosis. In our study, multivariate stepwise regression revealed that the independent predictors of fibrosis were steatosis grade, non-Hispanic black race, smoking, and systolic blood pressure.

Non-Hispanic black race as an independent predictor of fibrosis that may be a proxy for other socioeconomic and environmental factors not collected in the research effort. Although the pathogenesis of NAFLD is not fully understood, NAFLD is widely accepted to be a genetic-environment-metabolism-related disease[34]. Consumption of refined carbohydrates and sugar-sweetened beverages have been associated with NAFLD[35]. In a study that documented self-reported sugar-sweetened beverage intake among college students, black undergraduates were found to have a higher intake of sugared beverages than compared to their contemporaries[36]. Additionally, non-Hispanic blacks are reported to have suboptimal diet quality and to not meet national dietary recommendations with lower intakes of total vegetables, milk, and whole grains than whites[37]. Our findings may reflect the dietary and environmental differences among black adolescents and requires further investigation.

Smoking has been identified as an independent risk factor of NAFLD in adult patients[38,39]. The presumed pathogenesis is through the consumption of toxins in cigarettes that affect the antioxidant system, which includes cytochrome P450 and inflammatory cytokines[35]. Our smoking sub-group was adolescents and underpowered with a sample size of 6, so further investigation is needed to confirm smoking as a specific predictor for fibrosis.

Previous animal model study showed that the steatosis of any cause was associated with hepatic inflammatory changes and fibrosis by causing oxidative stress and mitochondrial dysfunction[40]. However, there were limited clinical evidence on the association between steatosis and fibrosis in general pediatric or adolescent population. Systolic hypertension is known as a primary clinical feature of metabolic syndrome, which were previously reported as independence risk factor of NAFLD[41].

Additionally, we compared the performance of three liver fibrosis indices for predicting steatosis (S1-S3) and cirrhosis (F4). PNFI was the only liver fibrosis index having a PPV and sensitivity greater than zero. Although it was only index that can be used to predict NAFLD, the performance on this dataset was moderately high with an accuracy of 85.6%. The superior performance of PNFI could derive from the fact that it is the only index developed by using the liver biopsy in the pediatric population[21] while other two indices (APRI and FIB4) were originally developed from the adult population[19,20], which could perform poorly in pediatric or adolescent population.

Table 2 Univariate Analysis of participant characteristics and steatosis grade

	Steatosis grade				Coefficient	*P* value
	S0 (*n* = 538)	S1 (*n* = 63)	S2 (*n* = 39)	S3 (*n* = 99)		
Age	14 (13-16)	14 (13-16)	15 (14-16)	15 (14-16)	0.0562	0.016[a]
Sex						
Male	273 (50.74%)	29 (46.03%)	20 (51.28%)	64 (64.65%)	0.1747	0.027[a]
Female	265 (49.26%)	34 (53.97%)	19 (48.72%)	35 (35.35%)		
Race						
Mexican American	82 (15.24%)	11 (17.46%)	7 (17.95%)	30 (30.3%)	0.3542	< 0.001[a]
Other Hispanic	42 (7.81%)	5 (7.94%)	1 (2.56%)	7 (7.07%)	-0.0903	0.549
Non-Hispanic White	178 (33.09%)	18 (28.57%)	13 (33.33%)	20 (20.2%)	-0.2008	0.019[a]
Non-Hispanic Black	128 (23.79%)	10 (15.87%)	11 (28.21%)	21 (21.21%)	-0.0440	0.640
Non-Hispanic Asian	60 (11.15%)	8 (12.7%)	4 (10.26%)	11 (11.11%)	-0.0026	0.983
Other Race-Including Multi-Racial	48 (8.92%)	11 (17.46%)	3 (7.69%)	10 (10.1%)	0.0666	0.618
Smoking	4 (0.77%)	1 (1.59%)	0 (0%)	1 (1.04%)	0.0732	0.868
Waist-to-height ratio	0.45 (0.42-0.51)	0.54 (0.47-0.59)	0.57 (0.48-0.62)	0.6 (0.55-0.66)	6.5565	< 0.001[a]
Waist-to-hip ratio	0.56 (0.52-0.6)	0.6 (0.57-0.65)	0.63 (0.57-0.68)	0.64 (0.61-0.69)	6.6835	< 0.001[a]
Body mass index					0.6128	< 0.001[a]
Underweight	4 (0.75%)	0 (0%)	0 (0%)	0 (0%)		
Normal	349 (65.48%)	22 (34.92%)	10 (25.64%)	10 (10.1%)		
Risk of overweight	97 (18.2%)	14 (22.22%)	8 (20.51%)	9 (9.09%)		
Overweight	83 (15.57%)	27 (42.86%)	21 (53.85%)	80 (80.81%)		
Days physically active at least 60 min	4 (2-6)	3.5 (1-5)	4 (2-5)	4 (2-5)	-0.0167	0.348
Hours/day watch TV or videos past 30 d	2 (1-4)	2 (1-4)	3 (1.75-5)	2 (1-4.25)	0.0421	0.070
Hours/day use computer past 30 d	3 (2-5)	4 (2-5)	5 (2-5)	4 (2-5)	0.0560	0.014[a]
Diet						
Energy (1000 kcal)	1.82 (1.43-2.45)	1.75 (1.26-2.28)	1.62 (1.33-2)	1.71 (1.34-2.38)	-0.0732	0.145
Protein (mg)	63.59 (48.32-86.06)	63.15 (40.29-77.27)	53.38 (37.92-80.78)	64.1 (49.36-87.48)	-0.0732	0.145
Carbohydrate (mg)	230.94 (180.54-301.73)	233.36 (154.21-296.51)	213.08 (169.63-253.71)	219.06 (173.09-290.58)	-0.0005	0.178
Total sugars (mg)	94.74 (67.04-133.77)	89.84 (54.32-140.86)	75.37 (51.43-97.67)	90.5 (63.35-127.21)	-0.0008	0.224
Dietary fiber (mg)	12.85 (9.25-17.36)	12.2 (8.77-18.3)	12.5 (9.24-16.79)	12.2 (8.8-16.4)	-0.0051	0.368
Total fat (mg)	75.09 (55.41-97.52)	66.13 (43.61-93.44)	62.68 (45.53-83.34)	71.58 (47.42-95.31)	-0.0016	0.143
Total saturated fatty acids (mg)	25.07 (17.39-35.43)	24.43 (11.47-32.25)	18.7 (11.7-30.89)	22.91 (14.97-31.11)	-0.0044	0.122
Cholesterol (mg)	197 (132.88-320.5)	165 (90.25-305.5)	162 (72.38-283.63)	199 (134-279.25)	-0.0003	0.254
Systolic blood pressure (mm Hg)	106 (100-114)	108 (103.5-114.5)	112 (104-120)	112 (104-120)	0.0254	< 0.001[a]
Diastolic blood pressure (mm Hg)	62 (54-68)	60 (51.5-68)	62 (55.5-70)	60 (54-66)	-0.0038	0.222
Triglycerides, refrig serum (mg/dL)[1]	74 (57-98)	79 (62-103)	78.5 (70-105.5)	98 (68-159)	0.0051	< 0.001[a]
Uric acid (mg/dL)	4.7 (4-5.6)	5.1 (4.15-6.05)	5.45 (4.65-6.15)	5.75 (4.7-6.7)	0.1984	< 0.001[a]
Aspartate aminotransferase (IU/L)[1]	18 (16-22)	18 (15.25-21.75)	16.5 (15-21)	20.5 (18-27)	0.0151	0.002[a]
Alanine aminotransferase (IU/L)[1]	12 (10-15)	15 (11.25-19)	14 (10-17.5)	20.5 (14-34)	0.0372	< 0.001[a]
Gamma glutamyl transferase (IU/L)[1]	12 (10-15)	12 (10-18.75)	15.5 (10-19)	18 (12-24)	0.0375	< 0.001[a]

Alkaline phosphatase (ALP) (IU/L)	130 (87-225.75)	121 (86.75-235)	135 (75.5-188)	126.5 (99-188)	-0.0003	0.537
Total bilirubin (mg/dL)[1]	0.4 (0.3-0.6)	0.3 (0.23-0.48)	0.4 (0.3-0.5)	0.4 (0.3-0.4)	-0.3419	0.009[a]
Total protein (g/dL)	7.3 (7-7.5)	7.3 (7-7.5)	7.35 (7.15-7.6)	7.35 (7.2-7.6)	0.3620	0.002[a]
Albumin, refrigerated serum (g/dL)	4.3 (4.1-4.5)	4.3 (4.1-4.5)	4.25 (4.05-4.45)	4.2 (4-4.4)	-0.5553	< 0.001[a]
Iron frozen, serum (μg/dL)	85 (61-113)	86 (58.25-105.75)	69 (49.5-85.75)	75 (56-103)	-0.0027	0.013[a]
Total iron binding capacity (μg/dL)	348 (317.5-382)	366 (342-392.25)	360 (326.25-406.5)	356 (322-385)	0.0015	0.092
Transferrin Saturation (%)	24 (17-33)	23 (15.25-30.75)	19 (13.5-26)	22.5 (15-30)	-0.0104	0.004[a]
Ferritin (ng/mL)	39.2 (24.85-59.85)	35.25 (18.75-57.5)	30.85 (14.65-60.15)	59.2 (35-93.12)	0.0038	< 0.001[a]
Total cholesterol (mg/dL)	150 (134-168)	158 (132.75-174)	152 (139.5-166.25)	157 (139.25-178.75)	0.0032	0.035[a]
Low-density lipoprotein cholesterol (mg/dL)	78.8 (64.8-94.6)	85.8 (69.15-107.45)	82.5 (70.1-97.8)	87 (70.6-103.6)	0.0041	0.019[a]
Direct high-density lipoprotein cholesterol (mg/dL)	53 (46-61)	50 (46-56)	48 (41.5-55)	44 (39-51)	-0.0238	< 0.001[a]
HS C-reactive protein (mg/L)[1]	0.49 (0.32-1.01)	0.72 (0.35-1.51)	0.95 (0.43-1.89)	1.76 (0.87-3.74)	0.0448	< 0.001[a]
Platelet count (1000 cells/uL)	258 (228-292)	269 (228.5-318.5)	273 (239-307)	282 (248-313)	0.0026	< 0.001[a]
Hemoglobin A1c (%)[1]	5.3 (5.1-5.5)	5.3 (5.1-5.45)	5.3 (5.1-5.6)	5.4 (5.2-5.5)	0.2280	0.054
Fasting glucose (mg/dL)	97 (93-101)	98 (93.25-101.75)	101 (94-103)	99.5 (96-103)	0.0219	0.017[a]
Insulin (pmol/L)	54.96 (39.84-79.38)	101.1 (71.58-130.8)	88.32 (62.28-118.14)	129.63 (75.66-185.46)	0.0086	< 0.001[a]
Homeostatic model assessment for insulin resistance	2.23 (1.58-3.32)	4.08 (2.96-5.47)	3.56 (2.64-4.96)	5.34 (3.08-7.78)	0.1976	< 0.001[a]

[1]Skewness > 3.
[a]$P < 0.05$. HS: High sensitivity.

Table 3 Predictors of steatosis grade in multivariate level

Predictors	Coefficient (standard error)	*P* value
Alanine aminotransferase (IU/L)[1]	0.3912 (0.1159)	0.001
Homestatic model assessment for insulin resistance	0.0684 (0.0247)	0.006
Waist-to-height ratio	3.2299 (0.0912)	0.001
Body mass index	0.2335 (0.0912)	0.011

[1]Log-transformed predictor. Number of observations = 307; Adjusted $R^2 = 0.37$;

There are several limitations of this study. Our study population is of United States adolescents and may not be reflective of non-American populations. Alcohol was not measured in the study population and also presumed to be zero because the population was United States adolescents. The legal age to drink in the United States is 21 but for some people drinking alcohol begins in adolescence[42]. Another limitation is subgroup sample size which was seen subgroups such as smoking, F3, and F4. Low statistical power reduces the chance of detecting a true effect[43]. Some variables not available in the NHANES include hormonal levels and Tanner stages of the participants. Hypogonadism and low testosterone level are associated with an increased risk for NAFLD and NASH[44]. Additionally, low sex hormone binding globulin (SHBG) can be viewed as a marker for NAFLD in women with oligomenorrhea and/or hirsutism[45]. Since these variables were not included in the NHANES database, they were not accounted for. Lastly, though seeing increasing utility in diagnostic value, TE has not been traditionally studied in adolescents.

Table 4 Univariate Analysis of participant characteristics and fibrosis stage

	Fibrosis stage				Coefficient	*P* value
	F0 - F1 (*n* = 693)	F2 (*n* = 26)	F3 (*n* = 12)	F4 (*n* = 9)		
Age	15 (13-16)	15 (13-17)	14 (13-15)	15 (14.75-17)	0.0106	0.276
Sex						
Male	356 (51.37%)	17 (65.38%)	6 (50%)	7 (77.78%)	0.0533	0.105
Female	337 (48.63%)	9 (34.62%)	6 (50%)	2 (22.22%)		
Race						
Mexican American	123 (17.75%)	4 (15.38%)	0 (0%)	3 (33.33%)	-0.0049	0.909
Other Hispanic	53 (7.65%)	1 (3.85%)	1 (8.33%)	0 (0%)	-0.0535	0.393
Non-Hispanic White	222 (32.03%)	3 (11.54%)	2 (16.67%)	2 (22.22%)	-0.0685	0.054
Non-Hispanic Black	148 (21.36%)	13 (50%)	8 (66.67%)	2 (22.22%)	0.1309	< 0.001[a]
Non-Hispanic Asian	78 (11.26%)	4 (15.38%)	0 (0%)	1 (11.11%)	-0.0222	0.669
Other Race-Including Multi-Racial	69 (9.96%)	1 (3.85%)	1 (8.33%)	1 (11.11%)	-0.0230	0.679
Smoking	5 (0.75%)	0 (0%)	0 (0%)	1 (11.11%)	0.3967	0.032[a]
Waist-to-height ratio	0.48 (0.43-0.55)	0.49 (0.44-0.61)	0.49 (0.4-0.61)	0.5 (0.42-0.68)	0.3746	0.042[a]
Waist-to-hip ratio	0.57 (0.53-0.62)	0.59 (0.54-0.69)	0.6 (0.52-0.64)	0.59 (0.5-0.7)	0.2804	0.215
Body mass index					0.0330	0.079
Underweight	4 (0.58%)	0 (0%)	0 (0%)	0 (0%)		
Normal	370 (53.78%)	11 (42.31%)	6 (50%)	5 (55.56%)		
Risk of overweight	126 (18.31%)	2 (7.69%)	0 (0%)	0 (0%)		
Overweight	188 (27.33%)	13 (50%)	6 (50%)	4 (44.44%)		
Days physically active at least 60 min	4 (2-5)	4 (2-5)	2.5 (0.5-6)	5 (2.5-6)	-0.0039	0.597
Hours/day watch TV or videos past 30 d	2 (1-4)	2 (1-4)	2.5 (2-4.5)	2 (0-3.5)	-0.0027	0.779
Hours/day use computer past 30 d	3 (2-5)	5 (3-5)	4 (2.5-5)	3 (0.75-5)	0.0062	0.519
Steatosis grade					0.0757	< 0.001[a]
S0	518 (74.86%)	13 (50%)	3 (25%)	4 (44.44%)		
S1	57 (8.24%)	2 (7.69%)	3 (25%)	1 (11.11%)		
S2	35 (5.06%)	2 (7.69%)	2 (16.67%)	0 (0%)		
S3	82 (11.85%)	9 (34.62%)	4 (33.33%)	4 (44.44%)		
Diet						
Energy (1000 kcal)	1.8 (1.4-2.42)	1.5 (1.37-2.11)	1.62 (1.4-1.75)	1.75 (1.32-2.4)	-0.0225	0.282
Protein (mg)	63.69 (46.81-85.41)	50.66 (42.74-94.29)	59.33 (45.9-76.55)	68.03 (49.61-73.78)	-0.0004	0.405
Carbohydrate (mg)	230.55 (174.26-299.84)	202.56 (152.11-255.23)	204.26 (177.87-238.7)	242.27 (186.12-305.52)	-0.0001	0.671
Total sugars (mg)	92.59 (64.25-133.63)	87.43 (58.07-120.32)	75.76 (62.31-94.74)	94.74 (85.8-123.02)	-0.0001	0.697
Dietary fiber (mg)	12.7 (9.25-17.1)	10.8 (7.62-17.64)	10.4 (9.02-14.29)	12.85 (10.8-16.96)	-0.0018	0.461
Total fat (mg)	74.07 (52.04-97.34)	55.7 (45.07-79.29)	65.98 (50.43-78.07)	69.09 (45.95-97.94)	-0.0007	0.091
Total saturated fatty acids (mg)	24.72 (16.8-34.81)	21.06 (14.84-29.88)	23.04 (18.87-27.64)	22.84 (18.35-28.35)	-0.0020	0.083
Cholesterol (mg)	197 (129.13-317.63)	150.5 (85-213.25)	162.5 (118.38-228.88)	146.5 (124.75-310.25)	-0.0002	0.065
Systolic blood pressure (mmHg)	106 (102-114)	108 (103.5-126.5)	108 (100.5-120)	116 (113-122)	0.0066	< 0.001[a]
Diastolic blood pressure (mmHg)	62 (54-68)	64 (53-71)	56 (50.5-65.5)	60 (56-68)	-0.0001	0.967

Triglycerides, refrig serum (mg/dL)[1]	78 (61-104)	67.5 (50-111)	88 (61.75-161)	88.5 (56.5-121.5)	0.0276	0.471
Uric acid (mg/dL)	4.9 (4.1-5.8)	5 (3.7-6)	4.7 (3.3-5.98)	5.75 (4.2-7.45)	0.0079	0.560
Aspartate aminotransferase (IU/L)[1]	18 (16-22)	18 (15-25)	15 (14-23)	29 (20-32)	0.0882	0.137
Alanine aminotransferase (IU/L)[1]	13 (10-17)	14 (9-20)	12 (9.5-15)	20.5 (15-37.5)	0.0738	0.046[a]
Gamma glutamyl transferase (IU/L)[1]	13 (10-17)	12 (9-16)	12 (10-19.5)	20.5 (14-32.5)	0.0047	0.018[a]
Alkaline phosphatase (IU/L)	129 (88-222.5)	121.5 (81-205)	187 (127.75-242.75)	113 (105-129.5)	-0.0001	0.470
Total bilirubin (mg/dL)[1]	0.4 (0.3-0.5)	0.3 (0.2-0.6)	0.4 (0.3-0.5)	0.45 (0.35-0.7)	0.0178	0.577
Total protein (g/dL)	7.3 (7-7.5)	7.15 (6.8-7.3)	7.3 (7-7.63)	7.2 (7.15-7.45)	-0.0156	0.745
Albumin, refrigerated serum (g/dL)	4.3 (4.1-4.5)	4.05 (3.8-4.4)	4.2 (4.03-4.3)	4.3 (4.1-4.65)	-0.0744	0.229
Iron frozen, Serum (μg/dL)	83 (59-112)	80.5 (47-88)	88 (68-106)	67.5 (58-120.5)	0.0001	0.777
Total iron binding capacity (μg/dL)	352 (322-387)	346 (314-378)	355 (315.25-375.25)	314 (310-327.5)	-0.0009	0.018[a]
Transferrin saturation (%)	23 (17-32)	22 (15-26)	28 (19.25-31.5)	20 (18-39)	0.0013	0.377
Ferritin (ng/mL)	39.3 (24.5-62.1)	45.55 (24.45-61.85)	56.25 (29-71)	102.35 (35.75-141)	0.0009	0.030[a]
Total cholesterol (mg/dL)	151 (134-171)	140.5 (136-156)	161 (143-175)	131 (119-147.5)	-0.0010	0.102
Low-density lipoprotein cholesterol (mg/dL)	81 (66.2-97.6)	77.5 (64.2-92.6)	88.2 (71.6-91.4)	57.2 (55.5-80.1)	-0.0013	0.082
Direct high-density lipoprotein cholesterol (mg/dL)	51 (44-59)	49.5 (44-58)	50 (46-62)	49.5 (39-56)	-0.0012	0.426
HS C-reactive protein (mg/L)[1]	0.57 (0.35-1.39)	0.83 (0.34-1.34)	0.72 (0.37-1.12)	0.97 (0.53-7.09)	0.0240	0.134
Platelet count (1000 cells/uL)	262 (230-297.5)	275.5 (242-302.5)	262.5 (226-277)	262.5 (234-275)	-0.0001	0.769
Hemoglobin A1c (%)[1]	5.3 (5.1-5.5)	5.3 (5.25-5.6)	5.45 (5.25-5.65)	5.35 (5.15-5.6)	0.4629	0.098
Fasting glucose (mg/dL)	98 (94-102)	99 (94-103)	101 (95.5-104.25)	92 (89.75-95.75)	-0.0031	0.490
Insulin (pmol/L)	64.83 (43.38-99)	70.26 (45.87-183.17)	87.06 (59.28-160.28)	51.42 (27.29-127.14)	0.0005	0.291
Homeostatic model assessment for insulin resistance	2.61 (1.71-3.96)	2.66 (1.96-7.9)	4.08 (2.34-6.66)	1.95 (1.1-4.95)	0.0101	0.383

[1]Skewness > 3.
[a]$P < 0.05$. HS: High sensitivity.

Table 5 Predictors of fibrosis stage in multivariate level

Predictors	Coefficient (standard error)	*P* value
Steatosis grade	0.0730 (0.0172)	< 0.001
Race: Non-Hispanic Black	0.1352 (0.0430)	0.002
Smoke	0.4065 (0.1845)	0.028
Systolic blood pressure (mmHg)	0.0040 (0.0019)	0.035

Number of observations = 643; Adjusted R^2 = 0.0598.

CONCLUSION

In conclusion, this study showed steatosis and advanced liver fibrosis in 27.2% and 2.7% of United States adolescents, respectively. ALT, BMI, HOMA-IR, and waist-to-height ratio were predictors of steatosis, while steatosis grade, smoking, non-Hispanic black race, systolic blood pressure were predictors of fibrosis. Environmental, dietary, and social history are important information to gather from adolescents as these factors can contribute to a risk of steatosis and fibrosis. Given the progressive nature of chronic liver disease, the evidence of steatosis or advanced fibrosis in younger age could lead to increased steatohepatitis and cirrhosis in young adults.

Table 6 Predictive performance of liver fibrosis indices

Liver fibrosis indices (Predictor)		Outcome	Predictive performance				
Index	Cutoff		Accuracy	PPV	NPV	Sensitivity	Specificity
APRI	0.7	F4	98.45%	0%	98.8%	0.0%	99.7%
FIB4	1.3	F4	98.61%	0%	98.8%	0.0%	99.8%
PNFI	9	F4	85.31%	3.26%	99.1%	37.5%	85.9%
PNFI	3	S1-S3	85.60%	83.33%	86.2%	59.7%	95.5%

APRI: Aspartate aminotransferase to platelet ratio index; FIB4: Fibrosis-4 index; NPV: Negative predictive value; PNFI: Pediatric non-alcoholic fatty liver disease fibrosis index; PPV: Positive predictive value.

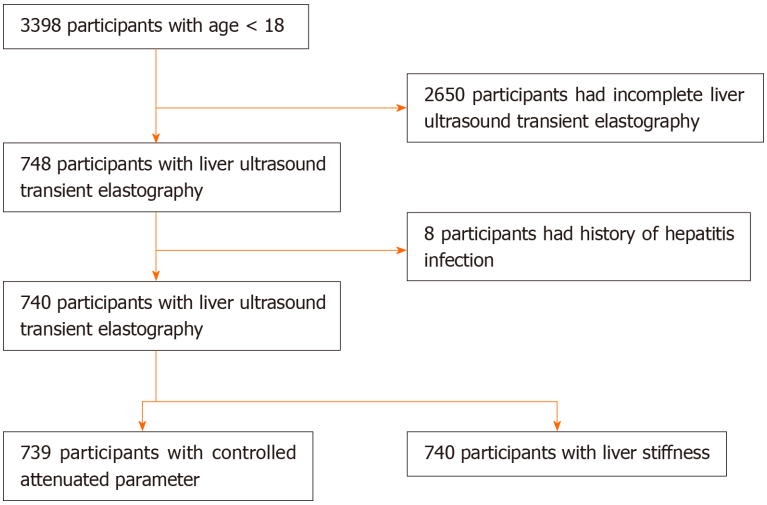

Figure 1 Study design flow chart.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in children and adolescents.

Research motivation

With the rise of obesity and metabolic syndrome among younger populations, NAFLD is a growing concern in adolescents.

Research objectives

The authors aimed to determine the prevalence and risk factors of steatosis and advanced fibrosis using transient elastography in the United States' adolescent population.

Research methods

The authors studied adolescent participants aged 13 to 17 years who underwent TE and controlled attenuation parameter using the National Health and Nutrition Examination Survey 2017-2018.

Research results

There is a high prevalence of steatosis (27.2%) in the United States' adolescent population, with 2.84% having advanced fibrosis. Risk factors of steatosis grade included alanine aminotransferase, insulin resistance, waist-to-height ratio, and body mass index. Steatosis grade, non-Hispanic black race, smoking history, and systolic blood pressure were significant predictors of fibrosis.

Research conclusions

Adolescents with steatosis or advanced fibrosis could progress to increased steatohepatitis and cirrhosis in young adults.

Research perspectives

Environmental, dietary, and social history are important information to gather from adolescents as these factors can contribute to a risk of steatosis and fibrosis. Given the progressive nature of chronic liver disease, the evidence of steatosis or advanced fibrosis in younger age could lead to increased steatohepatitis and cirrhosis in young adults.

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SYSTEMATIC REVIEWS

Safety of liver resection in patients receiving antithrombotic therapy: A systematic review of the literature

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Abstract

BACKGROUND

Little is unknown about the effect of chronic antithrombotic therapy (ATT) on bleeding complication during or after hepatectomy. In addition, the safety and effectiveness of chemical prevention for venous thromboembolism (VTE) is still controversial.

AIM

To clarify the effect of ATT on thromboembolism and bleeding after liver resection.

METHODS

Articles published between 2011 and 2020 were searched from Google Scholar and PubMed, and after careful reviewing of all studies, studies concerning ATT and liver resection were included. Data such as study design, type of surgery, type of antithrombotic agents, and surgical outcome were extracted from the studies.

RESULTS

Sixteen published articles, including a total of 8300 patients who underwent hepatectomy, were eligible for inclusion in the current review. All studies regarding patients undergoing chronic ATT showed that hepatectomy can be performed safely, and three studies have also shown the safety and efficacy of preoperative continuation of aspirin. Regarding chemical prevention for VTE, some studies have shown a potentially high risk of bleeding complications in patients undergoing chemical thromboprophylaxis; however, its efficacy against VTE has not been shown statistically, especially among Asian patients.

CONCLUSION

Hepatectomy in patients with chronic ATT can be performed safely without increasing the incidence of bleeding complications, but the safety and effectiveness of chemical thromboprophylaxis against VTE during liver resection is still

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controversial, especially in the Asian population. Establishing a clear protocol or guideline requires further research using reliable studies with good design.

Key Words: Liver resection; Bleeding complication; Antithrombotic therapy; Thromboembolic complication; Thromboprophylaxis

Core Tip: A total of 16 published articles on antithrombotic therapy and hepatectomy have been reviewed systematically. The articles showed that the risk of thromboembolic and/or bleeding events in patients with continued preoperative aspirin was not different from those in patients with no antithrombotic or interrupted antiplatelet drugs, although pharmacological prophylaxis of venous thromboembolism is still controversial, especially when performing hepatectomy in Asian patient populations.

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INTRODUCTION

Heart disease, cerebrovascular disease, and cancer are the three leading causes of death in the world. With the aging of society in recent years, patients with cerebrovascular and/or cardiovascular diseases are increasingly required to undergo non-cardiac surgery. Most of these patients receive antithrombotic therapy (ATT) in order to prevent thromboembolic events. The perioperative period in patients undergoing ATT is at high risk for both thromboembolism and bleeding, which can be very cumbersome for surgeons[1-3].

ATT is classified into two types of drugs: Antiplatelet drugs and anticoagulants. Antiplatelet drugs are frequently used for prevention of cerebrovascular or cardiovascular diseases, and can prevent thromboembolism by reduction of platelet aggregation. Antiplatelet agents include thienopyridine (*e.g.*, clopidogrel, prasugrel, or ticlopidine), aspirin, and type III phosphodiesterase inhibitor (*e.g.*, cilostazol)[4]. Anticoagulants, on the other hand, prevent coagulation of blood by suppressing the coagulation cascade. They are usually used for deep vein thrombosis, atrial fibrillation, acute coronary syndrome, and cardiac endoprostheses. Anticoagulants are also used for perioperative thromboprophylaxis of venous thromboembolism (VTE). Oral anticoagulants include warfarin, factor Xa inhibitors (*e.g.*, apixaban, rivaroxaban, edoxaban), and direct thrombin inhibitors (*e.g.*, dabigatran)[4,5]. The latter two types are called direct-acting oral anticoagulants (DOACs) or non-vitamin K antagonist oral anticoagulants (NOACs), and now increasingly used. Table 1 summarizes the type and the duration of action of each antithrombotic agent.

Minimizing intraoperative and postoperative bleeding complication is an important challenges in liver resection, and several technical improvement has been demonstrated, such as Pringle maneuver or sustained low central venous pressure (CVP)[6-8]. However, sustained low CVP during hepatectomy may increase the risk of thrombosis in ATT-received patients. Rigorous perioperative management of antithrombotics and strict hemostasis are requisite to prevent both thromboembolic and bleeding events. To date, there has been no consensus on the safety of hepatectomy and proper perioperative management of antithrombotics in patients undergoing ATT, and the optimal thrombotic prophylaxis for VTE remains unknown.

The aim of the current review is to clarify the effect of ATT on thromboembolic and bleeding complications in liver resection.

Table 1 Types, specific agents, and acting duration of commonly used antithrombotic drugs

Class of agents	Type	Specific agents	Duration of action
Antiplatelets			
	Thienopyridines	Clopidogrel (Plavix), ticlopidine (Panardine), prasugrel (Effient), ticagrelor (Brilinta)	5-7 d[1]
	Type III PDE inhibitor	Cilostazol (Pretal)	2 d
	Acetylsalicylic acid	Aspirin	7-10 d
	Other NSAIDs	Ibuprofen (Brufen, Advil), loxoprofen (Loxonin), diclofenac (Voltaren) *etc.*	Varies
Anticoagulants			
	Heparin (unfractionated)	Heparin	1-2 h
	Heparin (LMWH)	Dalteparin (Fragmin iv), enoxaparin (Clexane, s.c.), nadroparin (s.c.)	6-12 h[2]
	Vitamin K antagonist	Warfarin (Coumadin)	5 d
	Factor Xa inhibitor (s.c.)	Fondaparinux (Arixtra)	1-1.5 d
	DOACs		
	Direct thrombin inhibitor	Dabigatran (Pradaxa)	1-2 d
	Factor Xa inhibitors	Rivaroxaban (Xarelto), apixaban (Eliquis), edoxaban (Lixiana)	1-2 d

[1]In ticlopidine, duration of action is 10-14 d.
[2]In dalteparin, duration of action is 2-4 h. PDE: Phosphodiesterase; NSAID: Non-steroidal anti-inflammatory drug; DOAC: Direct-acting oral anticoagulant.

MATERIALS AND METHODS

Papers published between 2011 and 2020, which were written in English, were collected from Google Scholar and PubMed. The following key words were adopted for searching: "liver resection or hepatectomy" AND "antithrombotic therapy, aspirin, clopidogrel, antiplatelet therapy, anticoagulation, warfarin, DOAC, or NOAC" AND "bleeding or hemorrhage". Only articles which were published in the peer review journal were included in the current review. Eligible study types include prospective cohort studies, retrospective cohort studies, randomized clinical trials, or case-control studies, but case reports, reviews, or guidelines were not included.

Duplicate articles were first removed, and then articles were excluded systematically by reviewing each study carefully. Eligible articles were finally determined after the quality of each study was evaluated according to the study design. Complete data, including study design, sample size, publication year, type of surgery, type of antithrombotics, and surgical outcome, were extracted from the studies. Bleeding events included two categories; postoperative bleeding complications (BC) and increased surgical blood loss (SBL).

RESULTS

Study characteristics

Collection and screening of research were performed from December 2020 to January 2021 (Figure 1). The current review included a total of 16 published articles, with 8300 patients undergoing hepatectomy. There were no randomized clinical trials, but only case-control studies or cohort studies. Ten of the 16 studies were observational cohort studies, and only one was prospective studies; 6 studies were on the management of patients with chronic ATT[9-14] (Table 2) and 10 studies were on the pharmacological prevention for VTE (Table 3)[15-24]. Among studies regarding the management of chronic ATT, two studies were investigated using the propensity score matching method[9,12]. Nine of the 10 articles on pharmacological prophylaxis for VTE were observational studies; one was multicenter prospective and 8 were retrospective cohort studies.

Of the 6 studies on the management of patients receiving chronic ATT, three focused on the safety of continued perioperative aspirin during hepatectomy[9,12,13]. In 10 studies on pharmacological prevention for VTE, patients were primarily controlled by low-molecular-weight heparin during the perioperative period.

Table 2 Reported data concerning bleeding complications of liver resection in patients with antithrombotic therapy

Ref.	Year, type	Surgery type	Drug use and exposure	Bleeding events	TE, mortality
Naito *et al* [9]	2020, PSM	Liver resection (n = 425)	Patients with continued ASA (n = 63); Patients not on continued APT (control, n = 362); Post-PSM: 63 *vs* 63 matched cases	BC 4.8% in continued ASA *vs* 4.8% in control (P = 1.00); SBL was identical (P = 0.54)	TE 1.6% in continued ASA *vs* 4.8% in control (P = 0.62); Mortality 1.6% *vs* 1.6% (P = 1.00)
Fujikawa *et al*[10]	2017, RCS	Liver resection (n = 258) including 77 laparoscopic liver resection	Patients with ATT (n = 100); Patients without ATT (control; n = 158)	BC 3.0% in ATT *vs* 3.8% in control (P > 0.05); No BC in laparoscopic surgery; SBL was identical	TE 1.0% *vs* 1.3% (P > 0.05); No TE in laparoscopic surgery; Mortality 1.0% *vs* 0% (P = 0.350)
Ishida *et al* [11]	2017, CCS	HBP surgery (n = 886) including 520 liver resection	Patients with ACT (n = 39); Patients with APT (n = 77); Patients without ATT (control, n = 770)	BC 0.0% in ACT *vs* 1.3% in APT *vs* 3.4% in control (P = 0.32); SBL was identical (P = 0.99)	TE 0% *vs* 1.3% *vs* 0.8% (P = 0.75); Mortality 0% *vs* 0% *vs* 1.2% (P = 0.50)
Gelli *et al* [12]	2018, PSM	Liver resection (n = 1803)	Patients with continued ASA (n = 118); Patients not on continued APT (control, n = 1685); Post-PSM: 108 *vs* 108 matched cases	Overall BC 10.2% in continued ASA *vs* 12.0% in control (P > 0.05); Major BC 6.5% *vs* 5.6% (P > 0.05)	Mortality 5.6% *vs* 4.6% (P > 0.05)
Monden *et al*[13]	2017, CCS	Liver resection (n = 378)	Patients with continued ASA (n = 31); Patients not on continued APT (control, n = 347)	Major BC 0% in continued ASA *vs* 0.3% in control (P > 0.05); SBL 450 mL *vs* 360 mL (P = 0.735)	TE 3.2% *vs* 0% (P > 0.05); Mortality 3.2% *vs* 0.9% (P = 0.291)
Fujikawa *et al*[14]	2019, CCS	HBP surgery (n = 105) including 37 liver resection	Patients with DOAC (n = 35); Patients with WF (control, n = 80)	BC 2.9% in DOAC *vs* 0% in WF (P = 0.304); SBL was identical (P = 0.782)	No TE event in both groups; No mortality in both groups

RCS: Retrospective cohort study; mRCS: Multicenter retrospective cohort study; CCS: Case-control study; PSM: Case-control study with propencity-score matching; ATT: Antithrombotic therapy; APT: Antiplatelet therapy; ACT: Anticoagulation therapy; ASA: Aspirin; LAP: Laparoscopic; SBL: Surgical blood loss; BC: Postoperative bleeding complication; TE: Thromboembolism.

Safety of liver resection in patients receiving chronic ATT

In all 6 studies regarding the management of ATT-received patients, the authors generally demonstrated the safety of hepatectomy even in patients with chronic ATT. Among patients undergoing chronic ATT, the rates of major and overall BCs were 0%-6.5% and 1.3%-10.2%, retrospectively; the incidence of postoperative thromboembolic complication was 0%-3.2%. In all included studies, the rates of bleeding and thromboembolic complications between ATT-received patients and those without ATT were not significantly different (Table 2).

The safety of continued perioperative aspirin during hepatectomy was focused on in 3 case-control studies, including 2 studies using the propensity score matching method[9,12,13]. All three studies have shown that continued preoperative aspirin is not associated with increased intraoperative and postoperative bleeding events in patients with chronic antiplatelet therapy during or after hepatectomy. These studies suggested that continued preoperative aspirin in patients with chronic antiplatelet therapy is safe and should be considered preferable even when performing hepatectomy.

Safety of chemical thromboprophylaxis for VTE

In 10 articles regarding pharmacological prevention for VTE, 9 were observational cohort studies, including 8 retrospective and 1 prospective studies. The included studies generally showed potentially elevated risks of BC in patients receiving pharmacological thromboprophylaxis; the rates of overall and major BCs in the group receiving pharmacological thromboprophylaxis were 5.2%-26.6% and 1.6%-10.9%, respectively. Concerning the efficacy of thromboprophylaxis, 3 studies showed that the occurrence of VTE in patients receiving pharmacological thromboprophylaxis was significantly lower compared to the control group[15,20,24], but the other 7 studies, including 2 studies from Japan[18,19] did not demonstrate its effectiveness due to the small sample size (Table 3).

Analysis of these studies have demonstrated a potentially high risk of postoperative bleeding in patients undergoing pharmacological prevention for VTE, but the efficacy of pharmacological thromboprophylaxis after hepatectomy has not been shown, especially in Asian patient population.

Table 3 Reported data concerning the safety of thromboprophylaxis for venous thromboembolism during liver resection

Ref.	Year, type	Surgery type	Drug use and exposure	Bleeding events	TE, mortality
Ainoa *et al* [15]	2020, RCS	Liver resection (n = 512)	Patients with preop TP (n = 253); Patients with postop TP (control, n = 259)	BC 15.0% in preop TP *vs* 13.9% in control (P > 0.05)	VTE 1.2% *vs* 9.7% (P < 0.0001); PE 1.2% *vs* 9.3% (P < 0.0001)
Ejaz *et al*[16]	2014, RCS	Liver resection (n = 599)	Patients with TP (n = 454); Patients without TP (control, n = 145)	Not mentioned	VTE 5.1% in TP *vs* 3.4% in control (P = 0.42)
Nathan *et al* [17]	2014, RCS	Liver resection (n = 2147)	Patients with early TP (n = 1295); Patients with late or no TP (control, n = 852)	Major BC 1.7% in early TP *vs* 1.6% in control (P > 0.05)	VTE 2.1% *vs* 3.3% (P > 0.05); Overall mortality 1.9%
Eguchi *et al* [18]	2020, mPCS	Major HBP surgery (n = 133) including 74 liver resection	Patients with TP [LMWH (enoxaparin), n = 133, single arm]	Major BC 2.3%; Minor BC 5.2%	No PE event in whole cohort
Hayashi *et al* [19]	2014, RCS	Major HBP surgery (n = 349) including 138 liver resection	Patients with TP (n = 207); Patients without TP (control, n = 142)	BC 26.6% in TP *vs* 8.5% in control (P < 0.05); Rate of major BC is identical	VTE 2.9% *vs* 7.7% (P > 0.05)
Wang *et al* [20]	2018, CCS	Liver resection (n = 233)	Patients with TP (LMWH, n = 117); Patients without TP (control, n = 116)	Not mentioned	VTE 0.85% in TP *vs* 13.8% (P < 0.05)
Kim *et al*[21]	2017, RCS	Liver resection (n = 124)	Patients with extended TP [LMWH (enoxaparin), n = 124, single arm]	BC 1.6% in extended TP	No VTE in whole cohort
Doughtie *et al*[22]	2014, RCS	Major HBP surgery (n = 223) including 110 liver resection	Patients with preop TP (LMWH, n = 93); Patients without preop TP (control, n = 130)	Major BC 10.9% in preop TP *vs* 3.1% in control (P = 0.026); SBL was identical	VTE 1.1% *vs* 6.1% (P = 0.05)
Melloul *et al* [23]	2012, RCS	Liver resection (n = 410)	Patients with TP (n = 410, single arm)	Not mentioned	PE 6% (24/410) in TP
Reddy *et al* [24]	2011, RCS	Major liver resection (n = 419)	Patients with TP (n = 275); Patients without TP (control, n = 144)	RBC transfusion rate 35.0% in TP *vs* 30.6% in control (P = 0.36)	CR-VTE 2.2% in TP *vs* 6.3% in control (P = 0.03); PE 2.2% *vs* 4.2% (P = 0.35)

mRCT: Multicenter randamized controlled trial; RCS: Retrospective cohort study; mRCS; multicenter retrospective cohort study; LMWH: Low-molecular-weight heparin; TP: Thromboprophylaxis; LAP: Laparoscopic; CR: Clinically relevant; BC: Postoperative bleeding complication; VTE: Venous thromboembolism; PE: Pulmonary embolism; AOR: Adjusted odds ratio.

DISCUSSION

As far as we know, the current study is the first systematic review to investigate the effect of ATT on thromboembolic and bleeding complications in hepatectomy. The current study reviewed 16 published articles with special reference to ATT, in which a total of 8300 patients receiving hepatectomy were included. Concerning the effects of chronic ATT administration on bleeding events, most of the studies showed that hepatectomy can be performed safely in patients receiving chronic ATT, even if they continue to have aspirin preoperatively. Regarding pharmacological prevention for VTE, some studies have reported that patients undergoing pharmacological prophylaxis may be at increased risk of bleeding, but their efficacy against VTE has not been proven especially in the population of Asian patients.

Minimizing intraoperative and postoperative bleeding complication is one of the most important tasks in hepatectomy, and several technical improvement has been demonstrated, such as Pringle's procedure, the liver hanging maneuver, or the two-surgeon technique[25-27]. Pringle's procedure is generally used during transection of the liver parenchyma in order to control hepatic inflow; sustained low CVP is usually employed in order to control backflow bleeding from the hepatic vein[8]. However, sustained low CVP may expose the ATT-received patients to the increased risks of stroke or myocardial infarction. Rigorous perioperative management of antithrombotic agents and strict procedures of hemostasis are requisite in order to prevent both thromboembolic and bleeding complications.

Regarding the management of chronically ATT-received patients, guidelines regarding ATT management during non-cardiac surgery were recently updated and demonstrated that the prevention of thromboembolism is more significant than prophylaxis of bleeding, since it might cause severe sequelae or death[5,28-31]. To date, there are little consensus or evidence on the safety of hepatectomy and proper

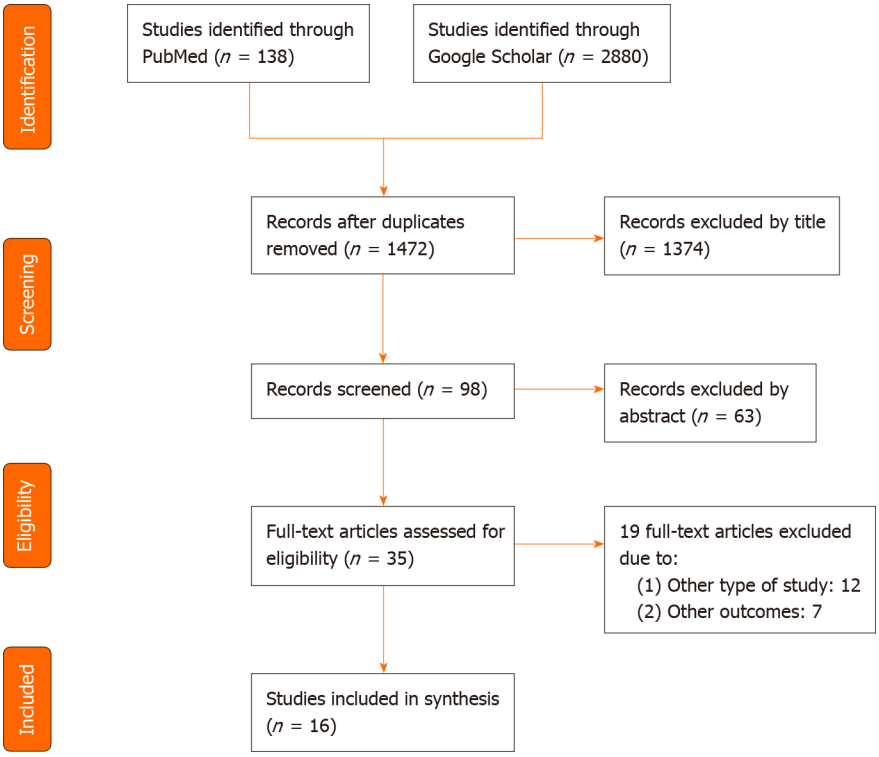

Figure 1 PRISMA flow diagram demonstrating articles selection process.

perioperative ATT management in ATT-received patients, and the optimal prevention for VTE also remains unknown.

Our hospital is a high-volume institution for referrals to patients with digestive cancer who are receiving ATT. Accordingly, we presently use a centralized management protocol in ATT-received patients undergoing digestive surgery including hepatectomy (Figure 2)[32], which was established and have been updated with reference to several guidelines and recently reported studies regarding perioperative ATT management for non-cardiac surgeries or endoscopic procedures[5,6,28-30]. The management consists of 3 ways according to ATT types; antiplatelets, warfarin, and DOACs. In patients with the risk of thromboembolism, preoperative aspirin monotherapy is sustained in antiplatelet-received patients, and warfarin is substituted by DOAC bridging (preferred) or heparin bridging. Regarding patients with DOACs, short-period discontinuation of DOACs (usually 1-2 d) is recommended and heparin bridging is usually not required, but heparin bridging might be considered if the thromboembolic risk is very high. Postoperatively, every antithrombotic drug is reinstituted as soon as possible.

Concerning the management of patients with antiplatelet drugs, some studies such as POISE-2 study have suggested that a slight increase in bleeding risk was observed in patients with continued antiplatelets during non-cardiac surgery[33,34], but most of other studies demonstrated that the bleeding events were not significantly increased [35,36]. Moreover, one large-scale retrospective cohort study was recently showed that the continued preoperative aspirin significantly reduced the rate of postoperative thromboembolism but was not associated with the occurrence of bleeding events[37]. In the current review, three studies showed that continued preoperative aspirin is not related to excessive SBL or increased occurrence of BC in patients with chronic antiplatelet therapy during or after hepatectomy[9,12,13]. Although the favorable management of antiplatelet-received patients during hepatectomy is still controversial, continued preoperative aspirin is one of the preferred options and should be considered.

In the clinical setting, when neurosurgeons or cardiologists judge the risk of thromboembolism as high, antiplatelet-recipient patients are sometimes managed by heparin bridging during perioperative discontinuation of antiplatelet drugs. This situation is probably because some cardiologists and surgeons are unaware of the preferred option of continued aspirin monotherapy for the perioperative management. The mechanism of heparin is different from that of antiplatelets, and heparin bridging is presently reported to be a significant risk factor for postoperative bleeding events

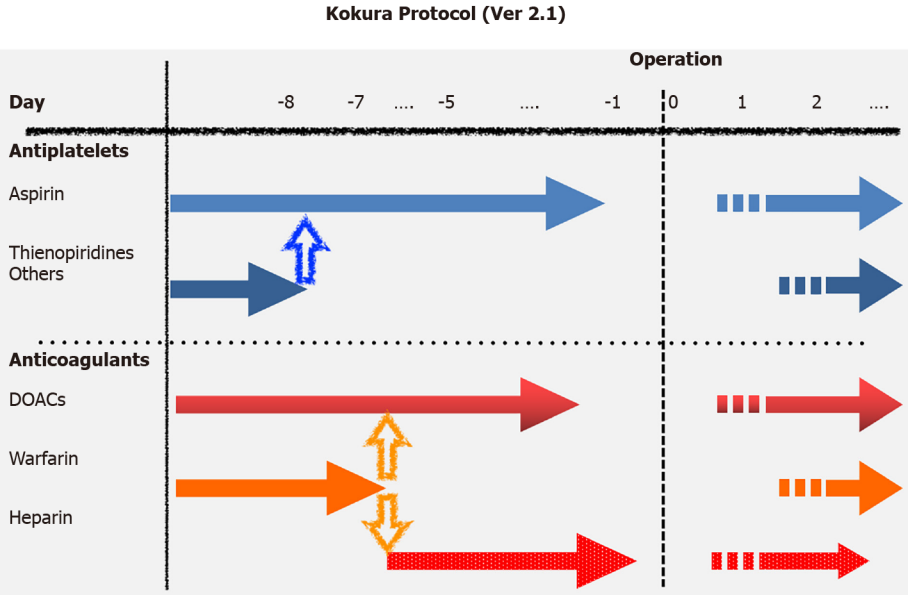

Figure 2 Recommended perioperative management protocol for patients undergoing antithrombotic therapy in case of hepatobiliary-pancreatic and gastrointestinal surgery. The management generally consists of 3 ways according to types of antithrombotic therapy; antiplatelet therapy, warfarin, and direct-acting oral anticoagulant (DOACs). In patients with thromboembolic risks, aspirin monotherapy is continued in patients receiving antiplatelet therapy, and warfarin is substituted by DOAC bridging (preferred) or heparin bridging. In case of DOAC, short-period discontinuation of DOACs (usually 1-2 d) without heparin bridging is generally recommended (with some modification needed if decreased renal function exists). Postoperatively, every antithrombotic agent is reinstituted as soon as possible (POD1-2). DOAC: Direct-acting oral anticoagulant.

[38,39]. Therefore, heparin bridging during antiplatelet discontinuation is not recommended and should not be used.

Concerning DOACs, only one report was included in the present review[14]. This study showed that perioperative short-period discontinuation of DOACs without heparin bridging was safe even for patients who undergo digestive surgery including hepatectomy, but patients who were managed by heparin bridging during DOAC discontinuation was at high risk of postoperative bleeding. Presently, DOACs are increasingly used for the prophylaxis of venous or arterial thromboembolic events. They are fast-acting drugs with their anticoagulant effect fading within 48 h after their withdrawal[28]. One large-scale multicenter prospective cohort study (the PAUSE study) was recently published, which examined outcomes in 3007 adult patients with atrial fibrillation who underwent DOAC therapy and received an elective non-cardiac procedure or surgery[40]. DOAC therapy was interrupted 1-2 d prior and reinstituted 1-2 d after the procedure or surgery. The occurrence of major bleeding 30 d after the procedure or surgery was 0.90%-1.85%, and arterial thromboembolic complication was occurred at the rate of 0.16%-0.60%. The study recommended that a centralized perioperative management of DOACs without heparin bridging can be performed safely for patients with atrial fibrillation. Although the PAUSE study included only a limited number of patients undergoing major gastroenterological surgery, the study included in the present review also suggested that the perioperative short-period cessation of DOACs without heparin bridging is the preferred management even for patients who receive major gastroenterological surgery including hepatectomy[14,37].

Regarding chemical prevention for VTE in hepatectomy, most of the studies included in the present review have demonstrated a potential risk of postoperative bleeding events in patients receiving pharmacological thromboprophylaxis, although its efficacy against VTE has not been shown, particularly in Asian patient population. VTE is fatal when it occurs during the perioperative period, and its prevention is of paramount importance. Although some guidelines in Western countries recommend pharmacological prevention for VTE during non-cardiac surgery[41-43], it is reported that there are racial differences in the rate of VTE between Western people and Asians [44]. In addition, in one systematic review regarding pharmacological prevention for VTE in Asian surgical patients[45], the risk of perioperative VTE in Asian patients is reported to be low even in the context of high risk for thromboembolism. The two large-scale cohort studies from Japan were recently showed that the incidence of clinically relevant VTE during or after major digestive surgery was 0-0.3%[37,46]. Currently, the safety and efficacy of pharmacological prevention with anticoagulation

drugs for VTE during hepatectomy is still controversial, particularly in Asian patient population. It is important to build evidence in order to classify risks individually according to each race is essential.

Summary and recommendations for future studies

Presently, the numbers of studies regarding the management of ATT during hepatectomy is limited. This patient population is expanding further, as the population ages and the prevalence of cardiovascular disease increases. Using reliable studies with good design, the definite guideline should be determined. Currently, one promising prospective multicenter cohort study was registered in the University Hospital Medical Information Network Clinical Trials Registry and is ongoing ["Study on the safety and feasibility of gastroenterological surgery in patients undergoing antithrombotic therapy (GSATT Study)", UMIN000038280]. In the future, the safety of ATT management during liver resection will be demonstrated by well-designed analyses like this study.

CONCLUSION

Hepatectomy in patients with chronic ATT can be performed safely without increase in the rates of bleeding complications, although the efficacy and safety of pharmacological prevention for VTE during hepatectomy remains controversial. Further investigation using reliable studies with good design must be required to establish definite protocol or guidelines.

ARTICLE HIGHLIGHTS

Research background

Little is unknown about the effect of chronic antithrombotic therapy (ATT) on bleeding complication during or after hepatectomy. In addition, the safety and effectiveness of chemical prevention for venous thromboembolism (VTE) remain controversial.

Research motivation

The goal of the present review was to clarify the effect of ATT on bleeding complications or increased surgical blood loss in hepatectomy.

Research objectives

The objective of the current systematic review was to investigate the effect of ATT on thromboembolism and bleeding in hepatectomy.

Research methods

Articles published between 2011 and 2020 were searched from Google Scholar and PubMed, and after careful reviewing of all studies, studies concerning ATT and hepatectomy were included. Data such as study design, type of surgery, type of antithrombotic agents, and surgical outcome were extracted from the studies.

Research results

Sixteen published articles, including a total of 8300 patients who underwent hepatectomy, were eligible for inclusion in the current review. All studies regarding patients undergoing chronic ATT showed that hepatectomy can be performed safely, and three studies have also shown the safety and efficacy of preoperative continuation of aspirin. Regarding chemical prevention for VTE, some studies have shown a potentially high risk of bleeding complications in patients undergoing chemical thromboprophylaxis; however, its efficacy against VTE has not been shown statistically, especially among Asian patients.

Research conclusions

Liver resection in chronically ATT-received patients can be performed safely without increase in the rate of bleeding complications, although the safety and efficacy of chemical thromboprophylaxis for VTE during liver resection is still controversial especially in Asian population.

Research perspectives

Further investigation using reliable studies with good design must be requisite to establish definite protocol or guidelines.

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META-ANALYSIS

Effects of intragastric balloon placement in metabolic dysfunction-associated fatty liver disease: A systematic review and meta-analysis

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Abstract

BACKGROUND

Metabolic dysfunction-associated fatty liver disease corresponds to a clinical entity that affects liver function triggered by the accumulation of fat in the liver and is linked with metabolic dysregulation.

AIM

To evaluate the effects of the intragastric balloon (IGB) in patients with metabolic dysfunction-associated fatty liver disease through the assessment of liver enzymes, imaging and several metabolic markers.

METHODS

A comprehensive search was done of multiple electronic databases (MEDLINE, EMBASE, LILACS, Cochrane and Google Scholar) and grey literature from their

article, revising the article and finally approving; de Oliveira CPMS and Bernardo WM conducted data analysis and interpretation and drafted the article .

Conflict-of-interest statement: Dr. Moura reports personal fees from Boston Scientific, personal fees from Olympus, outside the submitted work.

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inception until February 2021. Inclusion criteria involved patients with a body mass index > 25 kg/m^2 with evidence or previous diagnosis of hepatic steatosis. Outcomes analyzed before and after 6 mo of IGB removal were alanine aminotransferase (IU/L), gamma-glutamyltransferase (IU/L), glycated hemoglobin (%), triglycerides (mg/dL), systolic blood pressure (mmHg), homeostatic model assessment, abdominal circumference (cm), body mass index (kg/m^2) and liver volume (cm^3).

RESULTS

Ten retrospective cohort studies evaluating a total of 508 patients were included. After 6 mo of IGB placement, this significantly reduced alanine aminotransferase [mean difference (MD): 10.2, 95% confidence interval (CI): 8.12-12.3], gamma-glutamyltransferase (MD: 9.41, 95%CI: 6.94-11.88), glycated hemoglobin (MD: 0.17%, 95%CI: 0.03-0.31), triglycerides (MD: 38.58, 95%CI: 26.65-50.51), systolic pressure (MD: 7.27, 95%CI: 4.79-9.76), homeostatic model assessment (MD: 2.23%, 95%CI: 1.41-3.04), abdominal circumference (MD: 12.12, 95%CI: 9.82-14.41) and body mass index (MD: 5.07, 95%CI: 4.21-5.94).

CONCLUSION

IGB placement showed significant efficacy in improving alanine aminotransferase and gamma-glutamyltransferase levels in patients with metabolic dysfunction-associated fatty liver disease as well as improving metabolic markers related to disease progression.

Key Words: Intragastric balloon; Metabolic dysfunction-associated fatty liver disease; Homeostatic model assessment; Abdominal circumference; Body mass index

Core Tip: Metabolic dysfunction-associated fatty liver disease corresponds to the accumulation of fat in the liver and is linked with metabolic dysregulation. We evaluated the effects of the intragastric balloon in patients with metabolic dysfunction-associated fatty liver disease through the assessment of liver enzymes, imaging and several metabolic markers. Outcomes analyzed before and after 6 mo of intragastric balloon placement were alanine aminotransferase (IU/L), gamma-glutamyltransferase (IU/L), glycated hemoglobin (%) and other parameters related to metabolic disorders. This is the first systematic review and meta-analysis to assess the role of the intragastric balloon in the new definition of metabolic dysfunction-associated fatty liver disease.

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INTRODUCTION

The term nonalcoholic fatty liver disease, first proposed by Ludwig and collaborators in 1980[1] corresponds to a clinical entity that affects the histological structure and liver function triggered by the accumulation of fat in the liver unrelated to alcohol intake with a risk of developing nonalcoholic steatohepatitis and cirrhosis. It is estimated that this condition affects a quarter of the adult world population[2], and it will be the main cause of liver transplantation by 2030[3].

Recently, an international consensus panel of experts[4] proposed metabolic dysfunction-associated fatty liver disease (MAFLD) as a change in nomenclature and more appropriate term to reflect the pathophysiology and current knowledge of the

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disease rather than the outdated terms of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. The new definition is based on current knowledge of the role of metabolic dysfunction in the pathophysiology of fatty liver disease related mainly to obesity, type 2 diabetes mellitus and other metabolic disorders. Also, they provided diagnostic criteria to facilitate stratification and the subsequent management of patients along with new horizons for translational research and new treatments.

The natural history of fatty liver disease navigates through the initial stages of hepatic steatosis with progression to steatohepatitis and liver cirrhosis in certain chronic cases[5]. The treatment of these patients still represents a challenge[6]. Lifestyle changes and control of metabolic disorders are the mainstays of the therapeutic approach. Pharmacological therapies are promising but have not yet evidenced efficacy in regressing the inflammation and liver fibrosis associated with the evolution of the disease[7]. Bariatric surgery has gained notoriety, but the expansion of its indication as a form of treatment for MAFLD has been discussed in view of the added morbidity and irreversibility of different surgical modalities.

Research for alternative therapies is relevant in the treatment of MAFLD, with endoscopic bariatric and metabolic therapies, especially with the intragastric balloon (IGB), seen as a safe and less invasive treatment option[8-12]. The IGB is a widespread therapy for short-term control of obesity and its mechanism of action is based on the occupation of the gastric chamber, causing a delay in gastric emptying, an increase in the feeling of satiety and consequently a reduction in caloric intake. Currently, several models of IGB are available for clinical use, with variations in its design, volume, fluid *vs* air filled-balloons, implantation duration and efficacy[13].

This study aims to evaluate the impact of IGB placement on MAFLD through the assessment of liver enzymes, certain metabolic markers and imaging parameters.

MATERIALS AND METHODS

Protocol registration

This study was performed in conformity with the PRISMA[14] guidelines, and it was registered in the PROSPERO[15] database under the file number (CRD42020204485). The study was approved by the Ethics Committee of Hospital das Clínicas, Faculty of Medicine at The University of São Paulo.

Eligibility criteria

Data search was made without limitations of language or publication date. The eligibility criteria adopted were: (1) population: patients with a body mass index (BMI) > 25 kg/m^2 with evidence or previous diagnosis of hepatic steatosis; (2) intervention: endoscopic IGB placement; (3) comparator: the outcomes in baseline and post IGB moments; and (4) outcomes: alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), glycated hemoglobin, triglycerides, systolic blood pressure, homeostatic model assessment (HOMA-IR), abdominal circumference and liver volume were analyzed.

Studies that did not involve the use of an IGB for at least 6 mo of duration were excluded.

Search and study selection

We performed a search in electronic databases (MEDLINE, EMBASE, Cochrane, LILACS) and grey literature, from their inception until February 2021. As a search strategy, we used descriptors available from the United States National Library of Medicine Medical Subject Headings and other related terms that increased the sensitivity of search as described in Table 1. Two independent reviewers conducted the assessment of eligibility criteria. Disagreements were resolved by consensus or consultation with a third reviewer.

Data collection process

The data related to the analyzed outcomes were tabulated in an Excel table and included the IGB model used as well as the average volume of filling of the balloons and the number of calories in the diet associated with the treatment. In the comparison studies between IGB and diet, only data from the balloon intervention group were extracted, and not all outcomes were evaluated in all studies. When data of the published articles were insufficient, the corresponding authors were consulted by e-mail for further elucidation.

Table 1 Search strategy

Search strategy	
Medline	[(intragastric OR bariatric endoscopy OR balloon OR balloons OR bubble OR bubbles OR gastric balloon OR balloons)] AND [(mafld OR non alcoholic fatty liver disease OR nafld OR fatty liver OR nonalcoholic steatohepatitis OR nash OR nonalcoholic steatohepatitis OR alanine transaminase OR aspartate aminotransferase OR gamma-glutamyltransferase OR alkaline phosphatase OR fatty liver OR steatohepatitis OR steatohepatitis OR steatosis of liver OR visceral steatosis OR visceral]
MEDLINE, Embase, Cochrane, LILACS	[(intragastric OR balloon)] AND [(fatty liver)]
Grey literature	[(intragastric OR balloon)] AND [(fatty liver)]

Table 2 Grading recommendations assessment, development and evaluation certainty evidence assessment table

	Certainty evidence assessment							Study event rates (%)		
	Participants (studies) follow up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	With post-IGB	With pre-IGB	Risk difference with Pre-IGB
ALT	1114 (10 observational studies)	Not serious	Serious[1]	Not serious[2]	Not serious	Publication bias strongly suspected[3]	⊕⊕◯◯, Low	557	557	Mean 10.27 UI/L more (8.25 more to 12.29 more)
GGT	1014 (8 observational studies)	Not serious	Not serious	Not serious[2]	Not serious	None	⊕⊕⊕⊕, High	507	507	Mean 9.23 UI/L more (6.88 more to 11.58 more)
Hb1Ac	300 (6 observational studies)	Not serious	Not serious	Not serious[4]	Not serious	Publication bias strongly suspected[3]	⊕⊕⊕◯, Moderate	150	150	Mean 0.17 % higher (0.03 higher to 0.31 higher)
Triglycerides	564 (6 observational studies)	Not serious	Not serious	Not serious	Not serious	None	⊕⊕⊕⊕, High	282	282	Mean 38.58 mg/dL higher (26.65 higher to 50.51 higher)
Systolic blood pressure	468 (3 observational studies)	Not serious	Not serious	Not serious	Not serious	None	⊕⊕⊕⊕, High	234	234	Mean 7.27 mmHg higher (4.79 higher to 9.76 higher)
HOMA-IR	378 (5 observational studies)	Not serious	Serious[1]	Not serious	Not serious	None	⊕⊕⊕◯, Moderate	189	189	Mean 2.07 higher (1.64 higher to 2.49 higher)
BMI	912 (8 observational studies)	Not serious	Not serious	Not serious	Not serious	Strong association	⊕⊕⊕⊕, High	456	456	Mean 5.07 kg/m^2 higher (4.21 higher to 5.94 higher)
Waist	672 (7 observational studies)	Not serious	Not serious	Not serious	Not serious	None	⊕⊕⊕⊕, High	336	336	Mean 12.12 cm higher (9.82 higher to 14.41 higher)
Liver volume	32 (2 observational studies)	Not serious	Not serious	Not serious	Serious[5]	None	⊕⊕⊕◯, Moderate	16	16	MD 303.24 higher (56.66 lower to 663.15 higher)

[1]Heterogeneity > 50%.
[2]Indirect measurement of hepatic steatosis.
[3]Presence of Outlier.
[4]Surrogate endpoint.
[5]Wide confidence interval. Overall certainty of evidence definition: ⊕◯◯◯: Very low-Any estimate of effect is very uncertain; ⊕⊕◯◯: Low-Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; ⊕⊕⊕◯: Moderate-Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; ⊕⊕⊕⊕: High-Further research is very unlikely to change our confidence in the estimate of effect; MD: Mean difference; IGB: Intragastric balloon; HbA1c: Glycated hemoglobin; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; HOMA-IR: Homeostatic model; BMI: Body mass index.

Risk of bias and evidence quality

The risk of bias was assessed by the Risk of Bias in Non-randomized Studies-of Interventions tool[16]. The quality of evidence, expressed in high, moderate, low and very low, was assessed utilizing the objective criteria from Grading Recommendations Assessment, Development, and Evaluation (Table 2) using the GRADEpro-Guideline Development Tool software (McMaster University, 2015; Evidence Prime, Inc., Ontario, Canada)[17].

Statistical analysis

Our outcomes were continuous variables, and values of means and standard deviations were used for the statistical analysis. In studies that expressed the results in median and interquartile range, mathematical formulas were used for the data conversion[18].

The data of interest extracted from the selected studies were meta-analyzed using the RevMan software (Review Manager Software version 5.4-Cochrane Collaboration Copyright© 2020) using the inverse variance test. The mean values of each continuous outcome were calculated as well as the 95% confidence interval (CI). $P < 0.05$ were considered statistically significant, and the results were exposed through forest plots. Heterogeneity was calculated using the Higgins method (I^2)[19]. When heterogeneity < 50% was found, the fixed-effect model was used. In cases of heterogeneity > 50%, the funnel plot analysis was performed, and outlier cases were removed to maintain the analysis by a fixed effect. In cases where no outlier was evidenced, the analysis by the random effect model was performed. The correlation between outcomes was performed using the meta-regression using the Comprehensive Meta-Analysis tool version 2.2.057.

RESULTS

Study selection

The article selection process is shown in Figure 1. After applying the eligibility criteria, eleven articles were included in the qualitative analysis. Ten articles were included in the quantitative analysis, considering that one of the studies was a randomized controlled clinical trial. The individual results of each study are described in Table 3.

Risk of bias among the studies

Two studies presented moderate risk and eight studies presented low risk in the global analysis according to the Risk of Bias in Non-randomized Studies-of Interventions criteria. The study by Takihata *et al*[20] had a risk of serious bias in the classification of interventions because the patients themselves chose whether to participate in the IGB intervention group or the lifestyle modification (diet/physical exercise) group. The study by Nikolic *et al*[21] presented a moderate risk of lack of data due to the exclusion of participants due to a loss of follow-up in the study. The overall risk of bias in each study is detailed in Figure 2.

Meta-analysis

ALT (IU/L): Ten studies[20-29] with 508 patients were included in the meta-analysis of the outcome. The mean reduction in serum ALT values was 10.2 (95%CI: 8.12-12.3; $P < 0.01$) after 6 mo, favoring the use of the IGB. This analysis showed high heterogeneity ($I^2 = 56\%$), and the study by Bazerbachi *et al*[22] was identified as an outlier in the funnel plot analysis. After removing this study from the analysis, the heterogeneity remained at < 50% ($I^2 = 32\%$), maintaining the analysis by a fixed effect (Figure 3).

Table 3 Results of individual studies

Ref.	*n*	Balloon volume (cm^3)	ALT (UI/L)		GGT (UI/L)		HbA1c (%)		Triglycerides (mg/dL)		Waist (cm)		HOMA-IR		BMI (kg/m^2)		SBP (mmHg)	
			Pre-IGB	Post-IGB	Pre-IGB	Post-IGB	Pre-IGB	Post-IGB	Pre-IGB	Post-IGB	Pre-IGB	Post-IGB	Pre-IGB	Post-IGB	Pre-IGB	Post-IGB	Pre-IGB	Post-IGB
Forlano *et al*[25], 2010	120	500	39.3 (25.6)	24.4 (10.0)	37.5 (20.5)	24.5 (17.1)	-	-	-	-	-	-	-	-	43.1 (8.0)	38.8 (8.0)	-	-
Bazerbachi *et al*[22], 2021	21	-	91.6 (59.9)	39.4 (25.4)	-	-	7.7 (1.6)	6.5 (1.2)	-	-	128.9 (15.4)	119.7 (16.9)	-	-	43.2 (6.8)	37.9 (6.6)	-	-
Nikolic *et al*[21], 2011	33	600	30 (23.25)	27 (16.75)	31 (50.75)	21 (36.75)	4.7 (0.50)	4.6 (0.45)	124 (86.25)	124 (124.75)	122 (21.00)	110 (14.25)	-	-	41.4 (5.25)	35.6 (5.25)	-	-
Donadio *et al*[23], 2009	40	500	30.7 (14.0)	23.4 (9.3)	29.8 (19.1)	28.0 (28.1)	5.4 (0.5)	5.3 (0.4)	134.1 (67.8)	118.8 (66.5)	125.9 (18.6)	115.8 (17)	4.1 (2.1)	2.7 (1.6)	44.8 (8.9)	38.9 (6.8)	129.3 (14.0)	122.6 (10.4)
Stimac *et al*[29], 2011	166	600	34.7 (31.5)	26.5 (23.1)	33.3 (23.3)	24.7 (16.9)	-	-	118.6 (87.6)	81.0 (66.4)	127.8 (16.7)	113.3 (18.9)	-	-	41.6 (7.5)	35.8 (7.9)	130.9 (14.5)	124.2 (14.1)
Takihata *et al*[20], 2014	8	Variable	57.1 (55.6)	43.1 (48.8)	53.0 (25.4)	40.1 (19.3)	6.70 (1.43)	6.38 (1.49)	223.2 (194.8)	153.2 (80.6)	129.2 (8.3)	123.8 (12.3)	12.3 (10.9)	8.0 (7.3)	45.2 (5.9)	41.0 (6.2)	-	-
Folini *et al*[24], 2014	40	-	25.9 (10.31)	18.1 (5.96)	27.8 (27.57)	17.9 (12.21)	6.5 (1.17)	6.0 (0.74)	-	-	130.2 (13.96)	118 (13.01)	5.2 (2.23)	2.3 (1.66)	43.8 (6.62)	38.2 (6.19)	-	-
Ricci *et al*[26], 2008	65	-	31.5 (19.33)	24.0 (10.67)	31.0 (16.05)	23.5 (12.6)	-	-	-	-	-	-	4.71 (2.11)	3.10 (2.79)	-	-	-	-
Sekino *et al*[27], 2011	8	1000	74.2 (49.67)	56.7 (42.40)	57.00 (23.11)	41.25 (14.74)	6.30 (1.15)	6.31 (1.29)	251 (168.9)	163 (62.0)	-	-	6.74 (1.27)	3.27 (1.18)	-	-	-	-
Tai *et al*[28], 2013	28	500	49 (45.25)	22 (23.25)	-	-	-	-	149.0 (49.00)	88.5 (39.75)	101.9 (8.9)	90.6 (9.3)	-	-	32.4 (3.7)	28.5 (3.7)	136.8 (14.30)	125.9 (11.15)

IGB: Intragastric balloon; HbA1c: Glycated hemoglobin; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; HOMA-IR: Homeostatic model; BMI: Body mass index; SBP: Systolic blood pressure.

GGT (IU/L): Eight studies[20,21,23-27,29] with 479 patients were included in the outcome meta-analysis (Figure 4). The mean reduction in serum GGT levels was 9.41 (95%CI: 6.94-11.88; $P < 0.01$) after 6 mo of IGB use.

Glycated hemoglobin (%): Six studies[20-24,27] with 150 patients analyzed the effect of the IGB on glycated hemoglobin (Figure 5). The mean reduction in serum glycated hemoglobin values was 0.17% (95%CI: 0.03-0.31; $P = 0.02$) after 6 mo of IGB placement.

Triglycerides (mg/dL): Six studies[20,21,23,27-29] with 282 patients analyzed the effect

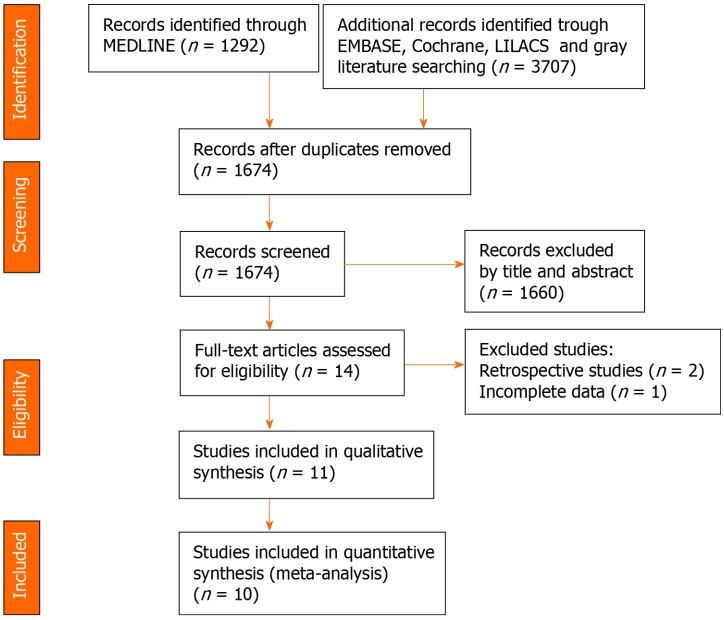

Figure 1 PRISMA flow diagram.

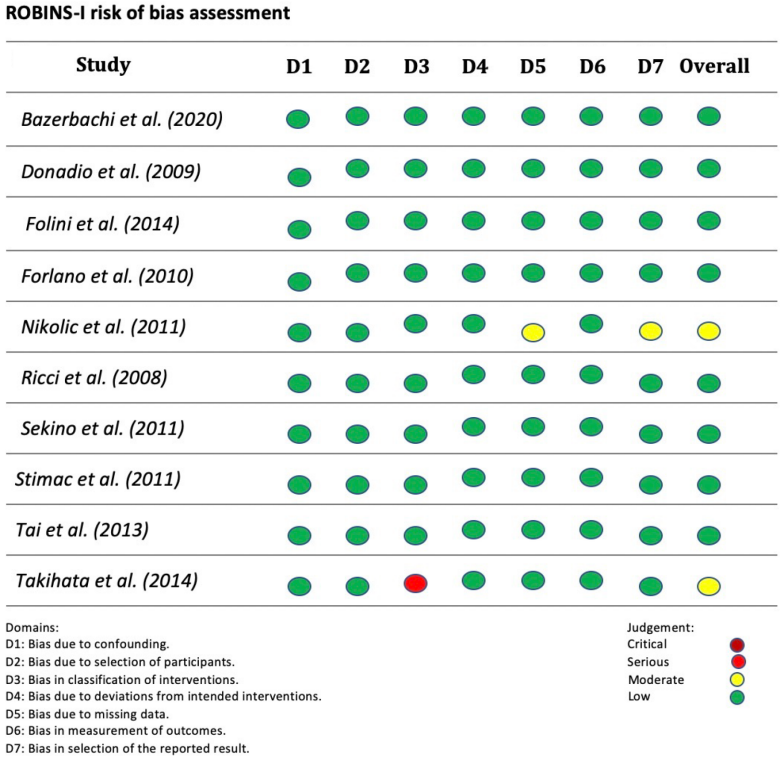

Figure 2 Risk of bias assessment (risk of bias in non-randomized studies-of interventions).

of the IGB on serum triglyceride levels (Figure 6). The mean reduction in triglycerides was 38.58 (95%CI: 26.65-50.51; $P < 0.01$) after 6 mo of use of the balloon.

Systolic blood pressure (mmHg): Three studies[23,28,29] with 234 patients analyzed the effect of the IGB on blood pressure levels (Figure 7). After 6 mo of IGB placement, the mean reduction in systolic blood pressure was 7.27 (95%CI: 4.79-9.76; $P < 0.01$).

HOMA-IR: Five studies[20,23-25,27], with 161 patients, were included in the outcome meta-analysis. The mean reduction in HOMA-IR values was 2.23 (95%CI: 1.41-3.04; $P < 0.01$) after 6 mo using the IGB (Figure 8).

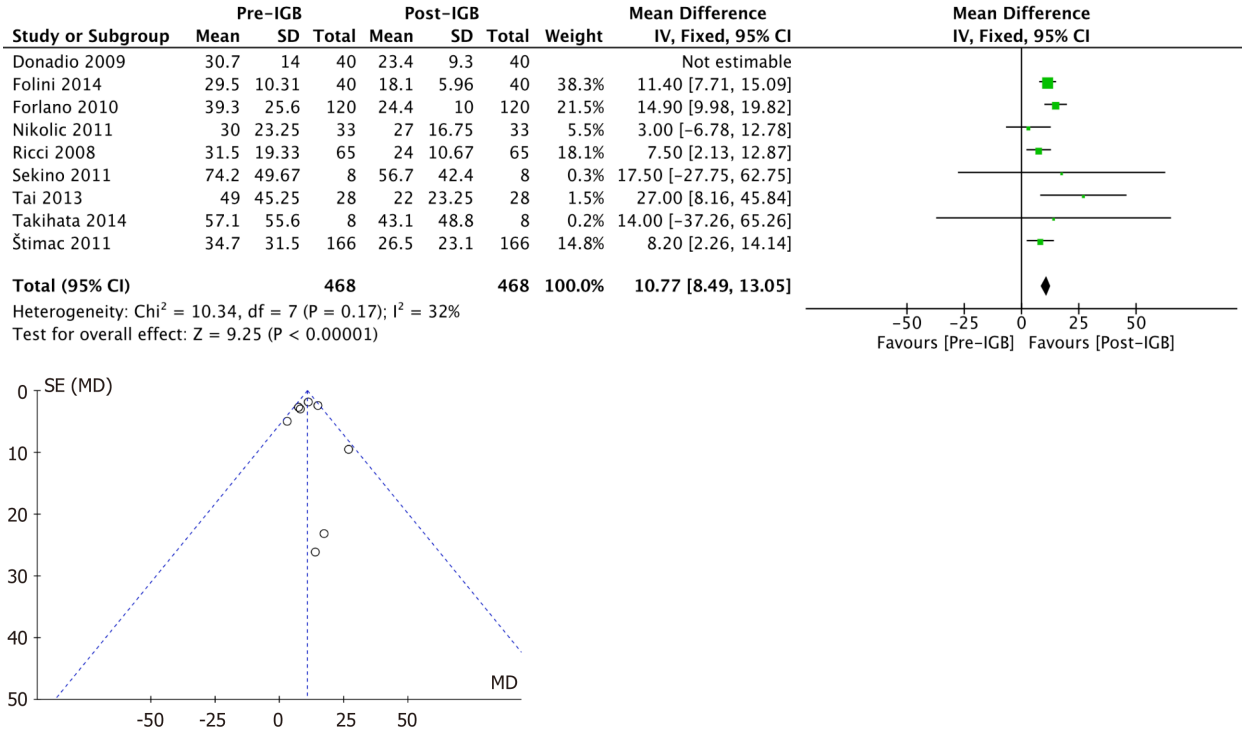

Figure 3 Forest plot of alanine aminotransferase and funnel plot without outlier. CI: Confidence interval; IGB: Intragastric balloon.

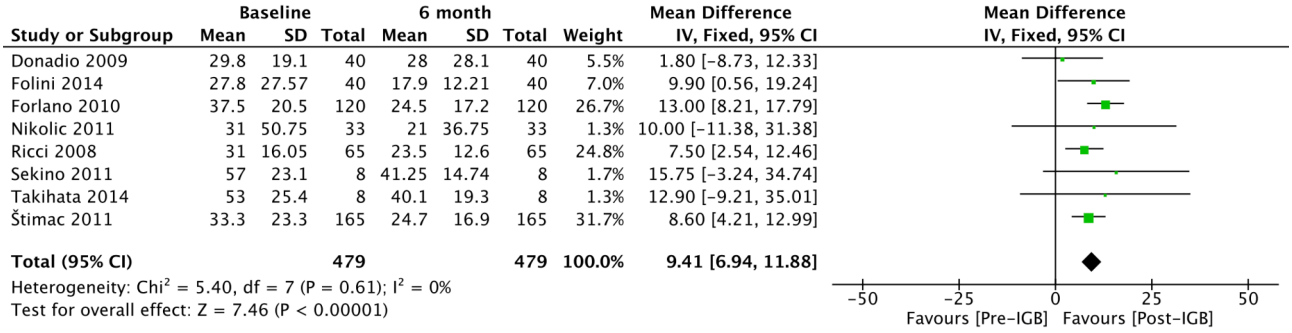

Figure 4 Forest plot of gamma-glutamyltransferase. CI: Confidence interval; IGB: Intragastric balloon.

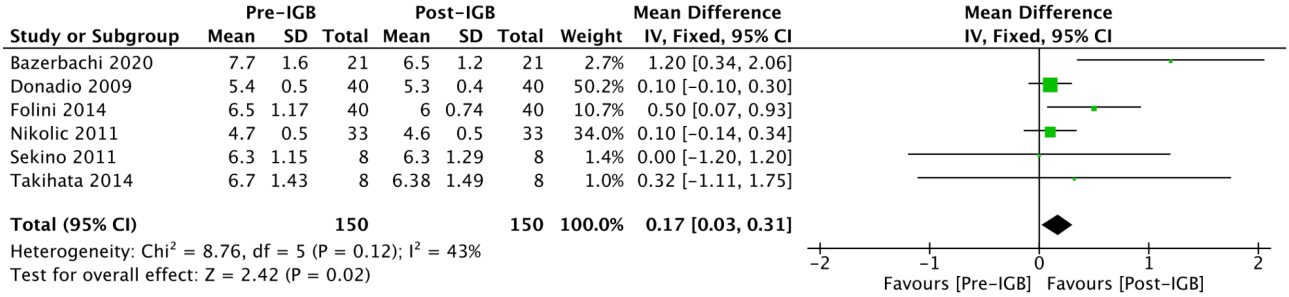

Figure 5 Forest plot of glycated hemoglobin. CI: Confidence interval; IGB: Intragastric balloon.

Abdominal circumference (cm): Seven studies[20-24,28,29], with 336 patients (Figure 9), were included in the outcome meta-analysis. The mean reduction in abdominal circumference was 12.12 (95%CI: 9.82-14.41; $P < 0.01$) after 6 mo of IGB use.

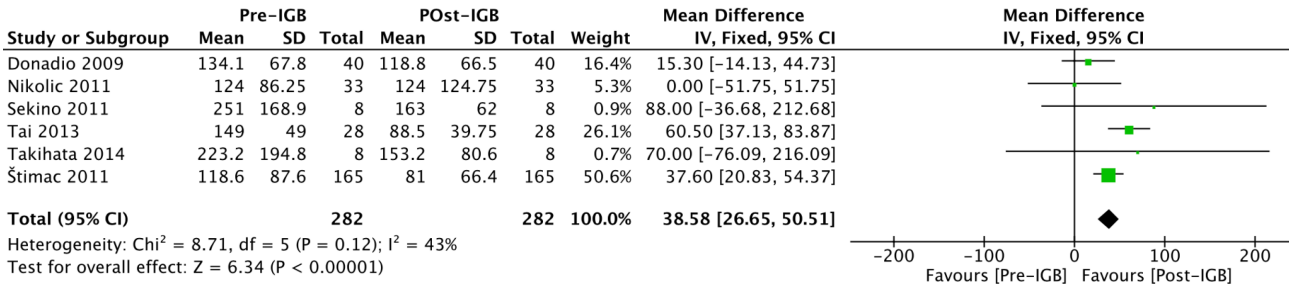

Figure 6 Forest plot of triglycerides. CI: Confidence interval; IGB: Intragastric balloon.

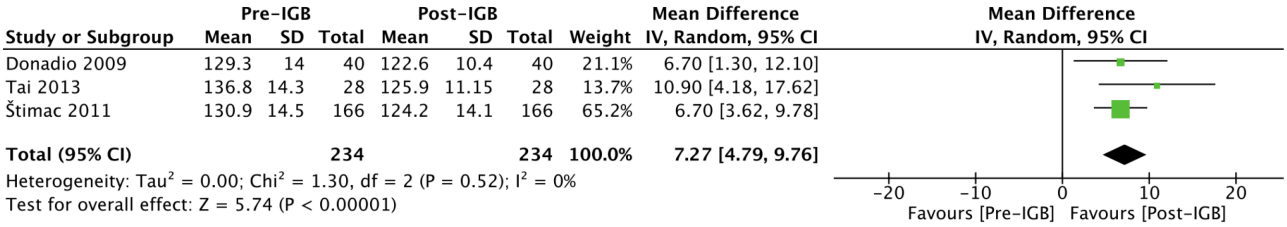

Figure 7 Forest plot of systolic blood pressure. CI: Confidence interval; IGB: Intragastric balloon.

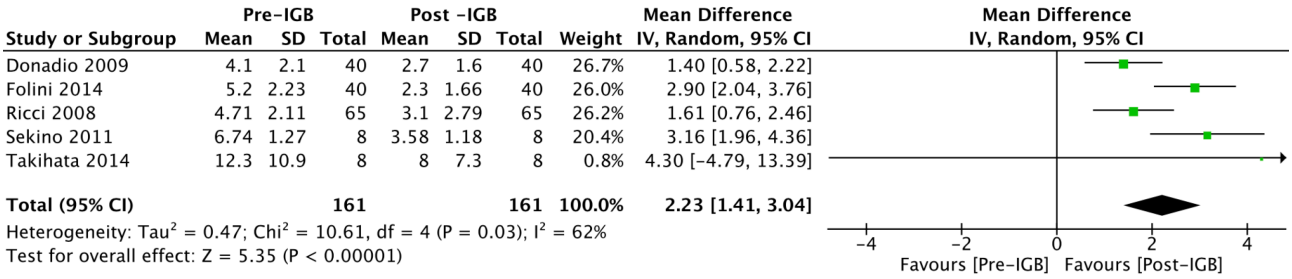

Figure 8 Forest plot of homeostatic model assessment. CI: Confidence interval; IGB: Intragastric balloon.

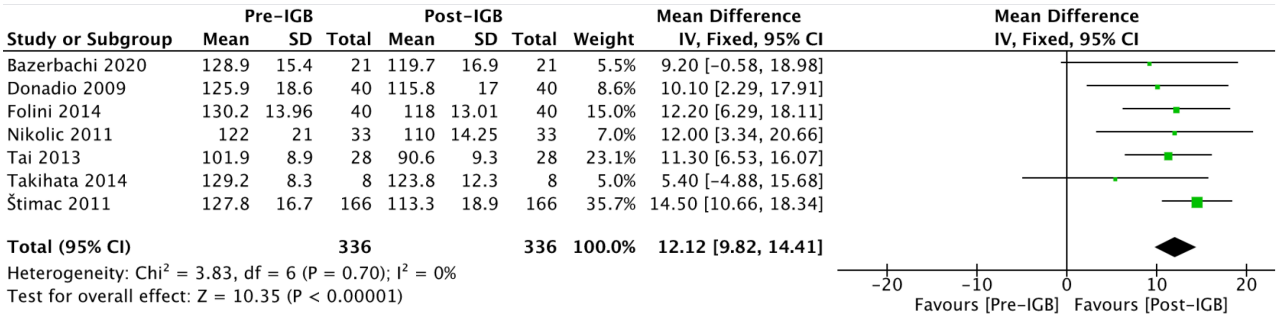

Figure 9 Forest plot of waist circumference. CI: Confidence interval; IGB: Intragastric balloon.

BMI (kg/m²): Eight studies[20-25,28,29], with 456 patients, were included in the outcome meta-analysis (Figure 10). The mean reduction in BMI was 5.07 (95%CI: 4.21-5.94; $P < 0.01$) after 6 mo of use of the IGB.

Liver volume (cm³): Two studies[20,27], with 16 patients, were included in the meta-analysis of the outcome (Figure 11). The mean reduction in liver volume was 303 cm³ (95%CI: -56.6-663.15; $P = 0.1$) after 6 mo of using the IGB but without statistical significance.

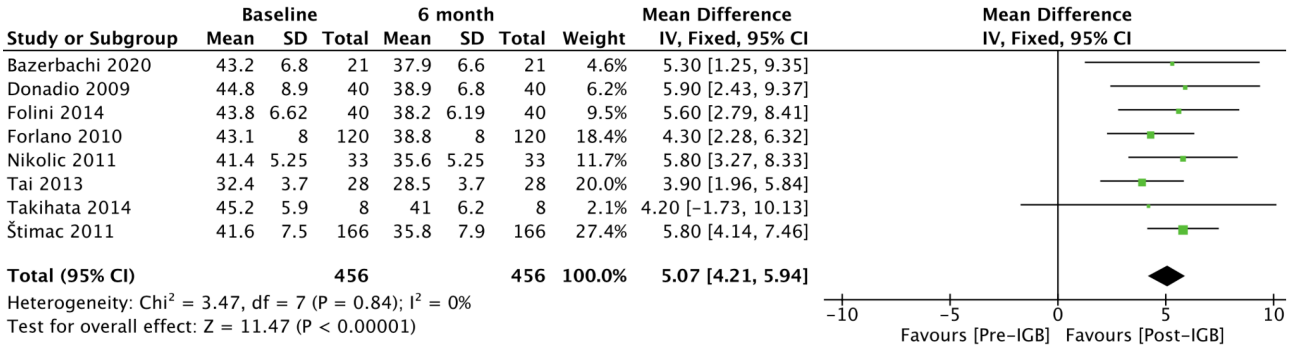

Figure 10 Forest plot of body mass index. CI: Confidence interval; IGB: Intragastric balloon.

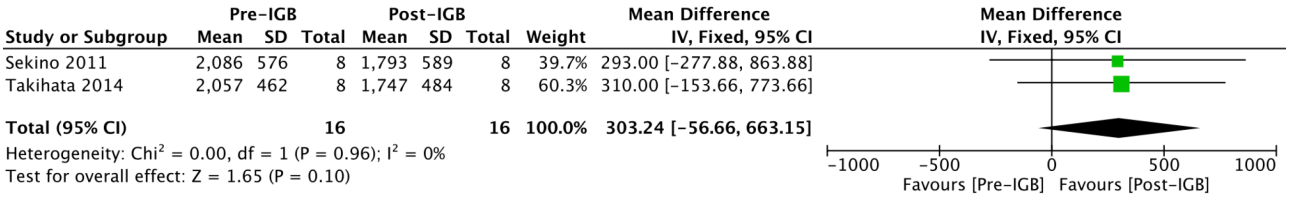

Figure 11 Forest plot of liver volume. CI: Confidence interval; IGB: Intragastric balloon.

Meta-regression

In the analysis by logistic meta-regression, there was no statistically significant correlation between the reduction in ALT and the reduction in BMI, with a $P = 0.37$. The graphical correlation between the outcomes is shown in Figure 12.

DISCUSSION

This is the first meta-analysis to assess the role of the IGB in the new definition of MAFLD. The IGB is an endoscopic bariatric and metabolic therapy for short-term management of obesity that has gained popularity due to its low rate of complications and reversibility[30]. Its mechanism of action is based on the occupation of space in the stomach causing a delay in gastric emptying, changes in gastric accommodation, neurohormonal effects, increased feelings of satiety and consequently a reduction in caloric intake[31]. A meta-analysis of randomized clinical trials published in 2020[13] evidenced that the IGB placement provided a loss of 17.98% of excess weight compared to the control group, showing to be an effective technique for weight loss. However, its metabolic effects were not evaluated.

The inclusion criteria for MAFLD showed that factors such as obesity, type 2 diabetes mellitus and metabolic disorders [increased waist circumference, increased blood pressure, lipidogram abnormalities, insulin resistance (IR) and increased C-reactive protein] were isolated variables related to progression to the most severe forms of liver disease under histopathological analysis[32,33]. Therefore, the control of progression factors is of fundamental importance in the management of these patients.

In the analysis of the metabolic parameters obtained by our study, we found results that show that IGB placement improves glycated hemoglobin, triglycerides, systolic blood pressure, abdominal circumference and HOMA-IR parameters. The improvement in such outcomes reflects a positive effect of IGB on metabolic dysfunction parameters, which are inclusion criteria in the new MAFLD classification and nomenclature.

The main relationship between obesity, fatty liver and metabolic syndrome appears to be in IR. IR is associated with a decrease in circulating adiponectin, a hormone secreted by adipocytes, that triggers fatty acid oxidation in the liver, favoring the increase and accumulation of visceral fat[34]. According to Bazerbachi *et al*[22], IGB has a weight-dependent pathway and a weight-independent pathway justifying the improvement in both the metabolic and inflammatory profiles of liver disease. The first is related to an improvement of IR in peripheral organs. The second, in turn, is

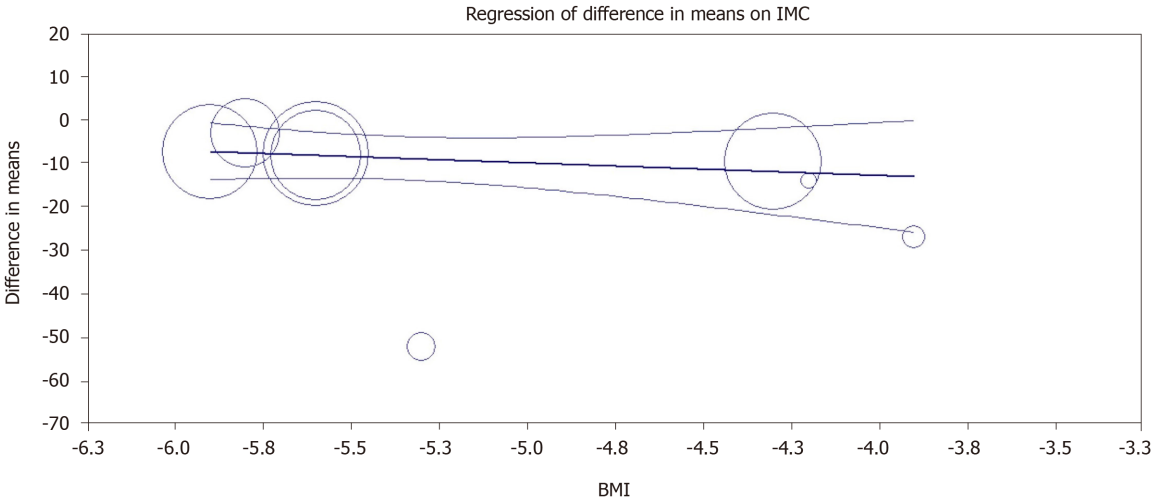

Figure 12 Meta-regression and the correlation between alanine aminotransferase and body mass index. BMI: Body mass index.

linked to a downregulation in ghrelin and hunger control, a reduction of postprandial glycemia and an improvement of the action of Sirtuin 1[35]. In this sense, the improvement of IR, represented by the evaluation of HOMA-IR[36], a mathematical model that assesses IR and functional capacity of pancreatic beta cells, seems to have a fundamental role in the positive impact of IGB on MAFLD.

In the meta-regression correlating the reduction in BMI with the reduction in liver enzymes, no statistically significant relationship was found between the two variables, showing that the improvement in ALT levels was an independent outcome of weight loss after the use of the IGB.

As demonstrated in the results of our meta-analysis, there were a statistically significant reduction in ALT and GGT levels, inferring a significant positive response in the progression of MAFLD. Although the histological evaluation by percutaneous liver biopsy is the gold standard in the evaluation of the degree of steatosis and steatohepatitis and the presence of fibrosis, this still presents limitations regarding its availability and risk of adverse events (AEs). The main AEs range from transient hypotension and pain to more serious complications such as bleeding, pneumothorax and death. A case series of 847 patients described by Filingeri *et al*[37] reported an incidence of post-procedural bleeding of approximately 2.4%.

Considering the risk of AEs, the use of alternative methods to assess clinical evolution and improvement, such as biomarkers and certain imaging methods, is necessary. The use of liver enzymes as an indirect marker of liver steatosis is controversial. Studies have shown that elevated liver enzymes can be used as a predictor of liver inflammation in obese individuals regardless of metabolic syndrome[38]. In patients undergoing bariatric surgery, the reduction in ALT and GGT is a predictor of improvement in lobular inflammation and liver fibrosis assessed in biopsies[39]. However, patients with advanced fibrosis may have normal transaminase levels[40].

Two of the studies found in our data search[10,22] demonstrated histopathological improvement in liver biopsies 6 mo after placement of IGB. Because they are studies with different designs, they could not be correlated in this meta-analysis. According to a randomized clinical trial[10] that included 18 patients, there was a statistically significant reduction in the nonalcoholic fatty liver disease Activity Score in the comparison between the use of IGB and sham procedure (decrease from score 5 to 2 with $P < 0.03$). A similar endpoint was found in the uncontrolled study conducted by Bazerbachi *et al*[22], which included 21 patients demonstrating histological improvement through nonalcoholic fatty liver disease Activity Score (decrease from score 4 to 1 with $P < 0.001$), an improvement in liver fibrosis measured by nuclear magnetic resonance and a reduction in ALT levels after 6 mo of IGB use.

In the assessment of the impact of IGB on image parameters of hepatic steatosis, the studies analyzed did not show linearity in the assessment methods. Folini *et al*[24] found a positive correlation between the improvement in the fraction of liver fat, measured by magnetic resonance imaging, and a reduction in GGT, BMI and waist circumference 6 mo after IGB placement. Similar results were evidenced by Bazerbachi *et al*[22], which found a reduction in hepatic fibrosis, measured on nuclear magnetic resonance elastography, after IGB use. In the meta-analysis of liver volume by

computed tomography, assessed by two studies involving 16 patients, a reduction of 330 cm^3 was observed after 6 mo of IGB placement but without statistical significance.

Regarding adverse effects, five studies[21,25,27-29] evaluated reported some AEs. The main ones being nausea, vomiting and abdominal pain, which were mostly controlled with symptomatic medications. Only three studies[21,25,29] reported early balloon withdrawal due to refractory symptoms. No study reported deaths or serious AEs. In a meta-analysis[41] including 6101 patients, nausea/vomiting and abdominal pain in 23% and 19.9% of patients, respectively, was described. Serious complications such as perforation and death were reported in 0.1% and 0.05%, respectively[41].

This study has some limitations. The short follow-up time (the studied outcomes were analyzed 6 mo after the insertion of the IGB) and the heterogeneity of the patients included in the studies shows how obesity is a plural disease that makes long-term results difficult to assess. Another limitation of our study corresponds to the indirect analysis of the improvement of hepatic steatosis employing liver enzymes, without a significant sample of histopathological analysis, considered as the gold standard as well as the existence of only one randomized controlled study on the subject. This showed the difficulty in including the biopsy in controlled studies due to its risks, costs and availability.

Because MAFLD is a disease with a high prevalence and complex pathophysiology that involves a multidisciplinary approach of the patients with dietary, pharmacological and often surgical interventions, the IGB should be considered as another tool in the therapy of this population. Its positive effects in the control of metabolic disorders, biomarkers of hepatic metabolism and histology of patients with MAFLD may play an important role in controlling this new worldwide epidemic.

CONCLUSION

The IGB showed significant efficacy in reducing liver enzymes in patients with MAFLD as well as improving metabolic parameters related to disease progression such as systolic blood pressure, triglycerides, HOMA-IR, waist circumference and glycated hemoglobin.

ARTICLE HIGHLIGHTS

Research background

Endoscopy has improved and has become the treatment of several diseases in recent decades. Bariatric endoscopy, through its various devices, helps in the treatment of obesity and its complications. Thus, the intragastric balloon (IGB) proves to be an effective and safe therapy for coping with this disease, and its indications have increased.

Research motivation

Metabolic dysfunction-associated fatty liver disease (MAFLD) corresponds to the accumulation of fat in the liver linked with metabolic dysregulation and has a high prevalence rate among the population. Unfortunately, no pharmacological therapy has yet shown efficacy in its treatment. In this sense, there is a need for new therapies to treat this new global epidemic.

Research objectives

We aimed to evaluate the effect of IGB in patients with MAFLD through the assessment of liver enzymes, imaging and metabolic markers in a systematic review of literature and meta-analysis.

Research methods

This systematic review was conducted according to the PRISMA guidelines and registered in PROSPERO international database. The search was performed in the electronic databases (MEDLINE, Embase, Cochrane, LILACS) and grey literature. The quality of evidence was assessed utilizing criteria from Grading Recommendations Assessment, Development, and Evaluation. The risk of bias was assessed by the Risk of Bias in Non-randomized Studies-of Interventions tool and the data were meta-analyzed using the RevMan software (Review Manager Software version 5.4-Cochrane

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Research results

Ten studies (non-randomized studies-of interventions) with 508 patients were meta-analyzed from an initial search of 1674 articles. The outcomes analyzed before and after 6 mo of IGB removal were alanine aminotransferase (IU/L), gamma-glutamyl-transferase (IU/L), glycated hemoglobin (%), triglycerides (mg/dL), systolic blood pressure (mmHg), homeostatic model assessment, abdominal circumference (cm), body mass index (kg/m^2) and liver volume (cm^3). After 6 mo of use, the IGB showed an improvement in alanine aminotransferase, gamma-glutamyltransferase, glycated hemoglobin, triglycerides, systolic blood pressure, homeostatic model assessment, abdominal circumference and body mass index. The liver volume analysis showed a non-statistically significant reduction.

Research conclusions

Our findings suggest that IGB had a significant improvement in liver enzymes (alanine aminotransferase and gamma-glutamyltransferase) in patients with MAFLD as well as improved metabolic biomarkers related to disease progression.

Research perspectives

Future studies should assess prolonged follow-up of patients after the intervention to analyze the long-term response to the improvements observed in the initial studies. A histological analysis using liver biopsies seems to be the best method of analyzing the effects of the IGB on the progression of MAFLD, and further studies should consider this method of evaluation.

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MINIREVIEWS

Evolution of liver transplant organ allocation policy: Current limitations and future directions

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Abstract

Since the adoption of the model for end-stage liver disease (MELD) score for organ allocation in 2002, numerous changes to the system of liver allocation and distribution have been made with the goal of decreasing waitlist mortality and minimizing geographic variability in median MELD score at time of transplant without worsening post-transplant outcomes. These changes include the creation and adoption of the MELD-Na score for allocation, Regional Share 15, Regional Share for Status 1, Regional Share 35/National Share 15, and, most recently, the Acuity Circles Distribution Model. However, geographic differences in median MELD at time of transplant remain as well as limits to the MELD score for allocation, as etiology of liver disease and need for transplant changes. Acute-on-chronic liver failure (ACLF) is a subset of liver failure where prevalence is rising and has been shown to have an increased mortality rate and need for transplantation that is under-demonstrated by the MELD score. This underscores the limitations of the MELD score and raises the question of whether MELD is the most accurate, objective allocation system. Alternatives to the MELD score have been proposed and studied, however MELD score remains as the current system used for allocation. This review highlights policy changes since the adoption of the MELD score, addresses limitations of the MELD score, reviews proposed alternatives to MELD, and examines the specific implications of these changes and alternatives for ACLF.

Key Words: Model for end-stage liver disease score; Acute-on-chronic liver failure; Regional sharing

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organ allocation in 2002, there have been numerous changes to policy in an effort to make organ allocation and distribution more fair and equitable. This review highlights policy changes since the adoption of the MELD score, addresses limitations of the MELD score, reviews proposed alternatives to MELD, and examines the specific implications of these changes and alternatives for acute-on-chronic liver failure.

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INTRODUCTION

Organ allocation for liver transplantation was revolutionized in 2002 by wide adoption of the model for end-stage liver disease (MELD) scoring system, which utilized objective criteria to facilitate equitable organ allocation. Although this system has improved fairness in prioritizing patients for transplantation, important disparities remain. In this review, we discuss current organ allocation policy and future directions through a historical lens, from the pre-MELD era through the development of MELD exception points, regional sharing, and implementation of the MELD-Na score. We conclude with an examination of limitations of the MELD scoring system in assessing mortality in certain patient groups and areas for improvement in current organ allocation policy.

OVERVIEW AND HISTORY OF MELD

Pre-MELD era

Prior to 1997, liver transplant priority was determined by hospitalization status and time on the waiting list. For example, a patient in the intensive care units (ICU) was given priority over a non-ICU hospitalized patient who was given priority over an outpatient. This system was based on subjective criteria that could be manipulated by hospitalizing patients or admitting to the ICU when there was no medical indication, thereby fraudulently giving a patient an advantage over others.

In 1998, United Network for Organ Sharing (UNOS) adopted the Child-Turcotte-Pugh (CTP) scoring system to stratify patients as Status 2A, 2B, or 3 for patients at high risk of death without transplantation, with Status 1 reserved for patients with acute liver failure. The CTP score incorporated objective data into waiting list priority, but still included subjective grading of encephalopathy and ascites which allowed for wide variability and the potential for inappropriately scoring the severity of a patient's condition. The CTP score was originally proposed in 1964 by surgeons Child *et al*[1] as a way to assess operative risk in patients undergoing surgical portosystemic shunt for variceal bleeding—patients were given a subclass score of A-C depending on bilirubin, albumin, ascites, hepatic encephalopathy, and nutritional status[1]. In 1973, Pugh *et al*[2] modified the scoring system by adding prothrombin time and removing nutritional status which became known as the CTP score[2]. In 2000, the United States Department of Health and Human Services released the Final Rule, which mandated that organ allocation should be based upon medical urgency that is determined by objective and reproducible data and that access to transplant should not be affected by geography[3].

Adoption of MELD score and donation service areas

The Mayo transjugular intrahepatic portosystemic shunt (TIPS) model was originally developed in 2000 as a scoring system to predict three-month mortality in patients with cirrhosis who underwent a TIPS procedure[4]. A year later this scoring system was shown to also be a reliable predictor of three-month mortality in patients with cirrhosis and became known as the MELD score[5]. The MELD score incorporated serum bilirubin, serum creatinine, international normalized ratio (INR) for prothrombin time, and etiology of liver disease. However, etiology of liver disease was

shown to have minimal impact on outcomes and was later removed from the scoring system[6].

The Final Rule led to the Organ Procurement and Transplant Network to implement the MELD score to prioritize patients awaiting deceased donor liver transplantation using only three objective lab values in its calculation—serum bilirubin, serum creatinine, and INR. In February 2002, donor liver allocation based on MELD score was implemented in the United States. The use of the MELD score led to more transplants for sicker patients and reduced waitlist mortality without reducing post-transplant survival[7]. However, distribution of donor livers prioritized patients within the local donation service area (DSA), followed by the UNOS region, and finally the nation. For example, if an organ became available, it was prioritized to the patient with the highest MELD score within that DSA. If the liver was not accepted by a transplant center within that DSA, it would be offered within the UNOS region, and then nationally. However, the differences in population size and demographics within DSAs and UNOS regions gradually led to significant geographic disparities in the MELD score at time of transplant, and therefore access to liver transplantation[7].

Policy changes to liver allocation and distribution since 2002

Since 2002, numerous changes to the system of liver allocation and distribution have been made with the goal of decreasing waitlist mortality and minimizing geographic variability in median MELD score at time of transplant without worsening post-transplant outcomes (Figure 1). Liver allocation refers to how waitlisted patients are prioritized by medical urgency based on the MELD score while liver distribution refers to the system by which donor livers are matched to patients on the waitlist based on geographic units. Each of these will be discussed below.

CHANGES IN LIVER ALLOCATION AND DISTRIBUTION IN THE UNITED STATES

Incorporation of serum sodium level (MELD-Na)

Multiple studies have shown that hyponatremia is an independent predictor of mortality in patients with cirrhosis[8-10]. Hyponatremia has also been shown to be a predictor of hepatorenal syndrome occurrence which is also associated with increased mortality[11]. In 2008, Kim *et al*[12] showed that adding serum sodium to the MELD score was a better predictor of mortality than MELD alone, making the argument that serum sodium should be added to the MELD score model[12]. The incorporation of serum sodium into the MELD score calculation was eventually adopted by UNOS in 2016. Studies evaluating the effectiveness of the MELD-Na score have shown the MELD-Na to be a more accurate predictor of 90-d mortality and that using the MELD-Na for liver allocation leads to a decrease in waitlist mortality[12-14].

Regional share 15

In 2005, Merion *et al*[15] showed mortality risk reduction in patients transplanted with a MELD score of 18 or greater with an increasing mortality reduction as the MELD score increased. But they also showed increased mortality in patients transplanted with a MELD score less than 14 compared to candidates who remained on the waitlist [15]. Due to these findings, the Regional Share 15 policy was implemented, which called for an organ to first be offered within the local DSA to patients with a MELD greater than 15 and then regionally before being offered locally to patients with a MELD less than 15.

Regional share for Status 1

Patients listed as Status 1 for liver transplantation are critically ill with acute liver failure and have a life expectancy of 7 d or less without transplantation. Under Regional Share for Status 1, patients listed as Status 1 would receive priority for transplant ahead of all other patients listed within an entire UNOS region. This policy change was implemented in December 2010 and was found to significantly increase the probability of transplantation within 7 d of listing as status 1 without negatively impacting waitlist mortality for non-status 1 patients in the same region[16].

Regional share 35 and national share 15

In 2012, it was shown that patients with a MELD score ^{3}35 had a waitlist mortality similar to patients listed with acute liver failure status 1, but only status 1 patients

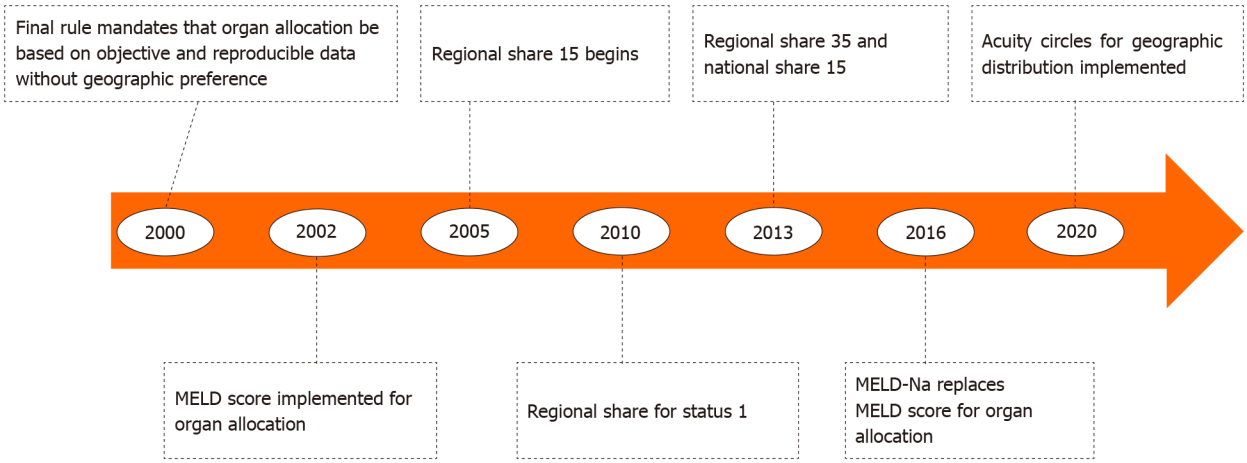

Figure 1 History of changes in organ allocation policy in the United States. MELD: Model for end-stage liver disease.

were eligible for regional sharing[17]. This lead to the Regional Share 35 and the National Share 15 policy change in 2013, which called for donor livers to be offered first to patients with a MELD score ³35 Listed within a region. If the liver was not accepted by a center then the distribution sequence was as follows: offered to patients with a MELD score ³15 within the DSA, offered to patients with a MELD score ³15 within the region, offered nationally to patients with MELD score ³15, before finally being offered locally to patients with MELD scores < 15. One year later, the Regional Share 35 policy was found to have the following effects: An increase in total transplants, 30% lower waitlist mortality for patients with MELD greater than 30, a decrease in in the number of unused organs, and no worsening of early post-transplant outcomes[18]. No difference was seen nationally when comparing post-transplant survival before and after implementation of Regional Share 35, however two regions did show significantly worse post-transplant outcomes after the policy was enacted[19].

Acuity circles distribution system

Despite the adoption of policy changes for donor liver distribution in the United States such as Regional Share for Status 1, Regional Share 35, and National Share 15, significant geographic variability in access to liver transplantation remained within the local-regional-national system of organ distribution with the median MELD score at transplant varying as much as 12 points in high *vs* low MELD score regions[20]. Spurred by lawsuits involving the lung transplant allocation system which prompted calls to eliminate the use of DSAs and UNOS regions as units of organ distribution, a new liver distribution system, known as Acuity Circles, based on concentric geographic circles around the donor site hospital was accepted in 2018 and implemented in 2020[21]. Acuity circles calls for a donor liver to first be offered to patients listed Status 1 within 500 nautical miles (nm) of the donor hospital. The organ is then offered to patients with a MELD score of at least 37 within 150 miles of the donor hospital, then to patients with a MELD score of at least 37 within 250 miles, and finally to patients with a MELD score of at least 37 within 500 miles. If the organ is not accepted for any of these patients, then it is allocated to patients with decreasing MELD score thresholds of 33, then 29, then 15 in expanding geographic circles at each MELD score tier as above before being allocated nationally, until finally being offered to patients with a MELD score under 15. As with prior policy changes, the new system was implemented to further minimize geographic disparities in access to liver transplantation.

WORLDWIDE ORGAN ALLOCATION

The MELD score is still used by many countries worldwide that perform a high volume of liver transplantations yearly. The MELD score was implemented for liver allocation in the United States in 2002 by UNOS. It was followed by North Italian Transplant (2006), Eurotransplant (2006), Canada (2006) and many others[22]. In Asia, South Korea became the first country to used MELD score for organ allocation in 2016

[23]. Some countries allow for a center-specific allocation policy, although that can only be applied in areas with high organ donation rates such as Scandinavia, Spain and Portugal[22,24].

Other countries have tried to combine recipient needs with donor availability. In 2007, France began using the French Liver Allocation Score which uses objective data of the recipient like MELD score, but additionally uses other data points such as donor-recipient distance and waiting time[24,25]. The United Kingdom began using a new allocation model in 2018 that aims to give urgent cases priority—the transplant benefit score uses donor and recipient parameters to determine optimal match[24].

LIMITATIONS OF THE MELD AND MELD-NA SCORE

The system of awarding MELD exceptions as described in the preceding section is helpful to account for conditions not addressed by the MELD calculation, however there are inherent limitations to the MELD model itself which will be discussed below.

Renal function assessment

The MELD score incorporates renal function into its calculation by using the serum creatinine value. However, patients with advanced cirrhosis often have significant muscle wasting which can lead to a "normal" creatinine level that underestimates the severity of their renal dysfunction[26,27]. Differences in muscle mass between men and women also leads to a disadvantage in organ allocation for women--their lower muscle mass leads to a lower creatinine level for equivalent renal function, leading to a lower MELD score[28,29]. Serum creatinine levels can also vary day-to-day in patients with ascites undergoing diuresis or paracentesis, and this variance is unlikely to actually reflect a true change in mortality risk[27]. Differences in the calculation of serum creatinine have also been shown to depend on the assay used by each laboratory[30].

The serum creatinine value in the MELD calculation also has a lower limit of 1 mg/dL and upper limit of 4 mg/dL, both of which have been called into question. The lower limit is in place to avoid negative values after logarithmic transformation in the MELD calculation[31], but this would assume that mortality risk is constant for all values below 1 mg/dL. The upper limit boundary was created so as to not raise the MELD score due to intrinsic kidney disease, however there is evidence that patients with a creatinine level greater than 4 mg/dL have a significantly higher mortality than those with a lower creatinine level[32].

Acute-on-chronic liver failure

Acute-on-chronic liver failure (ACLF) has been identified as a separate clinical entity from acute liver failure and acute decompensated cirrhosis and defined as "a syndrome in patients with chronic liver disease with or without cirrhosis, which is characterized by acute hepatic decompensation, organ failures, and a 28-d mortality greater than 15%[33,34]." The prevalence of ACLF is rising in the United States, particularly in the elderly[35,36]. ACLF is graded according to concurrent organ failures—ACLF grade 1 (ACLF-1) is single organ failure, ACLF grade 2 (ACLF-2) includes patients with two organ failures, and ACLF grade 3 (ACLF-3) includes patients with 3 organ failures or more[34]. ACLF-3 has a mortality without liver transplantation of 80% at 28 d and greater than 90% at one year[37].

The MELD score has been shown to be accurate for assessing mortality risk in decompensated cirrhosis, but ACLF presents a distinct entity with increased systemic inflammation and development of organ failures[37] and so the mortality risk of these patients is not completely demonstrated within their calculated MELD score. A study of the UNOS database showed that patients with ACLF-3 and MELD-Na score less than 25 had greater waitlist mortality than those without ACLF and a MELD-Na score greater than 35[38]. A recent study from the same group showed that ACLF-3 has a higher risk of waitlist mortality or delisting within 14 d compared to patients listed as status 1a, independent of their MELD score, however status 1a patients with acute liver failure have the highest chance of obtaining a liver transplant under the current organ allocation system[39]. The same study also found a rising 21-d mortality rate in patients with ACLF-3 compared to an unchanged mortality rate among status 1a listed patients[39]. A separate study from the UNOS database further demonstrated that utilization of MELD based regional sharing did not improve waitlist mortality among patients with ACLF-3[40].

Changing epidemiology of liver disease

MELD score was adopted as an accurate, objective, and reproducible tool to assess 90-d mortality risk in patients listed for liver transplant. Godfrey *et al*[41] looked to assess the predictive power of MELD score in assessing mortality risk since its adoption for organ allocation, finding that the MELD score's concordance with 90-d mortality was decreasing from 0.80 in 2003 to 0.70 in 2015[41]. The authors also found that the concordance of MELD score with mortality was lower in alcohol-related liver disease and non-alcoholic fatty liver disease while higher in patients with hepatitis C virus (HCV) related cirrhosis[41]. Given the shift from HCV-related cirrhosis to alcohol and nonalcoholic steato hepatitis-related cirrhosis as the leading indications for liver transplantation in the United States, these changes may be magnified in the years ahead. In addition to the changing epidemiology of liver disease, the emergence of ACLF as a distinct clinical entity, and the increasing reliance on MELD score exceptions, further studies are needed to determine if a MELD-based system can continue to be the most accurate, objective system for liver allocation.

ALTERNATIVES TO MELD SCORE ALLOCATION

Alternative scoring models have been proposed to the MELD score, as well as alterations to the calculation of the MELD score itself (Table 1). These alternative scoring systems attempt to address some of the issues with the MELD score that were addressed in the preceding section.

MELD-glomerular filtration rate assessment in liver disease

This scoring system aims to replace serum creatinine as a measure of renal function with a new calculation for glomerular filtration rate (GFR). The GFR assessment in liver disease (GRAIL) uses objective variables (creatinine, blood urea nitrogen, age, gender, race, and albumin) to better estimate renal function in patients awaiting liver transplantation[42]. GRAIL was developed by examining all adult patients with liver disease that underwent admission measurements of GFR using iothalamate clearance from 1985 to 2015[42]. Retrospective analysis showed that MELD-GRAIL-Na had the greatest difference compared to MELD-Na at increased disease severity—for a score ^{3}32 (observed 90 d mortality of 0.68), MELD-GRAIL-Na predicted mortality was 0.67 compared to MELD-Na predicted mortality of 0.51[43]. This scoring system would have resulted in a reclassified status for 16% of patients on the waitlist in 2015[43].

MELD-lactate

The MELD-lactate score incorporates serum lactate into the MELD calculation. This scoring model was developed by examining all patients with chronic liver disease in two health care systems in Texas from 2010-2015[44]. MELD-Lactate was shown to be a better predictor of in-hospital mortality compared to MELD and MELD-Na [area under the curve (AUC) 0.789 *vs* 0.776 *vs* 0.760; $P < 0.001$], with a more pronounced change in patients with a MELD < 15 (MELD-Lactate AUC 0.763 *vs* 0.674 for MELD) [45]. The MELD-lactate was also a better in-hospital mortality predictor when infection was the reason for hospitalization, however its performance was no different from MELD-Na in other situations[45].

MELD-plus

The MELD-Plus score uses the MELD-Na score along with additional variables found within the electronic medical record. This was developed by examining all cirrhosis related admission from 1992-2010 at Massachusetts General Hospital and Brigham and Women's Hospital and evaluating variables including demographic information, comorbidities using diagnosis codes, standard laboratory values, and current medication use[46]. Further analysis found that nine variables were the most effective predictors of 90 d mortality (bilirubin, INR, creatinine, Na, albumin, total cholesterol, white blood cell, age, and length of stay) and these were used to calculate the MELD-Plus score. A retrospective analysis showed the MELD-plus had improved 90 d mortality prediction compared to MELD-Na following a hospital admission [0.78 (95%CI: 0.75-0.81) *vs* 0.70 (95%CI: 0.66-0.73)][46].

ACLF

Patients with ACLF are defined by multi-organ failure and have increased mortality that is underestimated by the MELD score[47]. Scoring systems that may better predict

Table 1 Alternatives to the model for end-stage liver disease and model for end-stage liver disease-Na score

Test	Description	Comparison to MELD score	Ref.
MELD-GRAIL	Creatinine replaced with GRAIL	Improved 90-d mortality predictor in patients with severe disease (MELD-Na > 32), however similar to MELD-Na in patient with lesser disease severity	Asrani *et al*[42, 43], 2019
MELD-Lactate	Addition of lactate	Better predictor of in-hospital mortality when MELD < 15 or when infection is cause of hospitalization. Similar to MELD-Na in non-infectious admissions	Sarmast *et al* [44], 2020 Mahmud *et al* [45], 2021
MELD-Plus	Addition of albumin, total cholesterol, WBC count, age, and length of stay	Improved 90-d mortality predictor compared to MELD-Na, however can only be used after a hospital admission	Kartoun *et al* [46], 2017
CLIF-C ACLF	Score determined by six different organ systems failures, age and WBC count	Improved predictor of 28-d mortality compared to MELD-Na in patients with ACLF. However, only applicable for ACLF and not generalizable for decompensated cirrhosis	Jalan *et al*[51], 2014 Engelmann *et al* [52], 2018 Ramzan *et al* [53], 2020

GRAIL: Glomerular filtration rate assessment in liver disease; WBC: White blood cell; MELD: Model for end-stage liver disease; ACLF: Acute-on-chronic liver failure; CLIF: Chronic liver failure.

the mortality rate of these patients compared to MELD are being studied. The chronic liver failure–sequential organ failure assessment (CLIF-SOFA) score is a modification to the SOFA score which is used to predict outcomes in ICU level patients[48]. CLIF-SOFA includes sub scores (0 to 4) for each of its six organ components (liver, renal, neurologic, coagulation, circulation, respiratory) with higher scores indicating increased organ disease severity[49]. However, a meta-analysis showed MELD-Na to have a superior AUC compared to CLIF-SOFA for three month mortality in patients with ACLF[50].

A simplified scoring system with the same six organ components became known as the CLIF organ failure (CLIF-OF) score[37]. Further analysis showed that in addition to the CLIF-OF score, age and white cell count were also independently associated with mortality and these were combined with the CLIF-OF score to create the CLIF-C ACLF score[51]. The CLIF-C ACLF score was shown to be the most accurate predictor of 28-d mortality compared to CLIF-OF and MELD for ACLF patients (AUC 0.8 *vs* 0.75 *vs* 0.68, respectively)[52]. Another recent study found CLIF-C ACLF score ³70 at 48 h predicted mortality more accurately than MELD score[53]. These scoring systems may be superior to MELD-Na for liver allocation in patients with ACLF.

FUTURE DIRECTIONS

The number of patients awaiting liver transplantation continues to grow and outpace the amount of available organs, necessitating a fair and equitable organ allocation system. Since the creation of the MELD score in 2002, there have been many policy changes and alternatives systems proposed, however there still remains regional disparities. The recent implementation of acuity circles to address geographic distribution will need to be studied and assessed in the coming years. The success of this model will guide policy decision makers in the coming years.

MELD remains the standard scoring system to define disease severity and determine priority for transplantation, however many alternative scoring options have been discussed in this review as well but none have improved enough on the current standard to necessitate a change. Some countries have begun to explore systems that match recipient factors with donor factors to increase utilization of available organs, but more analyzation and assessment of efficacy and improvement will be needed prior to global implementation.

CONCLUSION

Liver transplant organ allocation models and policy have been changing dynamically

since the release of the Final Rule in 2000. These changes have led to improvements in liver organ utilization and making transplantation more equitable and fair for all patients, but many limitations and areas for improvement remain. Assessment of recent and past policy changes will be needed to continue to guide future direction for a more equitable liver allocation system.

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MINIREVIEWS

Antibiotic prophylaxis in patients with cirrhosis: Current evidence for clinical practice

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Abstract

Patients with cirrhosis show an increased susceptibility to infection due to disease-related immune-dysfunction. Bacterial infection therefore represents a common, often detrimental event in patients with advanced liver disease, since it can worsen portal hypertension and impair the function of hepatic and extra-hepatic organs. Among pharmacological strategies to prevent infection, antibiotic prophylaxis remains the first-choice, especially in high-risk groups, such as patients with acute variceal bleeding, low ascitic fluid proteins, and prior episodes of spontaneous bacterial peritonitis. Nevertheless, antibiotic prophylaxis has to deal with the changing bacterial epidemiology in cirrhosis, with increased rates of gram-positive bacteria and multidrug resistant rods, warnings about quinolones-related side effects, and low prescription adherence. Short-term antibiotic prophylaxis is applied in many other settings during hospitalization, such as before interventional or surgical procedures, but often without knowledge of local bacterial epidemiology and without strict adherence to antimicrobial stewardship. This paper offers a detailed overview on the application of antibiotic prophylaxis in cirrhosis, according to the current evidence.

Key Words: Cirrhosis; Quinolones; Spontaneous bacterial peritonitis; Liver transplantation; Trans-jugular intrahepatic portosystemic shunt; Variceal bleeding

Core Tip: Antibiotic prophylaxis represents a cornerstone for the management of several complications of decompensated cirrhosis, as spontaneous bacterial peritonitis

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and variceal bleeding. Short-term antibiotic prophylaxis is often applied in many other settings during hospitalization of patients with cirrhosis, such as before interventional or surgical procedures, but often without knowledge of local bacterial epidemiology and without strict adherence to antimicrobial stewardship.

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INTRODUCTION

Progress has been made on the pathogenetic and prognostic role of bacterial infection (BI) in many clinical settings of liver cirrhosis. Bacterial translocation from the intestinal lumen is now considered key factor for the development and worsening of portal hypertension[1]. Moreover, cirrhotic patients, especially at advanced disease stages, experience an impaired immune-surveillance, with reduced response to pathogens and a contemporary "exhausted" systemic inflammation[2]. Both the high susceptibility to BI and the exaggerated systemic response trigger hepatic and extra-hepatic organs dysfunction, favoring the development of acute-on-chronic liver failure [3], and a sudden worsening of portal hypertension. Therefore, it is not unusual that an episode of BI impairs the natural course of the disease, increasing morbidity, mortality, and the risk of drop-out from the liver transplantation (LT) waiting list[4-6].

The development of aggressive, tailored strategies against BI has become a cornerstone in several fields of hepatology. It has been demonstrated that every hour of inappropriate antibiotic use was associated with 1.9 higher odds of death in patients with cirrhosis and septic shock[7]. Therefore, a timely, adequate antibiotic stewardship, defined as the optimal selection, dosage, and duration of antimicrobial treatment, saves lives.

To date, among pharmacological options, antibiotic prophylaxis appears the most effective preventive measure[8]. Indeed, its wise use has improved prognosis in many settings, such as spontaneous bacterial peritonitis (SBP) or acute variceal bleeding (AVB), becoming standard of care[9].

Nevertheless, the wide and prolonged use of systemic antibiotics (not only for prophylaxis) has brought lights and shadows in cirrhosis. Indeed, there has been the spread of multidrug resistant (MDR) bacteria, a huge healthcare problem that involves many fields of medicine with significant heterogeneity and prevalence across countries and centers, but exerting a highly negative prognostic impact in the setting of decompensated cirrhosis[10]. Moreover, *Clostridioides difficile* infection has been increasingly seen in cirrhotic patients, with prolonged hospitalization and higher in-hospital mortality when compared with non-cirrhotic patients with similar burden of comorbidities[11-13]. Moreover, the onset of such infection raises an already known intestinal dysbiosis, whose prevalence aligns with the severity of liver dysfunction. This may increase the risk of a refractory infection or impair the effectiveness of several treatments, as fecal microbiota transplantation[14].

Several other issues, such as the optimal length of prophylaxis, the preferable antibiotic class to use, and potential drug-drug interactions, remain still unexplored areas. These factors may explain the relatively low adherence to antibiotic prophylaxis in some fields. In a recent survey from France[15], almost all physicians prescribed antibiotics during AVB or after an episode of SBP (97.7% and 94.8%, respectively), but 1 out of 4 did not adhere to primary prophylaxis of SBP, without significant differences between workplaces (general *vs* university hospitals). In a recently published paper from the United States, investigating potential harmful prescriptions in patients with cirrhosis[16], nearly half (48.0%) of the patients with prior SBP filled an antibiotic prescription for secondary prophylaxis, but only 8.8% consistently filled this prescription.

Apart from these areas, antibiotic prophylaxis may be applied in many other settings during hospitalization of patients with cirrhosis, such as before interventional or surgical procedures. Therefore, this paper offers a detailed overview on the

application of antibiotic prophylaxis in cirrhosis, according to current evidence.

SEARCH METHODS

PubMed/Medline until December 2020 was searched in accordance with the Preferred Reporting Items for Systemic Reviews and Meta-Analyses[17] to identify all relevant medical literature included under the following search text terms: ("cirrhosis" OR "liver cirrhosis") AND ("antibiotic prophylaxis" OR "prophylaxis") for each of the following items: SBP, variceal bleeding, gastric varices, radiofrequency ablation (RFA), trans arterial chemoembolization, endoscopic retrograde cholangiopancreatography, LT, acute liver failure, and alcoholic hepatitis. Only studies involving patients over 18 years of age and in the English language were included. In addition, a full manual search was performed of all relevant review articles and the retrieved original studies.

SBP

According to current guidelines[9,18], primary prophylaxis should start in patients with Child–Pugh score ≥ 9 and serum bilirubin level ≥ 3 mg/dL, impaired renal function or hyponatremia, and ascitic fluid protein lower than 15 g/dL, in view of previous randomized controlled trials (RCTs)[19-21]. A meta-analysis published in 2012 on three studies confirmed the beneficial role of primary prophylaxis in preventing SBP but not in reducing mortality[22]. Recently, an updated Cochrane meta-analysis did not show any gain in survival, in either primary or secondary prophylaxis[23], but the studies were at high risk of bias. Meta-analysis further clarified that, currently, no antibiotic seemed to be superior to others[23,24].

Moreau *et al*[25] investigated the role of norfloxacin in Child-Pugh class C cirrhotic patients. In this RCT, 291 patients (95% without prior SBP) were included independently of ascitic fluid protein level and then randomized to norfloxacin (400 mg/d administered for 6 mo) *vs* placebo. The primary endpoint (*i.e.* 6-mo survival) was not different between cohorts, neither was the incidence of SBP. When LT was considered as a competing risk of death or survival, patients given norfloxacin and having low ascitic fluid proteins displayed a significantly better outcome (cumulative 6-mo probability of death: 15.5% *vs* 24.8%, $P = 0.045$). Notably, patients on norfloxacin therapy were also at lower risk of developing BI, *gram-negative* BI, and MDR infections during therapy. That said, in clinical practice, primary prophylaxis seems to be reasonable for high-risk patients (*i.e.* those with low ascitic fluid proteins and advanced disease), especially if they are waiting for LT.

The rationale behind secondary prophylaxis is the high recurrence rate in patients who recover from SBP (69% within a year)[26]. In a seminal RCT, Ginés *et al*[27] demonstrated that norfloxacin (400 mg/d) decreased SBP recurrence to 20%[27]. As a consequence, current guidelines recommend secondary prophylaxis with norfloxacin (400 mg/d) until death or LT after the first episode of SBP[9,18]. Although the previously reported meta-analysis did not strongly support this measure, due to heterogeneity across studies and a high risk of bias[23], secondary prophylaxis is routinely adopted worldwide.

Nevertheless, clouds are still on the horizon, as well as grey areas in this field. First, it has been questioned whether fluoroquinolones, widely investigated in such patients due to their potential ability in reducing the translocation of *gram-negative* bacteria from the gut lumen, still remain the drugs of choice. Indeed, there has been a changing epidemiology of BI in cirrhosis from *gram-negative* to *gram-positive* rods (especially in hospitalized patients), with increasing prevalence of *Enterococci*. Therefore, quinolones effectiveness after hospital-acquired SBP or after MDR-related SBP appears unclear. Moreover, warnings about their metabolic and cardiovascular side effects were added to previously known effects on joints and nervous system. Apart from trimethoprim-sulfamethoxazole, which has been proposed as a possible second-line drug, or first-line choice in quinolones-intolerant patients[28], no effective alternatives have been available between systemic antibiotics; head-to-head comparisons between quinolones and other drug classes, even in specific settings, are urgently needed. The use of other molecules such as rifaximin, which is poorly absorbed in the gastrointestinal tract with high intraluminal levels and already used for prophylaxis of hepatic encephalopathy, is a promising alternative[29] and warrants further investigation through dedicated trials. Moreover, there is some concern about the possible increase in MDR organisms after long-term antibiotic use, but this has not been confirmed in recent studies[25,30].

Lastly, adherence to life-long therapy represents a major issue, as mentioned above. A recent multicenter RCT demonstrated non-inferiority of prophylaxis with ciprofloxacin 750 mg once a week when compared with norfloxacin 400 mg/d in terms of SBP occurrence in a relatively small group of patients with low ascitic fluid protein and previous history of SBP[31]. If these results can be confirmed, without determining increased incidence of MDR rods, this new antibiotic schedule may be of help in clinical practice. In summary, patients with cirrhosis at highest risk of SBP development may require primary antibiotic prophylaxis, especially when awaiting LT. Secondary prophylaxis is recommended in view of stronger supporting evidence. Until now, quinolones remain the drugs of choice.

VARICEAL BLEEDING

The beneficial role of antibiotic prophylaxis has been widely demonstrated in patients with decompensated cirrhosis and AVB. The rationale behind antibiotic prophylaxis is that a relevant percentage of bleeding episodes can be due to infection-related worsening of portal hypertension and coagulopathy. Moreover, infection is a causative factor in early variceal rebleeding[32]. A meta-analysis of 12 RCTs, including 1241 patients, confirmed the beneficial role of antibiotic prophylaxis in terms of overall mortality, mortality from BIs, and overall incidence of BIs[33].

Two major issues have to be addressed in the AVB setting. First, whether one class of antibiotics could be considered more effective than the others. A RCT conducted by Fernández *et al*[34] showed that patients who received norfloxacin had a higher rate of BI than those receiving cephalosporin, quinolone resistance being a major cause of infection breakthrough in these patients. The abovementioned meta-analysis[33] did not show any superiority of a specific class of antibiotics over the others, since these were all superior to the placebo; nevertheless, the beneficial effect seemed to be more pronounced in trials using cephalosporins (relative risk: 0.16, 95% confidence interval: 0.05-0.48), followed by quinolones (relative risk: 0.27, 95% confidence interval: 0.18-0.39). Therefore, current Guidelines recommend the use of intravenous (i.v.) cephalosporins (*i.e.* ceftriaxone 1 gr/d) as the best prophylactic therapy in AVB[35,36]. In clinical practice, the choice also has to take into account local epidemiology, setting of bleeding (*i.e.* out- *vs* in-hospital bleeding), and patient's individual features [previous antibiotic therapy; previous known infections or colonization(s)].

Second, the need for universal prophylaxis. Data from a propensity-matched cohort of 381 patients with AVB[37] showed that Child-Pugh A patients had a negligible risk of infection (2% *vs* 1%) and mortality (2.5% *vs* 0.4%), regardless of prophylaxis. The risk of infection rose in Child-Pugh class B patients, being significantly different in those receiving prophylaxis (6% *vs* 14%), even if mortality did not change (5% *vs* 7%). Finally, antibiotics significantly reduced both BI (19% *vs* 39%) and mortality (35% *vs* 62%) in Child-Pugh C patients. Therefore, current guidelines advocate prospective studies to assess properly the effectiveness of antibiotic prophylaxis in compensated patients[35].

In the setting of elective variceal band ligation, antibiotic use is less common. The rationale behind prophylaxis is the risk of bacteremia, which occurs in 3%-6% of cases, but it becomes clinically relevant only in a minority. A recently published systematic review and meta-analysis investigated this topic including 1001 procedures in 587 patients from 19 studies[38]. Overall, the frequency of bacteremia was 17% and 6% after sclerosis and band ligation, respectively. Comparing elective *vs* emergency procedures, the authors showed a significant difference for sclerosis (13% *vs* 22.5%) but not for band ligation (7.6% *vs* 3.2%). In summary, data do not currently provide strong recommendations about routine antibiotic prophylaxis for elective variceal therapy[35,39]. Few data are available on the effectiveness of antibiotic prophylaxis for elective fundal variceal obturation with cyanoacrylate. A study from China[40] showed that sepsis occurred with a relatively low frequency (0.64%), whereas the risk was four-fold higher in the emergency setting. A further prospective RCT from China, including 107 patients undergoing elective cyanoacrylate obturation, showed that 53 who received cefotiam 2 gr i.v. before endoscopy experienced a lower incidence of post-operative complications, even if differences on infectious complications were not exhaustively reported[41]. Finally, a small study from Thailand compared cyanoacrylate injection in urgent *vs* elective setting, showing a negligible rate of peri/post-procedural infectious episodes in the former group (0% *vs* 20%)[42].

In summary, antibiotic prophylaxis remains a cornerstone for decompensated cirrhosis with AVB. According to available data, its use may be not routinely used in

the non-urgent setting.

INTERVENTIONAL PROCEDURES

Trans jugular intrahepatic portosystemic shunt (TIPS) has been increasingly adopted in patients with cirrhosis, especially for the treatment of refractory ascites and variceal bleeding. Sepsis or bacteremia are quite common complications of TIPS placement, occurring in 2%-10% of cases[43,44]. Stent infection (*i.e.* endotipsitis) is a rare condition, caused by either *gram-positive* or *gram-negative* bacteria and can occur early (*i.e.* within 3 mo) after stent placement, or in a later period[45,46]. A single-center randomized study on 105 patients showed a non-significant reduction of post-interventional infections (20% *vs* 14%) after prophylactic administration of cephalosporin (cefotiam, 2 g i.v.). At multivariate analysis, multiple stenting, maintenance of central venous line, but not severity of underlying liver disease, had a significant impact on post-TIPS infection[47]. The same group further demonstrated that different antibiotic dosages for prophylaxis (single dose of ceftriaxone, 1 gr *vs* 2 gr i.v.) were not associated with different outcomes in terms of post-procedural infections in 82 patients undergoing elective TIPS (2.6% BI occurrence within 1 wk, in both groups)[48]. That said, current guidelines do not suggest the routine use of antibiotic prophylaxis for TIPS placement[49,50], mainly because strong evidence for this is still lacking[51]. Nevertheless, this must be weighed against the risk of serious post-procedural septic events. Therefore, antibiotic prophylaxis may be considered at least for expected technically difficult procedures or in patients with previous biliary interventions.

Considering endotipsitis, there is no evidence for adopting long-term prophylaxis given the rarity of the condition and the absence of robust microbiological data. Lastly, it has been proposed that antibiotic prophylaxis may be considered in patients having a diagnosis of a thrombosed TIPS, before invasive procedures (*e.g.*, gastrointestinal endoscopy), but larger studies are needed to properly assess this[46].

Endoscopic retrograde cholangiopancreatography (ERCP) is a commonly used procedure for many benign and malignant diseases of the biliary tract. A systematic review of nine RCTs showed that antibiotic prophylaxis reduced bacteremia in patients undergoing elective ERCP, but in the subgroup of patients with uncomplicated ERCP, the effect of antibiotics was less pronounced[52]. Therefore, American guidelines recommend antibiotic prophylaxis for prevention of cholangitis in cases of biliary duct obstruction and incomplete drainage[53]. Endoscopic procedures in patients with primary sclerosing cholangitis fall in this special group, due to multiple strictures and frequent prevalence of bacteriobilia, therefore antibiotic prophylaxis is recommended[54,55].

RFA and trans-arterial chemoembolization (TACE) are interventional procedures for the treatment of hepatocellular carcinoma. RFA has been classified as a clean procedure in such patients, not requiring routine antibiotic administration[56]. The incidence of post-procedural abscess is equal to 0.8%, according to available case series [57,58].

Thermal ablation determines heat-induced coagulative necrosis of the tumor. Therefore, bacterial superinfection may be a quite common complication, due to bacterial colonization of the necrotic area; moreover, thermal injury can connect biliary ducts with the ablation zone, creating a route for contamination from enteric bacteria in patients with underlying altered biliary anatomy (*e.g.*, choledocho-jejunostomy, prior endoscopic sphincterotomy). Current evidence therefore suggests that antibiotic prophylaxis may be used in such patients[59-63].

The rationale of TACE is to reduce arterial feeding to a malignant nodule, adding local chemotherapy, such as doxorubicin. A recent retrospective, single-center study from the United States analyzing the outcome of 171 patients who underwent 253 TACE without antibiotic prophylaxis[64] reported no infectious complications. A meta-analysis on four studies reported no significant difference between patients undergoing antibiotic prophylaxis and patients without[65], but interventional techniques were not homogeneous across studies and some endpoints (*e.g.*, post-procedural fever) may unmask inflammatory response rather than true infectious complications. Local instillation of antibiotic particles during interventional procedures has recently been proposed[66] but requires further investigations.

Yttrium^{90} embolization is a relatively novel interventional technique for the treatment of hepatocellular carcinoma or liver metastases. Few data are currently available about antibiotic prophylaxis in this setting, also in view of heterogeneous patients' characteristics, such as presence or absence of cirrhosis. A recently published

survey from 45 European centers confirmed different strategies regarding antibiotic prophylaxis, which was routinely adopted in 8% of cases[67]. However, as for chemoembolization, patients with a history of biliary endoscopic or surgical interventions seemed to be those who may receive antibiotic prophylaxis[68].

In summary, antibiotic prophylaxis is not routinely recommended for elective interventional procedures in patients with cirrhosis. It should be carefully considered in high-risk patients, such as those with bilio-enteric anastomosis, whereas it should be routinely adopted in patients with primary sclerosing cholangitis undergoing ERCP.

LT

Infection remains a major cause of morbidity and mortality in liver transplant recipients, with a significant burden on short-term post-operative graft and patient survival. Length of surgery, prior transplant or abdominal surgery, severity of liver disease at time of transplantation, and post-operative complications represent the most important risk factors for post-LT surgical site infection (SSI). The pathogens most commonly associated with early SSIs are *Escherichia coli, Klebsiella, Enterobacter, Acinetobacter*, but also *Enterococci*[69,70].

Theoretically, the main role of pre-operative prophylaxis would be to prevent SSI. Although a Cochrane meta-analysis, after including only one RCT (at high risk of bias), concluded that benefits and harms of prophylactic regimens were difficult to assess[71]; antibiotic prophylaxis has been widely used before LT, being justified by high infection rates (even during ongoing prophylaxis) and complexity of surgery.

Data on the type and length of peri-operative LT prophylaxis are scant. In a survey from 61 European LT centers, Vandecasteele *et al*[72] reported that the type of antibiotic prophylaxis was heterogeneously chosen among centers. An extended spectrum antibiotic regimen was reported in the majority of cases (73%) for elective LT. Notably, 25% centers reported a change in prophylactic schedule (in terms of drug class and length) for the sickest candidates (*i.e.* those with acute-on-chronic liver failure). The survey further demonstrated that one-third of centers used to change antibiotic prophylaxis in the presence of LT for candidates with acute liver failure (ALF).

Current American guidelines recommend the use of piperacillin–tazobactam, or cefotaxime plus ampicillin as routine prophylaxis during LT[73], considering cefuroxime, metronidazole, clindamycin, or quinolones as important alternatives in candidates with allergy to B-lactams. Notably, the guidelines highlight correct timing of prophylaxis (60 min before surgical incision for most antibiotics) and the need to repeat the dose in cases of prolonged surgery and suggest against the routine use of vancomycin, since it may increase the risk of post-transplant MDR rods. Pre-transplant surveillance for ruling-out colonization(s), as well as updates on local bacterial epidemiology, represent further important measures for tailoring prophylaxis to prevent antibiotic failure and reduce MDR development[74,75]. The length of antibiotic prophylaxis remains debated, with heterogeneous courses ranging from 24 h to 5 d. Recently, a RCT from the United States compared short-course (*i.e.* intraoperative doses) and 72-h extended course in 97 adult LT recipients[76]. The authors did not find any difference in prevalence of SSI (19% *vs* 27%) or overall infection (35% *vs* 37%) between groups, providing evidence in favor of a shorter antibiotic schedule. Larger studies are warranted to confirm properly these hypotheses. Recently, antibiotics have been investigated as factors potentially changing post-surgical ischemia-reperfusion injury. In mice, antibiotics prior to LT reduced the gut microbiota, decreasing the inflammatory response and promoting homeostatic responses[77]. These data were confirmed in a retrospective group of LT recipients, confirming that pretreatment with antibiotics was associated with improved hepatocellular function and a decreased incidence of early allograft dysfunction. Further data are needed to confirm properly the effectiveness of antibiotic therapy in LT recipients, beyond its preventive role against SSI.

SPECIAL CONDITIONS

Severe alcoholic hepatitis

Patients with severe alcoholic hepatitis (sAH) are prone to develop infection due to their severe state of immunosuppression[78]. BI accounts for nearly 80% of overall

invasive infections, although growing attention has been paid to fungal infection, especially Aspergillosis. The prevalence of BI at hospital admission and during hospitalization is up to 30% and 60%, respectively[79,80]. Urinary tract and airways are the most common infectious sites in such a cohort, the latter being highly prevalent after corticosteroid treatment, probably due to an increasing need for mechanical ventilation and intensive care management.

Corticosteroid therapy has been proven effective in improving short-term survival in sAH and currently represents the first-choice medical therapy.

Given the high prevalence of BI at baseline, and the theoretical immunosuppressive role of corticosteroids, several studies investigated whether they would increase infectious risk, and whether infection occurring during corticosteroid therapy would significantly impair survival[81]. A study on a large cohort of patients with sAH confirmed an increasing rate of BI during corticosteroid treatment (23% *vs* 12% at baseline)[82], but the actual role of corticosteroids was difficult to ascertain. Considering prognosis, a landmark study from France[79] demonstrated that the probability of being infected after/during corticosteroids reduced the survival benefit given by medical therapy. A further meta-analysis on 12 studies involving 1062 patients did not show a higher short-term risk of death for infection in those receiving corticosteroids, when compared with those receiving a placebo[83].

That said, antibiotic prophylaxis has been proposed in such a setting. Vergis *et al*[82] demonstrated that an infection occurring prior to corticosteroid introduction has a more favorable course if the antibiotic is continued also during steroid therapy. Moreover, the use of prophylactic antibiotics (prescribed in 45% of cases) was associated with a lower risk of death than that in patients who did not receive prophylactic antibiotics (13% *vs* 52%)[82]. Summarizing the available data, infection is highly prevalent in patients with sAH, both in those receiving steroids and not. The impact of steroids as a potential risk factor for infection is currently debated and not supported by robust data. An ongoing clinical trial (NCT02281929) assessing the prophylactic role of amoxicillin-clavulanic acid will probably clarify this point.

ALF

In a similar fashion to sAH and acute-on-chronic liver failure, ALF is characterized by a severe state of immunosuppression. Moreover, the rapidly evolving scenario of ALF, including the changing neurological status and need for circulatory support and mechanical ventilation, makes diagnosis of BI even more difficult. The prevalence of BI is nearly 30%-34%, according to recent studies[84,85]. Severity of the underlying condition and presence of cerebral edema seem to be associated with infection development. Occurrence of infection is obviously associated with worse outcome in ALF, since it may further derange hepatic and extra-hepatic organ(s) failure and may delay or contra-indicate LT. Recently, a retrospective analysis of a large United States cohort by Karvellas *et al*[86] did not show any significant improvement with administration of antibiotic prophylaxis in 600 patients with ALF, if compared with the 951 patients who did not receive antibiotics. Indeed, there was no significant difference in the probability of having bloodstream infection based on receiving prophylaxis (12.8%) or not (15.7% $P = 0.12$). Notably, the timing of prophylaxis was not homogeneous, nor were the clinical characteristics between cohorts, such as type of prophylaxis (47% extended spectrum beta-lactam, 39% vancomycin, 27% fluoroquinolones, and 20% third and fourth generation cephalosporins). Other strategies, such as selective bowel decontamination, did not show any significant benefit either [87]. In summary, current guidelines say that, even the routine use of prophylactic antibiotics does not increase survival in such patients, a strict surveillance for infection should be provided in order to start antibiotic therapy as early as possible[88,89]. Prophylaxis should be considered in cases where illness progression is considered likely, as in those with worsening encephalopathy, signs of systemic inflammation, or awaiting LT[90,91]. The choice of antibiotic class is even more debated, probably due to heterogeneous epidemiology across studies and the relevant number of culture-negative infections. That said, the high prevalence of pneumonia[87], as well as the presence of indwelling catheters and invasive procedures should be taken into account.

Table 1 Current recommendations and uncertainties regarding antibiotic prophylaxis in patients with cirrhosis

Procedure/clinical setting	Antibiotic prophylaxis	Areas of uncertainties
Spontaneous bacterial peritonitis	Primary prophylaxis recommended in decompensated patients with low ascitic fluid proteins. Secondary prophylaxis recommended	Second-line antibiotics. Quinolone resistance. Rifaximin. Secondary prophylaxis after MDR infection
Variceal bleeding	Prophylaxis recommended in acute bleeding from esophageal/gastric variceal bleeding	Prophylaxis in compensated (*e.g.*, Child-Pugh A) patients having acute variceal bleeding. Prophylaxis in elective endoscopic therapy of gastric/esophageal varices
Endoscopic retrograde cholangiopancreatography	Routine prophylaxis not recommended. Prophylaxis is recommended in patients with incomplete drainage and in those with primary sclerosing cholangitis	
Transjugular intrahepatic portosystemic shunt	Prophylaxis should be considered in difficult procedures	Prophylaxis in patients with thrombosed transjugular intrahepatic portosystemic shunt undergoing invasive procedures
Radiofrequency ablation. Trans-arterial chemoembolization. Radioembolization	Routine prophylaxis not recommended. Advisable in patients with prior interventions on biliary tree	Intra-procedural antibiotic instillation
Liver transplantation	Routine prophylaxis is recommended	Length of prophylaxis
Severe alcoholic hepatitis receiving steroids	Prophylaxis would be preferable	Length of prophylaxis, antibiotic class
Acute liver failure	Prophylaxis is advisable in high-risk patients, or those waiting for liver transplant	Antibiotic class

CONCLUSION

BI represents a common complication in patients with cirrhosis due to disease-related immune dysfunction. In this setting, antibiotic prophylaxis plays a major role, especially in high-risk patients. Type and length of prophylaxis are supported by low quality data in several fields of hepatology and LT (Table 1) and are currently heterogeneously adopted across centers. Since unnecessary prophylaxis or prolonged schedules may increase the risk of anaphylaxis and development of MDR rods, a wise adherence to current recommendations and a rigorous application of antibiotic stewardship are of utmost importance. Other important remarks should be offered to the reader. First, this paper does not include prophylaxis against invasive fungal infection, which is another serious complication in cirrhosis, having an increasing prevalence and a dreadful outcome[92]. Second, although we have focused on systemic antibiotic prophylaxis, growing evidence on non-antibiotic prophylaxis against BI in cirrhosis has to be mentioned. The role of rifaximin, a nonabsorbable antibiotic, has been largely demonstrated for patients with prior episodes of hepatic encephalopathy. Other emerging selective gut decontamination modalities, including prebiotics and probiotics, and fecal microbiota transplant are in the pipeline[93]. Future studies are therefore warranted to investigate whether these modifications to gut microbiota will reduce the occurrence of BI (especially SBP), acting as prophylactic strategies. Moreover, the preventive role of non-selective beta blockers and albumin has to be robustly confirmed, according to underlying liver function and setting[94,95].

Finally, we strongly encourage an updated review of local bacterial epidemiology in clinical practice, and a strong liaison with infectious disease specialists, pharmacologists, microbiologists, and epidemiologists, in order to use tailored prophylaxis regimens, because the right prevention works better than a cure.

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MINIREVIEWS

Kidney transplant from donors with hepatitis B: A challenging treatment option

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Abstract

Utilizing kidneys from donors with hepatitis B is one way to alleviate the current organ shortage situation. However, the risk of hepatitis B virus (HBV) transmission remains a challenge that undermines the chance of organs being used. This is particularly true with hepatitis B surface antigen (HBsAg) positive donors despite the comparable long-term outcomes when compared with standard donors. To reduce the risk of HBV transmission, a comprehensive approach is needed. This includes assessment of donor risk, optimal allocation to the proper recipient, appropriate immunosuppressive regimen, optimizing the prophylactic therapy, and post-transplant monitoring. This review provides an overview of current evidence of kidney transplants from donors with HBsAg positivity and outlines the challenge of this treatment. The topics include donor risk assessment by adopting the nucleic acid test coupled with HBV DNA as the HBV screening, optimal recipient selection, importance of hepatitis B immunity, role of nucleos(t)ide analogues, and hepatitis B immunoglobulin. A summary of reported long-term outcomes after kidney transplantation and proposed criteria to utilize kidneys from this group of donors was also defined and discussed.

Key Words: Hepatitis B virus; Organ donor; Recipient allocation; Kidney transplant; Transmission; Long term outcomes

Core Tip: Low-risk hepatitis B surface antigen (HBsAg) positive kidney donor, defined by a negative test of hepatitis B virus (HBV) DNA being allocated to immune-recipients with anti-HBs at least 10 mIU/mL is a key factor in overcoming the risk of HBV transmission. The risk may be further eliminated with optimal nucleos(t)ide analog prophylaxis. Blood tests for HBV DNA, HBs Ag, and liver function tests

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should be routinely monitored after transplantation and when there is a change of immunosuppression. The excellent long-term outcomes being reported suggested that the outcomes of this treatment option are promising. This will lead to broader use of organs with positive HBsAg.

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INTRODUCTION

Kidney transplantation (KT) is the preferred treatment for patients with end-stage kidney disease (ESKD). It is associated with reduced mortality and improved quality of life when compared to dialysis therapy[1]. However, the number of ESKD patients awaiting KT far exceeds the number of organ donations globally and leads to a problem of organ shortage. This major barrier has led to a prolonged waiting time and subsequently excess mortality of patients in the waiting list pool[2]. There are several proposed rationales to solve the problem of organ shortage[3]. One possible solution is to expand the donor pool by utilizing "extended donor criteria organs". Such organs include those from donors with hepatitis B virus (HBV) infection.

The prevalence of chronic HBV infection varies greatly by geographical region, ranging from 0.4% to 1.6% in the region of the Americas, 1.2% to 2.6% in Europe, 1.5% to 4.0% in Southeast Asia, 2.6% to 4.3% in the Eastern Mediterranean, 5.1 to 7.6 % in the Western Pacific, and 4.6% to 8.5% in Africa[4]. Discarding all kidneys from donors with markers of HBV infections may substantially harm the donor pool in endemic areas since the prevalence in donors is similar to that of the general population. Thus, one challenge is determining the optimal use of kidneys from such donors. The best utilization may involve allocating such kidneys to transplant candidates at low risk of acquiring a donor-transmitted hepatitis B infection. Prophylactic therapy and appropriate monitoring will further eliminate the risk of HBV transmission.

According to current guidelines, there is an increasing trend of accepting non-liver organs from total hepatitis B core antibody-positive [anti-HBc (+)] donors to be used in any recipient regardless of HBV immune status without prophylaxis due to the negligible risk of de novo infection. However, utilizing kidneys from donors with positive hepatitis B surface antigen (HBsAg) [HBsAg (+)] remains controversial, and it is generally suggested that such organs be discarded[5-7]. In this review, we aim to summarize the current evidence regarding the use kidneys from HBsAg (+) donors with an emphasis on the risk of HBV transmission, liver related morbidities, and the outcomes of KT.

SCREENING TEST FOR HBV INFECTION IN ORGAN DONORS

Screening for HBV infection usually relies on a panel of serologic tests. The test for HBsAg is widely distributed. However, it can fail to detect disease during a 35-44 d window period after inoculation or occult infection defined as detectable viral DNA in absence of HBsAg[8]. Another importance serologic screening test for previous HBV exposure is anti-HBc. In acute hepatitis B infection, immunoglobulin M (IgM) antibody to hepatitis B core antigen (IgM anti-HBc) becomes positive after 4 wk to 6 wk of exposure indicating recent infection and active viral replication whereas total hepatitis B core antibody (anti-HBc) appear at the onset of symptoms and persists for life. Hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe) are additional tests to identify viral replicative activity as HBeAg positivity which indicates active viral replication (*i.e.*, usually a viral load > 10000 IU/mL). In contrast, anti-HBe positivity indicates the presence of the non-replication phase (*i.e.*, a viral load < 10000 IU/mL). Lastly, hepatitis B antibody (anti-HBs) is a marker of immune status due to either naturally- or vaccine-acquired immunity[9].

In the situation of deceased kidney donation, HBs Ag and anti-HBc are generally accepted as cost-effective screening tools. The results should be integrated with additional essential information of the donors to assess the risk of donor-derived infection as previously described[10-12]. Some transplant centers in endemic areas routinely add on anti-HBe and HBeAg to the donor screening platform as biomarkers of high viral replication and infectivity activity relating to a high viral burden[13-15]. However, serologic testing alone still has limitations due to the long window period and the lack of sensitivity to detect occult infections, raising concerns over risk misclassification[16,17]. In clinical practice, isolated anti-HBc is commonly observed. This may occur in several clinical settings. First, the early window period of acute hepatitis B. Second, a resolved HBV infection with waning of anti-HBs titer. Third, a false positive anti-HBc. This setting is commonly found in an area with a low prevalence of HBV infection. Fourth, an occult chronic HBV infection with low viremia and undetectable HBsAg. The latter can occur with a poor test quality or when there is a mutation of HBsAg[18,19].

To improve the sensitivity of screening tests, the nucleic acid test (NAT), which is usually in the form of an HIV/HCV/HBV multiplex, has been proposed as an optional donor screening test. This test is advantageous, because it shortens the windows period to 20-22 d compared to 35-44 d by conventional serology[8]. Although NAT seems a promising solution, obstacles to its implementation include whether it is cost-effective in a particular healthcare setting, the logistic challenges, the long turn-around times (*i.e.*, as much as 8 h), the technical proficiency required, and the reliability of an in-house developed test[8,20]. In low prevalence settings, such as the United States , the concern is that the benefit may not outweigh the disadvantage that can lead to loss of organ donor, and have suggested that routine use of NAT screening was unnecessary[8]. However, a look-back study demonstrated adding NAT to routine screening by serologic testing enhanced the physician's confidence in using organ with discordant results [*i.e.*, anti-HBc(+)/NAT(-)], and adding NAT led to a gain in overall organ utilization after policy implementation[21]. Currently, this test is gradually becoming accepted in national policies in several countries. For example, the US Public Health Service 2020 guideline revision suggests performing NAT for HIV, HBV, and HCV in organ donors in all donor transplants[22]. While guidelines in the Transplantation Society of Australia and New Zealand suggests performing NAT in donors with HBsAg positivity, anti-HBc positivity, or HBsAg and anti-HBc negativity with increasing behavioral risk for HBV infection[6]. However, the decision to use NAT in individuals depends on the context of each setting or country. For practical purposes, we suggest that all serologic tests for HBV (HBsAg, anti-HBc, HBeAg, anti-HBe) as well as other essential infectious markers are done at the donor hospitals. In parallel, a universal NAT test for HBV (usually in combination with HIV and HCV) should be conducted at the central or regional organ allocation center and the result should come back before or at the time of organ retrieval.

RISK OF HBV TRANSMISSION AND INFECTION AFTER KT

Donor factors and the role of HBV DNA

The risk of donor-transmitted HBV infection is lower in kidney transplant recipients compared with liver transplant recipients with similar serologic marker positivity[23]. Specific HBV receptor recognition may play important roles in this hepatotropism phenomenon[24]. The demonstration of persistent HBV viral genome in the liver and peripheral blood mononuclear cells of patients with acute and chronic HBV infection after the clearance of HBsAg in the blood has led to an awareness of possible HBV reactivation in the immunocompromised host. This notion was supported by previous studies that showed the presence of HBV covalently closed circular DNA and total DNA in the serum of patients with negative HBsAg[25,26].

Important behavioral risk factors to acquire HBV (and other coincidental infections such as HIV and HCV) should be carefully reviewed when assessing the risk of HBV transmission from the donors. Patients who have strong risk factors for HBV/HCV/HIV combination should be tested for HBsAg, HBV NAT, and then HBV DNA by a test with the highest sensitivity and specificity. A previous study had suggested a test with a lower detection limit of less than < 0.1 ng/mL for HBsAg and 10 IU/mL for HBV DNA[27]. Donors with HBV infections are generally categorized into two groups according to their serologic status. The first donor group is the anti-HBc positive group in which the rate of transmission appears to be negligible according to the recipient's protective immunity status. The overall seroconversion rate was 3.24% (mostly anti-

HBc seroconversion). HBsAg seroconversion rate from this study was shown to be 0.28% with no symptoms of hepatitis and no excess mortality[28]. The second donor group is the HBsAg positive group where the HBV transmission remains a challenging problem[5]. In the current era, interesting information regarding the use of kidneys from HBsAg (+) donors is increasing. Previously, it was generally believed to discard the use of these kidneys. However, several recent studies and guidelines suggested that kidneys from HBsAg (+) donors can be carefully considered to be transplanted to appropriate recipients after careful consideration of the risk and benefit with informed consent[5,29]. The role of NAT in reducing the window period of serological test in combination with a careful evaluation of the donor behavioral risk factors has been increasingly emphasized[30]. KT from living HBsAg (+) donors can be donated to anti-HBs (+) recipients with protection who have no abnormalities of liver function test, no history of liver disease within the previous 28 days, and who are not living in the area of possible mutation strain of HBV[31].

It is important to note that fulminant hepatitis B infection had been reported in a naïve recipient who received kidneys from donors with HBsAg (+)/HBeAg (+) donors [32]. Since this report, HBeAg and anti-HBe were routinely checked in HBsAg (+) donors to ensure a low infectivity rate of HBV before performing KT[14,15,33]. Use of antiviral medications to treat HBV add benefit to the treatment plan to use organs from HBsAg (+) donors. Unlike liver transplantation, KT from this type of donor can be associated with a functional cure of HBV. The functional cure was defined by a state of sustained loss of HBsAg with or without anti-HBs seroconversion which was usually associated with good clinical outcomes[34]. A recent study performed 83 living KTs from HBsAg (+) donors to HBsAg (-) recipients. Before the transplant, 28% of the donor in the latter study were HBV DNA (+) and 24% of the recipients had no anti-HBs. All recipients in the latter study received hepatitis B immunoglobulin (HBIG) and antiviral medication as prophylaxis treatment. The results showed that this treatment was associated with excellent graft and patient survival without excess HBV transmission when compared with the control group[35]. In recent years, tests for HBV DNA have increasingly become popular. Several studies revealed that the prevalence of hepatitis B viremia in HBsAg (+)/HBeAg (-) donors ranged from 2.3%-28.3%[14,15,35]. Chancharoenthana *et al*[14] reported that kidney transplants from HBsAg (+)/HBV DNA (-) (< 20 IU/mL) donors to 20 immune recipients (anti-HBs > 100 mIU/mL) was safe and was not associated with any HBV viremia, hepatitis or death despite the absence of antiviral prophylaxis. The other two studies reported excellent outcomes of transplanting kidneys from HBsAg (+)/HBV DNA (-) donors to a total of 146 recipients with anti-HBs > 10 mIU/mL. Those studies have also shown excellent outcomes with no evidence of HBV transmission[36,37]. It was interesting to note that there was one out of 58 recipients of HBsAg (+)/HBV DNA (-) donor who developed HBsAg seroconversion one month after transplantation. That patient had received HBV vaccination, but with low (non-protective) anti-HBs titer (4.6 mIU/ml). However, this patient did not develop clinical evidence of hepatitis and has acquired anti-HBs seroconversion which may be due to prophylactic therapy lamivudine and HBIG in the study protocol[15].

Recipient factors and the role of protective immunity

In principle, the recipients who received kidneys from donors with hepatitis B should have protective anti-HBs. Several guidelines and studies have suggested that an anti-HBs > 10 mIU/mL was protective[5,33,36,37]. It is important to note that HBV transmission may not necessarily lead to clinical evidence of HBV infection. This was clearly shown in one study that performed transplantation of HBsAg (+) kidney to four immunized patients with an anti-HBs ranged from 63 mIU/mL to > 1000 mIU/mL. The results showed that there was no HBsAg seroconversion, although the anti-HBc IgG was positive in all 4 cases at six months despite the presence of anti-HBs positivity. This study showed evidence of HBV transmission by the kidney grafts without any clinical manifestations of HBV infection[38].

For KT, it was unclear whether a higher level of anti-HBs concentration was associated with a higher level of protection of HBV transmission as was shown in liver transplantation. Immunity to hepatitis B was crucial to prevent donor-derived infection. However, it was suggested that an anti-HBs concentration of > 10 mIU/mL was protective[39]. It was shown in studies of transplanting kidneys from anti HBc (+) donors to 50 recipients with anti-HBs > 10 mIU/mL, that there was no anti-HBc IgM or HBsAg seroconversion[40]. Tuncer *et al*[36] and Asuman *et al*[37] reported that kidney transplants from HBsAg (+) donors to 146 recipients with anti-HBs > 10 mIU/mL were not associated with any *de novo* HBV infection or active liver diseases. A study in 43 recipients of HBsAg (+)/HBV DNA (-) donor with patients with higher

anti-HBs level (> 100 mIU/mL) found that there was neither anti-HBc nor HBsAg seroconversion and there was no evidence of HBV DNAemia[14]. However, a recent study of kidney transplants from HBsAg (+) donors to 83 HBsAg (-) recipients with varying degrees of anti-HBs did not support the importance of high anti-HBs concentration[35].

There was variation in the definitions of HBV transmission *via* transplantation of non-liver organs[5]. In the setting of kidney transplants from HBsAg (-) donors to immune protective recipients (Anti-HBs > 10 mIU/mL), definitions of HBV transmission may include anti-HBc IgM seroconversion, HBsAg seroconversion, and HBV DNAemia. *De novo* HBV infection can occur as a consequence of HBV transmission with clinical evidence of acute or chronic liver disease associated with HBV.

Differences in the reported rate of HBV transmission and/or infection after kidney transplant may be related to the different targets of protective anti-HBs concentration. Subclinical infection presenting with anti-HBc seroconversion was observed with kidney transplants from both anti-HBc (+) and HBsAg (+) donors[14,15,35,41]. In addition, the need for higher levels of immunity is related to global variation in HBV genotypes. The genotype predominance by region is A in North America, B in Europe, C in Asia and Australia, and D in the middle east and central Asia[42,43]. Most commercially available HBV vaccines were developed using genotype A2. Although cross-protection against other genotypes is observed, it has been suggested that a higher antibody concentration (> 50 mIU/mL) might be required[43]. However, the immune benefit may be lost in cases of HBV antigenic variation due to mutation in the 'a' determinant region of HBsAg[43,44]. In this case, the protective effect of HBIG is also lost. One case of fulminant hepatitis B in a kidney transplant recipient with vaccine-acquired immunity and an HBV infection of the D2 genotype with an escape mutation at G145R (glycine to arginine, G145R) was reported after the recipient had received a kidney from an HBsAg (+) donor, despite the recipient having received HBIG and NA prophylaxis[45]. Although such cases are rare, they may lead to fatal complications.

MONITORING OF HBV INFECTION AFTER TRANSPLANTATION

For kidney transplant recipients, The American Association for the Study of Liver Diseases (AASDL) suggested periodic assessment of serum ALT, HBV DNA, and HBsAg during immunosuppressive therapy. Reactivation of HBV infection was defined by detectable HBV DNAemia or positive HBsAg seroconversion. In addition, hepatitis flare was defined by rising of serum ALT more than 3 times the baseline level and > 100 U/L with evidence of hepatitis B reactivation[19].

The optimal frequency of monitoring for HBV infection in a susceptible individual is still varied. The Infectious Disease Community of Practice of the American Society of Transplantation advised monitoring liver enzymes, HBsAg, and HBV DNA every 3 mo for at least 12 mo post-transplantation. Subsequent management was based on the evolution of test results over the first year[46]. In the case of naïve recipient receiving Anti-HBc (+) kidney without antiviral prophylaxis, the European guidelines recommend monitoring for HBsAg, and HBV DNA at least during the first year. Also, most of the recipients from donors with HBV infection were suggested to receive lifelong monitoring[47].

Besides, all kidney transplant recipients who have a resolved infection of HBV (defined by positive anti-HBc serology) should be aware of a possibility of HBV reactivation during a course of intensive immunosuppression particularly rituximab [48]. Kim *et al*[49] studied HBV reactivation in a cohort of 499 kidney transplant recipients. 86.6 % of those recipients were anti-HBs (+) and 29.6% received kidneys from donors with positive anti-HBc IgG. No recipients received kidneys from donors with positive HBsAg. The authors reported that the incidence rate of hepatitis B reactivation was 2% during a follow-up period of 6.7 years. HBV reactivation was observed at the median time of 2.8 years (range 1.4-11.5). A high incidence of reactivation was observed in recipients with ABO incompatibility, who received plasmapheresis, received acute rejection therapy, and received induction therapy with rituximab[49]. These findings provided evidence that HBV reactivation can occur at any time after KT. As such, HBV reactivation may be the consequence of either donor-derived infection or the resolved recipient infection.

THE ROLE OF PROPHYLAXIS THERAPY

Vaccination and revaccination protocol

Anti-HBs play a key role to minimize the risk of HBV transmission. Hepatitis B vaccination should be given to naïve recipients or previously immune recipients who have anti-HBs concentration below 10 mIU/mL[39]. Also, the KDIGO guideline suggested a concentration of Anti-HBs below 100 mIU/mL can be rapidly lowered down to a non-protective level and may require a booster dose at this step[50]. Differences in suggestions may be due to a concern that patients with chronic kidney disease may have impaired anamnestic response to viral infection. This can lead to an insufficient immune response to HBV, and suppression of memory T and B cells that may result in a low or absence of antibody titer[44]. As an antibody concentration is likely to wane over time, monitoring of anti-HBs concentration should be done at least yearly. A further booster dose of vaccine may be required. This can be prescribed by either a single-shot high dose (40 μg) or a total complete course with a follow-up level at 4-wk after a complete course of treatment[39,51]. One study found that 95% of immune recipients with waning titer can be successfully boosted with a full course of hepatitis B. However, 10% of patients might have delay response of titer up to 6 mo after treatment completion. Higher antibody concentration was observed in patients who had a shorter duration of dialysis and positive anti-HBc status[52].

A high dose of HBV vaccine was suggested to patients with ESKD who were receiving hemodialysis therapy. A protocol of three or four high-dose (40 μg) hepatitis B vaccine series with a target level of 10 mIU/mL at 4 wk post-treatment was suggested. Also, a second three doses of vaccination were suggested if the anti-HBs could not reach the desired level[51]. Similarly, the CDC recommended Recombivax™ vaccine at 0, 1, and 6 mo or Engerix B™ at 0, 1, 2, and 6 mo[53]. Despite this approach, at least 30% of hemodialysis patients were still not successfully immunized[54].

The strategies to improve vaccine efficacy may be related to the type, dose, and route of administration. Besides the use of commercially available hepatitis B vaccine derived from genotype A2, a vaccine specifically derived from common genotype in the specific geographical area will add a layer of protection. This practice has been investigated in Korea and Japan where Type-C derived vaccine (Bimmugen™) was being given. The proof of this concept will take up to a decade[43,55]. To those who were not responding to conventional vaccine protocol, a subcutaneous injection route was reported to be associated with increased responsiveness (70 by intramuscular, 74 by subcutaneous)[54]. Also, a third-generation vaccine containing pre s/s epitope vaccine has been reported to be associated with good immunogenicity and responsiveness in a healthy individual[56]. The results of this third-generation vaccine when administered to patients with ESKD are further required to fill the practice gap.

Despite a debate, there was a suggestion to keep anti-HBs concentration more than 100 mIU/mL. Reactivation after KT has been reported in patients with antibody titer less than 100 mIU/mL[57]. In another study, no anti-HBc or HBsAg seroconversion was developed in patients who had received a booster vaccine to keep levels above 100 mIU/mL[52]. Due to the low-risk nature of the interventions, KDIGO suggested re-evaluating anti-HBs annually and administering re-vaccination if anti-HBs were found to be below 10 mIU/mL[50].

Antiviral medications (nucleos(t)ide analogues) and HBIG

Another modality to prevent HBV transmission *via* kidney transplant organs was the use of antiviral medications and HBIG. HBIG provides passive immunity for a high concentration of anti-HBs that are aimed to act as neutralizing antibodies to HBV[58]. Most prescriptions of HBIG were used in combination with antiviral nucleos(t)ide analogs (NAs) that aim to prevent recurrent infection of HBV after liver transplantation. This regimen was found superior to HBIG or NA alone[59]. However, the optimal dose of HBIG to be used for kidney transplant recipients from donors with HBV was not clearly known.

NAs are a group of antiviral medications that directly suppress HBV virus replication. Lamivudine was the most popular prophylaxis agent being used globally [5]. However, its efficacy was hampered by small number of lamivudine-resistant hepatitis B. Therefore, other drugs with a high genetic barrier such as entecavir were considered as a better alternative[19]. This was especially noteworthy in selected patients who were at risk of exposure to a lamivudine-resistant strain of HBV, including those who received kidneys from the donors previously treated by lamivudine. From a meta-analysis of 12212 chronic naïve hepatitis B patients, the prevalence of lamivudine-resistant HBV was 8% in China, 0-6.6% in other Asian

regions, 0%-4.5% in South America, 1%-3% in Europe, and 0.71% in the United State [60]. The incidence of lamivudine-resistance can be increased with longer durations of exposure (as high as a fifty percent increase after 2 years)[61]. In chronic hepatitis B liver transplant recipients, high genetic barrier nucleos(t)ide analog combined with HBIG was superior to lamivudine combined with HBIG in the prevention of recurrent HBV infection (disease recurrent rate 1.0% compare to 6.1%)[62].

Serologic markers of HBV infection may have some impact on the choice to prescribe antiviral medications (NA). Anti HBs (> 10 mIU/mL) and positive recipients can receive kidney transplants from anti-HBc (+) donors without a need for prophylaxis antiviral medications due to the negligible risk of HBV transmission. In contrast, naïve recipients who received kidneys from anti-HBc (+) donors should receive lamivudine prophylaxis without HBIG for at least 1 year[5]. In the setting of HBs Ag (+) donors, recipients with protective anti-HBs (> 10 mIU/mL) were considered suitable to receive the allocation of kidney grafts. Further risk should be assessed by the result of the NAT test and HBV DNA measurement. If the result of nucleic acid for HBV was negative and HBV DNA was undetectable (by a method with a detection limit as low as 20 copies per mL), preventive strategies varied from no NA prophylaxis (in the setting of no potent induction therapy), or prescription of NA alone without HBIG. If the anti-HBs is > 100 mIU/mL, one can proceed to KT without NA prophylaxis[14]. However, if HBV DNA was not measured by a method of optimum low detection limit or the result of HBV DNA cannot be obtained due to any reason, NA may be prescribed to make the risk of HBV transmission as low as possible.

In the setting of HBsAg (+)/HBV DNA (+) donor, most authors prescribed universal NA prophylaxis with or without HBIG as a prophylaxis regimen among patients with different levels of anti-HBs concentration[14,15,35]. However, the optimal dose of HBIG in this setting was not clearly known. One important issue in this setting was the use of potent induction therapy. A study in 24 immunized recipients (mean anti-HBS 452 ± 384 mIU/mL), 89% who received induction therapy including anti-thymocyte globulin, found that three of them had detectable HBV DNAemia. This HBV DNAemia occurred although all patients received six months of lamivudine therapy. Fortunately, none of those patients developed liver failure[13]. Thus we have suggested that the use of HBsAg (+)/HBV DNA (+) donors to patients with immunized anti-HBs should be exercised with due caution as this group still carries a significant risk of de novo HBV infection particularly in the recipients who receive potent induction therapy[13].

Use of HBsAg (+)/DNA (+) donors to recipients with naïve or anti-HBs < 10 mIU/mL was the group with the highest risk being reported. A study in 20 naïve recipients who received prophylactic NA or HBIG or combination showed that the incidence of acute liver injury, anti-HBc seroconversion, and HBV DNAemia was 20 %, 10%, and 10% respectively[35]. Thus the use of this treatment option should be restricted to patients with an urgent need for KT (exhausted multiple vascular access, with ongoing uremia despite adequate hemodialysis prescription).

Another interesting issue is the use of HBsAg positive donors to recipients with HBsAg positive serology. A few studies[63-66] have reported favorable outcomes of this treatment option provided that the recipients received antiviral treatment before transplantation. Also, there is a suggestion that the recipients with positive HBsAg should have the result of liver biopsy that did not show evidence of cirrhosis. However, it should be noted that the number of patients being reported with this option was small. Additional information for this setting may be required.

Figure 1 showed the important factors associated with the risk of HBV transmission in the setting of KT from donors with HBV. Figure 2 showed a practical approach to the use of kidneys from donors with positive HBsAg.

One important use of NAs was in the setting of treatment with rituximab. This monoclonal antibody acted directly against CD 20 which can lead to impaired immunoglobulin production[67]. There was a study that showed HBV reactivation in kidney transplant recipients with resolving hepatitis B infection[48]. We believe that NA should be prescribed to kidney transplant recipients, who receive kidney allograft from donors with HBV, who have been treated with rituximab either as anti-rejection therapy or induction therapy in ABO-incompatible recipients.

LONG TERM OUTCOMES AND SURVIVAL

Regarding HBsAg (-) anti-HBc (+) donors, historic studies found that there were no

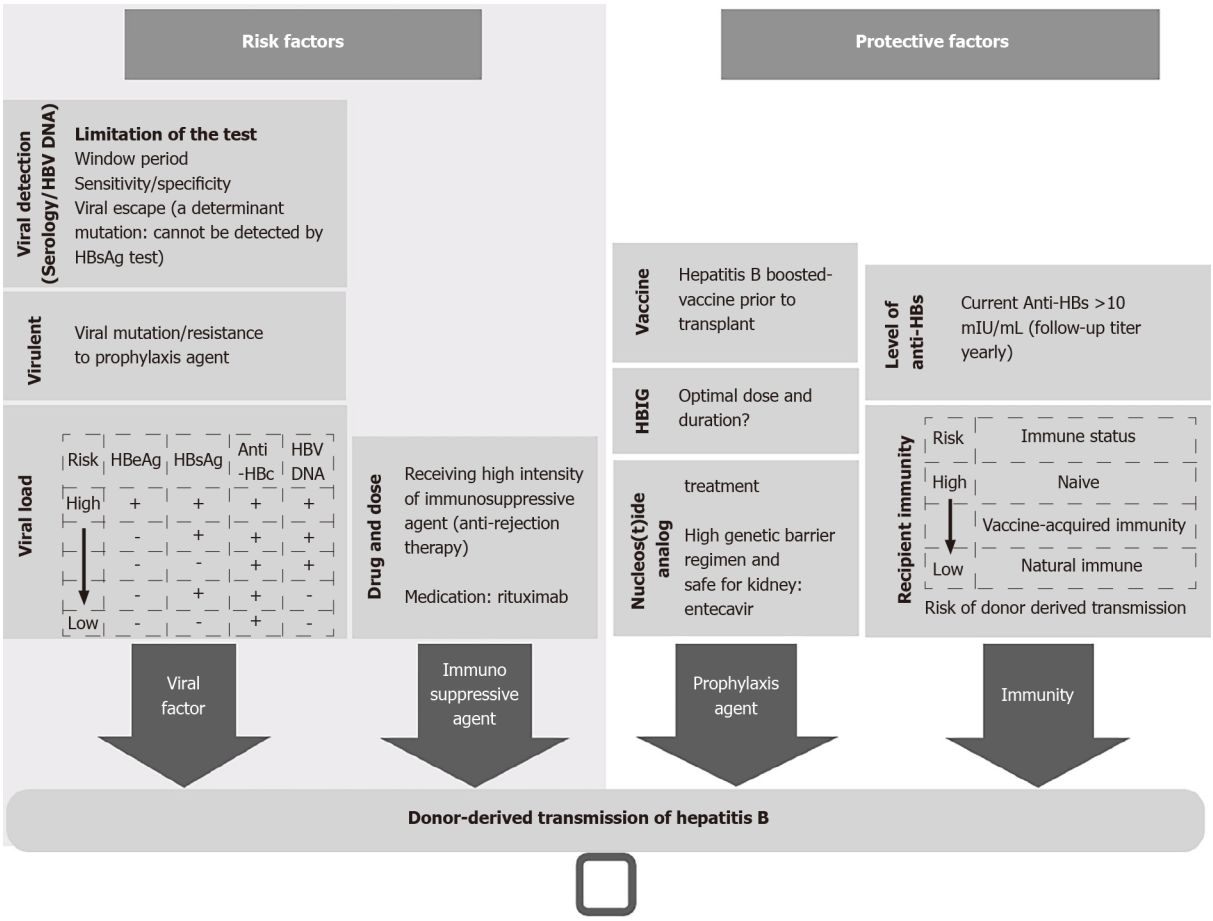

Figure 1 Risk factors of donor derived transmission of hepatitis B virus and proposed protective factors. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; Anti-HBc: Hepatitis B core antibody; Anti-HBs: Hepatitis B surface antibody.

HBsAg seroconversion and no excess risk of morbidity and graft failure[68]. Subsequent studies that examined the outcomes in children have shown a similar result in terms of patient survival and graft survival[69]. A quantitative review of nine studies found the seroconversion rates of HBsAg, anti-HBc, anti-HBs were 4/1385, 32/1385, and 5/1385 recipients. Those numbers were considered to be very low and the authors conclude that HBsAg (-) anti-HBc (+) kidneys can be transplanted safely to patients with ESKD[28].

The amount of information on KT from HBsAg (+) donors is much less than anti-HBc (+) donors. However, the results of long-term outcomes being reported showed favorable outcomes when compared with donors with no markers of HBV with proper prophylaxis regimen[13-15,37]. Our result of HBsAg (+) donor to anti-HBs (> 10 mIU/mL) recipient reported a ten-year actuarial graft survival rate of 84.6% and patient survival rate of 92.8% (with no hepatitis and hepatoma) provided that the recipients received no induction therapy[33].

The previous report of fulminant hepatitis B infection in the setting of HBsAg (+) /HBeAg (+) donor to anti-HBs (-) recipients has been a major concern. However, our review of published articles from 2005 onwards (Supplementary Table 1) has shown there were a total of at least 412 KTs from HBsAg (+) donors to HBsAg (-) recipients. This treatment option was associated with good outcomes. First, in 20 HBsAg (+) donors to anti-HBs (-) recipients, there was 1 death from liver disease, and there were 2 HBV transmissions (2 HBsAg seroconversion). Next, in 392 HBsAg (+) donors to anti-HBs (+) recipients, there were two deaths and four HBV DNAemias. One death occurred in a patient with HBV mutation that escaped from the protective effect of anti-HBs. Another death was associated with liver failure which was reported to be due to nonadherence to lamivudine. There was one HBsAg seroconversion (with HBV DNAemia) associated with lamivudine resistance. The final three HBV DNAemias were reported from a single study. This study reported that the mean anti-HBs of the recipients was 452 ± 384 mIU/ml. However, all of the latter three patients could be successfully treated with lamivudine therapy. No excess risk of liver failure was reported[13,15,70]. It was important to note that most HBV DNAemias being observed

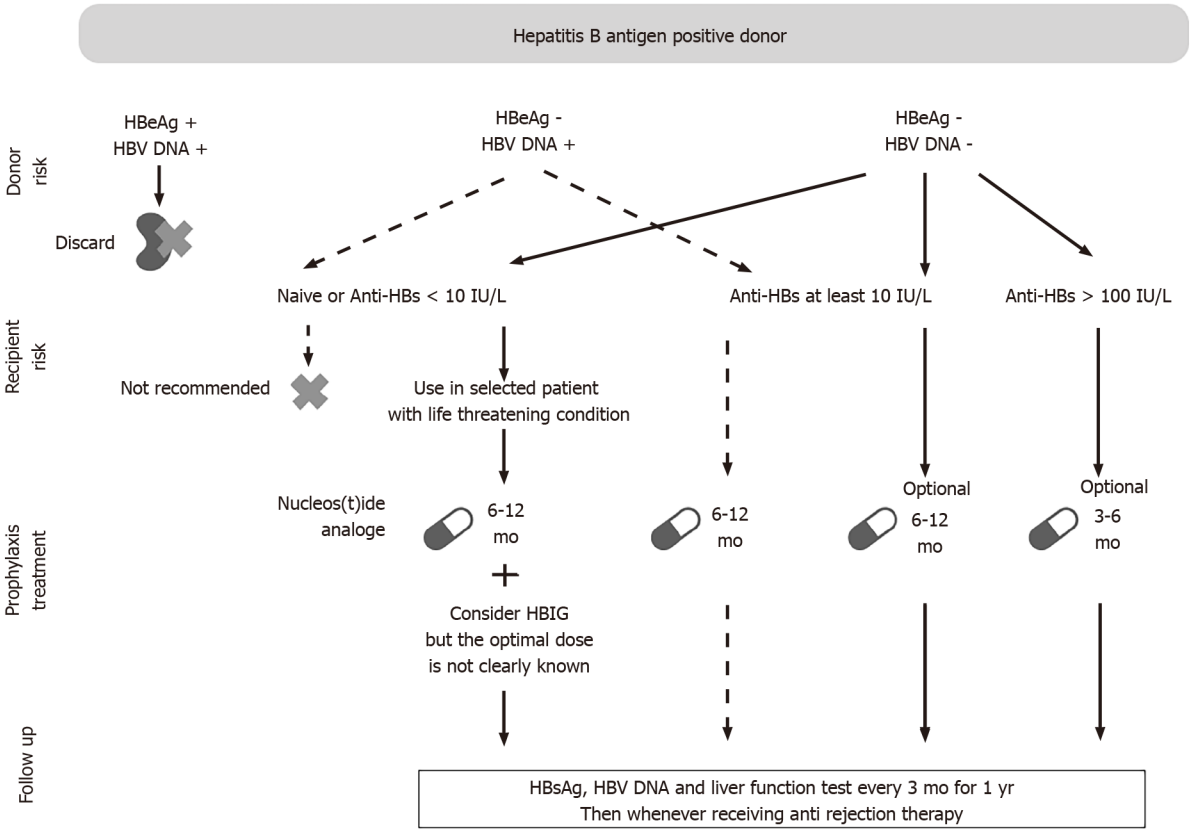

Figure 2 Proposed management for several types of donor and recipient pairs according to the results of hepatitis B virus serology test and DNA markers. HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; Anti-HBs: Hepatitis B surface antibody; HBIG: Hepatitis B immunoglobulin; HBsAg: Hepatitis B surface antigen.

were usually observed with lamivudine resistance or non-adherence. These HBV DNAemias occurred despite the presence of anti-HBs > 100 mIU/ml. These results suggested that kidney transplants from HBsAg positive donors to appropriate recipients was a cost-effective option when compared with keeping the potential recipient in the waiting list pool[71].

RISK BENEFIT OF TRANSPLANTATION AND PROPOSED CRITERIA FOR HBsAg (+) DONOR UTILIZATION

As has been mentioned earlier, organs from HBsAg (+) donors are generally suggested to be discarded[72]. However, with careful individual risk and benefit assessment, these organs may be utilized safely and serve as an alternative treatment to shorten waiting time rather than stay on a usual transplant waiting list. Shortened waiting time was also beneficial in improving 10-year graft survival in both living and deceased donor KTs[73]. Moreover, recipients can benefit from excellent graft survival without excess risk of liver disease as aforementioned[33,35].

It has long been shown and recently confirmed that kidney transplants promoted both longer life expectancy and better quality of life at a lower cost relative to staying on dialysis treatment[74,75]. In order to gain comparable survival benefit to kidney transplant, an intensive home hemodialysis has to be attained which would be a much higher effort than an in-center standard hemodialysis and this option is not feasible in some countries[76]. A recent economic study using data from USRDS showed that kidney transplants using standard donors were a cost saving procedure compared to remaining on dialysis. The same study also showed that kidney transplant using high risk donors were cost-effective[77]. All of the above studies have highlighted the benefit of expanding the donor pool by using kidneys from donors with HBsAg positivity.

Utilized kidneys from HBsAg (+) donors not only direct benefits to the potential recipients, but also the national society. However, the criteria for utilization of kidneys from donors with HBsAg positivity has not been well described. We would like to describe our proposed criteria to define three groups of potential recipients. The first group is patients with urgent need to receive KT. Urgent condition included patients with exhausted vascular access for hemodialysis, patients with ongoing uremia despite adequate dialysis prescription, and patients who cannot remain in the dialysis treatment (hemodialysis or CAPD) due to any reason. The second group is the recipients with positive HBsAg[6,30,46]. The third group is patients being registered as active waiting list who have waiting time longer than the median time to receive a kidney in each national society. The potential recipients should be discussed about the willingness to receive a kidney from donors with HBsAg positivity. They may choose not to take this opportunity and continue to wait for HBsAg negative donors. Examples of the use of kidneys with increased risk of blood borne viral infection has been previously described[78]. A short summary of prophylaxis regimen and special requirements for recipients of kidneys from HBsAg (+) donors is discussed below.

Our rationale for proposal of the third criteria is related to the following information. Data from OPTN (organ procurement and transplantation network) showed that the median time to receive a kidney for a new transplant candidate in waiting list is 4.5 years[77,79]. In US, waiting-listed patients were associated with 5%-7% increase in mortality which continues to increase in older waiting-listed patients. As reported in Matas *et al*[80], there was 2% mortality rate in those aged between 18-34 years which increased to 8% for patients over 65. Utilizing kidneys from HBV infection donors can be one strategy to shorten the recipients' waiting time. This can help to decrease the mortality rate of waiting list candidates and downsize the waiting list pool.

Due to the risk of infection transmission before undergoing KT, recipients should be fully informed and consent must be obtained from each individual. In addition, all potential recipients should be vaccinated that aim to achieve anti-HBs at least > 10 mIU/mL. The potential recipients should not have HCV coinfection nor other cause of chronic liver disease which may worsen after KT. All recipients of HBsAg (+) donors should receive anti-viral medication, especially in the situation when the result of HBV DNA cannot be obtained before actual transplantation. HBIG may be considered for recipients with non-protective anti HBsAb level and/or in the situation of unknown HBV DNAemia of the donor. A protocol for close surveillance of viral reactivation and liver disease must be implemented. For HBsAg (+) recipient candidates, they must be treated with NA and evaluated by a specialist in liver disease. Untreated patients result in a higher mortality rate, with liver-related complications[19]. The AASDL recommends further evaluation of HBV DNA, ALT and to undergo staging with biopsy or elastography to determine whether advanced fibrosis or cirrhosis is present in order to assess the need for simultaneous liver KT[22,72].

It is an ethical challenge to allocate kidneys from donors with positive HBsAg to potential recipients with anti-HBs < 10 IU/ml. In our opinion, this treatment option should be limited to recipients with urgent criteria under a careful management that includes HBIG, antiviral medication and a careful protocol. Wang *et al*[35], demonstrated the possibility of this option (see section: Antiviral medications). However, these transplants should be performed by experienced teams.

CHALLENGING PERSPECTIVE

KT from donors with HBsAg (+) donors is not a risk-free procedure. A careful allocation to appropriate recipients can be successfully performed. NAT for HBV is now accepted to be a useful screening test. The result of a sensitive HBV DNA test is of prime importance in the organ allocation and the design of the prophylactic protocol. The rate of HBV transmission from this treatment option was reported to be low and manageable. HBV reactivation can occur in resolved HBV infection. Thus, a regular monitoring schedule for HBV is an essential part of post-transplant care. Differentiation between donor-derived HBV infection and reactivation of recipient strain HBV infection may be difficult. We believe that the use of kidney organs from donors with HBV infection in the area where the national organ donation rate is less than the rate of endemic HBV infection is a better alternative than discarding the organs.

CONCLUSION

Within this era of several newer antiviral medications, the presence of positive HBsAg in potential organ donors should not preclude the use of kidney organs. Several additional steps and experienced transplant teams are specifically required to prepare waiting list candidates who are willing to receive a kidney from such donors. These steps should be regularly assessed for each individual during his or her registration as active waiting list to receive KT from deceased donors. However, the criteria that we have described in this review, can also be applied to patients who are planning to receive living (related) KT as well.

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MINIREVIEWS

Unpacking the challenge of gastric varices: A review on indication, timing and modality of therapy

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Abstract

Upper gastrointestinal bleeding from oesophageal or gastric varices is an important medical condition in patients with portal hypertension. Despite the emergence of a number of novel endoscopic and radiologic therapies for oesophagogastric varices, controversy exists regarding the indication, timing and modality of therapy. The aim of this review is to provide a concise and practical evidence-based overview of these issues.

Key Words: Upper gastrointestinal bleeding; Portal hypertension; Gastric varices; Variceal band ligation; Variceal obliteration; Sclerotherapy; Transjugular intrahepatic porto-systemic shunt; Balloon-occlusion retrograde transvenous obliteration

Core Tip: Gastric varices are an uncommon source of bleeding in patients with portal hypertension. Although evidence supports acute bleeding treatment and secondary prophylaxis using interventional endoscopy or radiology, there is still lack of data to support primary prophylaxis for all patients. If treatment is required, both interventional endoscopy and radiological approaches should be considered. Interventional endoscopy using endoscopic ultrasound-guided combination coil and cyanoacrylate obliteration appears to be the optimal approach based on the current literature.

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INTRODUCTION

Incidence

The incidence of gastric varices (GV) is 15%-20% from endoscopic epidemiological studies of portal hypertension[1,2]. Unlike oesophageal varices that tend to be present in the lamina propria mucosae and superficial submucosa, GV lie deep in the submucosa and as such can be difficult to differentiate from prominent gastric rugae with standard endoscopy. Endoscopic ultrasonography studies have demonstrated that a proportion of GV are undiagnosed on standard diagnostic endoscopy[3,4]. However, this may not be clinically significant, as the size of the varix is one of the characteristics that predict risk of haemorrhage, and larger GV are less likely to remain undetected by standard endoscopy.

Both GV and oesophageal varices develop as a consequence of portal hypertension. Portal hypertension may lead to reversal of flow through the portal circulation, with two common outlets - *via* the coronary (gastric) vein to the right and left gastric veins, and *via* the splenic vein to the short and posterior gastric veins[5]. The former supply the distal oesophagus and cardia of the stomach where transmitted pressures and increased flow lead to formation of oesophageal and cardio-oesophageal varices. The latter supply the fundus whereby increased pressures and flow through this system leads to the formation of fundal varices. In a haemodynamic study of oesophageal and and GV by Watanabe *et al*[5], 78% of patients with portal hypertension had the majority of collateral flow through the left and right gastric veins, likely accounting for the difference in incidence between oesophageal and GV.

In the same study, GV were demonstrated to bleed at lower portal pressures than oesophageal varices, largely due to the higher prevalence of gastro-renal shunts in those with GV. These shunts decompress the portal system. This finding has since been confirmed in further studies[6,7].

GV CLASSIFICATION

Sarin and Kumar[8] seminal paper on the anatomical classification of GV from 1989 remains the most widely accepted method for describing GV. They are divided into two groups, with further sub-classification (Figure 1): (1) Gastroesophageal varices (GOV) – are continuation of oesophageal varices that extend beyond the gastroesophageal junction. These are divided into: (a) Type 1 (GOV1) – those that extend along the lesser curve of the stomach. These account for 75% of all GV[2]; (b) Type 2 (GOV2) – those that extend along the greater curve of the stomach into the fundus; (2) Isolated GV (IGV) – occur in the absence of oesophageal varices and are sub-classified into: (a) Type 1 (IGV1) – located in the fundus and do not extend to the cardia. They are also called fundal varices; (b) Type 2 (IGV2) – can occur anywhere in the stomach (*i.e.*, body, antrum, pylorus). These are rare, occurring in < 5% of those with GV.

The use of this classification system has been shown to predict risk of bleeding and guides management. GOV1 varices behave similarly to oesophageal varices, and so the treatment paradigm for prophylaxis and acute variceal haemorrhage is the same for oesophageal varices. IGV1 and GOV2 varices are more difficult to control when they bleed compared with GOV1 varices and portend a poorer prognosis[2].

An alternate classification, published by Hashizume *et al*[9], is a more detailed examination of GV describing the form (tortuous, nodular or tumorous), location (anterior, posterior, lesser curve or greater curve of cardia, or fundic), and colour (red or white) of the varix (Figure 2). Similar to classification systems for oesophageal varices, this classification is aimed at stratifying patients at highest risk of bleeding. In a stepwise logistic regression analysis, those with varices in the anterior or greater curve of the cardia, nodular appearance (*i.e.*, larger size), or red colour spot had the highest predicted risk for bleeding[9]. In another study, which focused on patients with fundic varices, increased size and presence of a red spot increased risk of

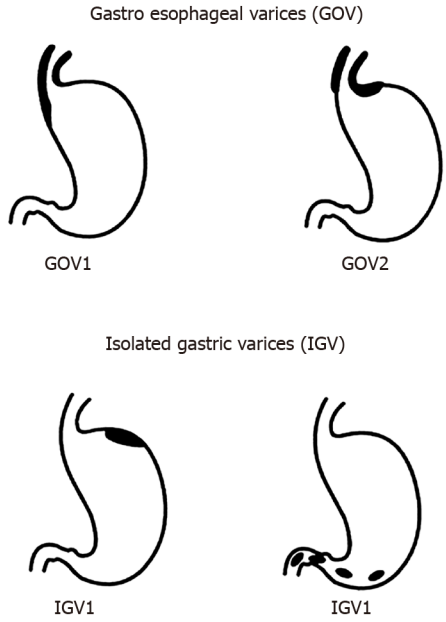

Figure 1 Classification of gastric varices according to Sarin *et al*[2]. Citation: Sarin SK, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: A long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; 16: 1343-1349. Copyright © The Authors 1992. Published by John Wiley and Sons.

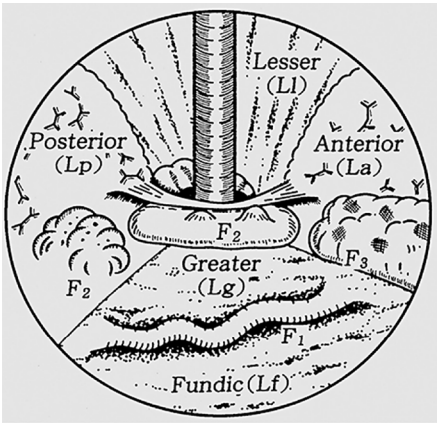

Figure 2 Classification of gastric varices according to Hashizume *et al*[9]. Citation: Hashizume M, Kitano S, Yamaga H, Koyanagi N, Sugimachi K. Endoscopic classification of gastric varices. *Gastrointestinal Endoscopy* 1990; 36: 276-280. Copyright © The Authors 1990. Published by Elsevier.

haemorrhage[10]. Advanced liver disease, as determined by the Child-Turcotte-Pugh classification, was an additional risk factor for bleeding[10]. Other classification systems exist[11], however are used less frequently than those described by Sarin and Kumar[8], and Hashizume *et al*[9].

ACUTE GASTRIC VARICEAL HAEMORRHAGE

Bleeding from GV is considered definite if there is active spurting or oozing from the varix or an adherent clot or fibrin plug on the varix. GV bleeding should be considered the cause of upper gastrointestinal bleeding when a GV without high-risk stigmata is present in the absence of oesophageal varices or an alternate source of bleeding[12].

Pre-endoscopic management

Pre-endoscopic management follows that for oesophageal variceal bleeding, namely use of splanchnic vasoconstrictors (*i.e.*, terlipressin or octreotide) to reduce portal pressures, prophylactic antibiotics (*e.g.*, ceftriaxone), and a restrictive transfusion protocol[13,14]. The same medical treatment is instituted in patients with a presumed

diagnosis of variceal haemorrhage with known portal hypertension who present with symptoms of upper gastrointestinal bleeding.

Endoscopic management

As outlined above, GOV1 varices are considered an extension of oesophageal varices and so at the time of haemorrhage should be treated in the same manner as bleeding oesophageal varices [*i.e.*, endoscopic variceal band ligation (EVBL), Figure 3][2,12-14]. One small prospective randomized controlled trial (RCT) reported numerically higher haemostasis and lower re-bleeding rates in the subset of patients with GOV1 haemorrhage treated with endoscopic variceal obturation (EVO) rather than EVBL [15], although this did not reach statistical significance. Both treatments may be considered equally efficacious for GOV1 varices.

Current guidelines support the use of cyanoacrylate injection – either as *N*-butyl-2-cyanoacrylate (*e.g.*, Histoacryl®) or 2-octyl-cyanoacrylate (*e.g.*, Dermabond) – for acutely bleeding fundic varices (GOV2 and IGV1), in a procedure termed EVO[13,14]. Cyanoacrylate is a tissue adhesive that rapidly polymerizes upon contact with water/blood, leading to a change in the liquid composition to one of a hard brittle acrylic plastic. Majority evidence for its use stems from uncontrolled retrospective and prospective studies[16], with one RCT demonstrating a statistically non-significant increased haemostasis rate when compared to alcohol-based sclerotherapy (89% *vs* 62%)[17]. Haemostasis rates for cyanoacrylate glue injection are 80%-100%, with re-bleeding rates of 10%-60%[16]. Complications are rare, with fever and pain being the most common, while the most feared is embolization of the glue into systemic beds that can lead to ischaemia in those tissues (*e.g.*, stroke, myocardial infarction, splenic infarction, pulmonary embolus).

Thrombin injection has been utilized as an alternative to cyanoacrylate for EVO for almost three decades; however, like cyanoacrylate, evidence for its use is taken from small, uncontrolled studies[16]. It appears safe, with few adverse procedure-related outcomes, and haemostasis and re-bleeding rates similar to cyanoacrylate.

Sclerotherapy, the injection of a sclerosant agent into the varix, has gone out of favour for the treatment of gastric variceal haemorrhage due to unacceptably high re-bleeding rates of up to 90%-100%[16]. This is often due to ulceration at the point of injection resulting from the high volume of sclerosant required to obliterate GV, with a large amount of sclerosant flowing away from the variceal bed *via* co-existent gastro-renal shunts that occur with high prevalence in patients with GV[16]. Adverse events include fever, and retrosternal and abdominal pain.

IGV-2 varices are rare; hence little evidence exists as to the optimal endoscopic management. In general, it is accepted that they should be treated according to GOV2/IGV1 varices with EVO.

Salvage therapy

Balloon tamponade with Sengstaken-Blakemore, Minnesota or Linton-Nachlas tubes can be utilized in patients with bleeding from GOV1, GOV2 and IGV1 varices. They will not be effective for IGV2 varices, given the ectopic location of the culprit lesion. The Linton-Nachlas tube may be preferred in gastric variceal haemorrhage if available, as the gastric balloon has greater volume capacity[12].

Transjugular intrahepatic portosystemic shunt (TIPS) (Figure 4) is an effective salvage therapy for patients with endoscopically-uncontrollable bleeding oesophageal varices, however its utility in refractory gastric variceal haemorrhage is less clear[12]. Although haemostasis rates exceed 90%, re-bleeding is reported to occur in 15%-30% [18-20] and concerns remain over post-TIPS encephalopathy, which can be recalcitrant to standard medical therapy and necessitate revision of the TIPS. Several hypotheses have been proposed to explain the risk of re-bleeding[21,22]; 'Proximity' theory – feeding vessels to GV lie further away from a TIPS shunt than feeding vessels to oesophageal varices, hence the shunt is less effective in decompressing GV; 'Throughput' theory – gastro-renal shunts that occur in high frequency in association with bleeding GV compete with the TIPS for portal flow and can continue to feed the gastric variceal bed; 'Recruitment' theory – development of new feeder vessels after proximal embolization of a GV.

In retrospective comparison studies, Mahadeva *et al*[23] found TIPS to be more effective in preventing re-bleeding when compared with EVO in acute gastric variceal haemorrhage, whilst Procaccini *et al*[24] found no difference between the two modalities.

Balloon-occlusion retrograde transvenous obliteration (BRTO) and its modifications (coil-assisted or plug-assisted retrograde transvenous obliteration) aim to sclerose a varix without treating portal hypertension. BRTO is often reserved for use in patients

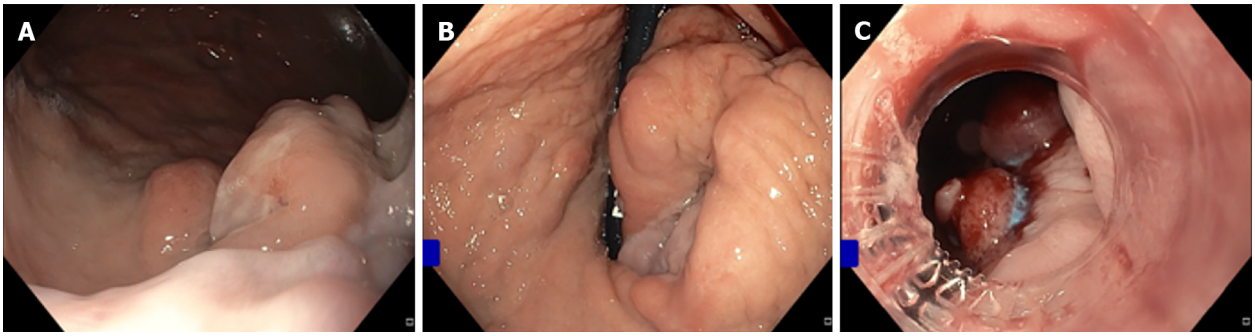

Figure 3 Endoscopic variceal banding of gastroesophageal varices 1. A: Pre-banding forward view of varix; B: Pre-banding retroflexed view of varix; C: Immediately post-banding of varix.

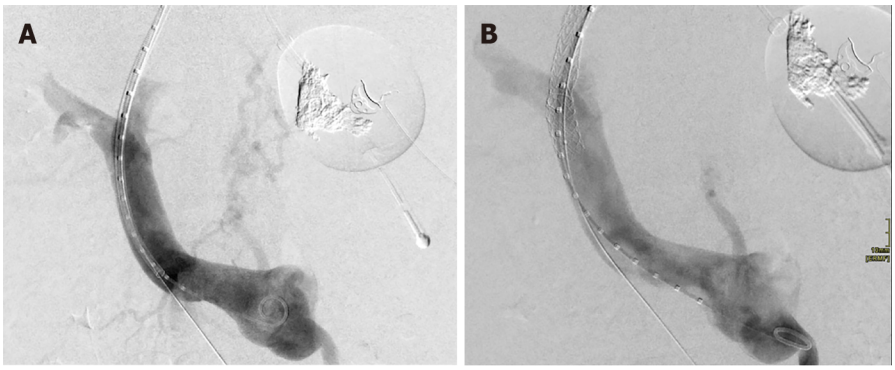

Figure 4 Transjugular intrahepatic portosystemic shunt decompressing gastric varix. A: Pre-transjugular intrahepatic portosystemic shunt (TIPS) with contrast toward gastric variceal bed; B: Post-TIPS with no contrast flow toward gastric variceal bed.

with anatomy not amenable for TIPS or where TIPS is contraindicated (*i.e.*, past history of hepatic encephalopathy or advanced synthetic liver dysfunction), and is reliant on the presence of a gastro-renal shunt for technical feasibility. Similar to TIPS, it is highly effective in achieving haemostasis with success rates > 90%[21,25]. A meta-analysis of uncontrolled studies reported a clinical success rate of 97%, defined as no GV recurrence or re-bleed of acutely bleeding GV or no bleed in the case of at-risk GV that have never-bled[26]. The main sclerosant used for BRTO in reported studies was ethalonamine oleate, in 94% of cases, and the most common side effect was haematuria, occurring in 70%[26]. Given ethalonamine oleate is a known cause of haemolysis, the common occurrence of haematuria is somewhat expected from consequent haemoglobinuria. The antidote for this is parenteral administration of haptoglobin. Although this review by Park *et al*[26] included patients who underwent BRTO for acute treatment of variceal haemorrhage, primary prophylaxis and secondary prophylaxis, a breakdown of indication was not provided and subgroup analysis for this purpose not available. Another, more recently published meta-analysis[27] found that there was no significant difference in immediate haemostasis rates between the two procedures, but a higher re-bleeding rate post-TIPS [relative risk (RR) 2.61, 95% confidence interval (CI) 1.75–3.90, $P < 0.01$], higher post-procedural hepatic encephalopathy rate post-TIPS (RR 16.11, 95%CI: 7.13–36.37, $P < 0.01$), statistically non-significant higher rate of ascites in the BRTO group and statistically non-significant worsening in Child-Pugh status in those who received TIPS. Apart from a small pilot study out of Seoul, Korea[28] that randomly assigned 14 patients with acutely bleeding GV to up-front TIPS or BRTO, there have not been any head-to-head RCTs to ascertain the difference in safety and efficacy between these procedures in patients with acutely bleeding GV. A disadvantage of BRTO is that it can lead to the development or worsening of non-GV (oesophageal or ectopic) as portal blood flow is diverted through alternate pathways, or exacerbation or new development of ascites due to raised portal pressures. This is not an issue post-TIPS, which effectively decompresses the portal system.

SECONDARY PROPHYLAXIS

Non-selective beta-blockers

No controlled study has demonstrated efficacy of non-selective beta-blockers (NSBB) for secondary prophylaxis following gastric variceal bleeding. In a RCT by Mishra *et al* [29], EVO was far more effective in preventing re-bleeding than propranolol, with a relative risk reduction of 80% and absolute risk reduction of 35%. Hung *et al*[30] and Chen *et al*[31] explored the adjunct use of propranolol or carvedilol, respectively, to 3-4 weekly EVO alone following gastric variceal haemorrhage in an RCT setting, albeit with no placebo arm. Neither found a difference in gastric variceal re-bleeding rates between the two groups. Of note is that both studies were conducted in the same institution with similar inclusion and exclusion criteria, except the more recent study by Chen *et al*[31] included all patients with any form of gastric variceal bleeding, whilst Hung *et al*[30] only included patients with fundic variceal haemorrhage (GOV2 or IGV1). The study by Chen *et al*[31] did demonstrate a significant reduction in all-cause upper gastrointestinal re-bleeding in the group assigned to carvedilol (28% *vs* 48%, $P = 0.03$), driven by a reduction in bleeding from portal hypertensive gastropathy, and oesophageal and gastric ulcers. However, the authors also reported on a higher incidence of adverse events in the carvedilol group (53% *vs* 15%, $P < 0.001$), due to more frequent dizziness and exertional dyspnoea. Despite this, no patient in the carvedilol group discontinued therapy. The lack of efficacy with NSBB following gastric variceal haemorrhage is postulated to be due to the fact that GVs bleed at lower portal pressures, with NSBB having little effect on preventing flow of blood to the culprit variceal bed *via* co-existent gastro-renal shunt[30,31].

Endoscopic therapy

Although endoscopic ultrasound (EUS) guided injection of cyanoacrylate reduces embolic complication rates, as a result of reduced volume of cyanoacrylate injected, its use during acute gastric variceal haemorrhage is limited in most centres due to access to endoscopists with expertise in EUS. However, it is an emerging therapy for secondary prophylaxis. A two-part observational comparative study by Lee *et al*[32] compared fortnightly EUS-guided injection of cyanoacrylate in patients presenting with acute bleeding from any type of GV with "on demand" therapy, whereby standard endoscopy and injection of cyanoacrylate was only undertaken at the time of re-bleeding. A significant reduction in re-bleeding was demonstrated in the active endoscopic treatment group (35% *vs* 70%, $P = 0.0006$). There was no impact on mortality, likely due to the small number of patients in the study. In a similar cohort trial design, Bick *et al*[33] found that there was a lower gastric variceal and all-cause upper gastrointestinal re-bleeding rate (9% *vs* 24%, $P = 0.045$ and 19% *vs* 50%, $P < 0.001$, respectively) in those managed with EUS-guided cyanoacrylate injection compared with standard endoscopy guided injection. It is important to note that the standard endoscopy cohort had a higher mean MELD (17 *vs* 13, $P = 0.004$) and lower incidence of IGV1 varices (8% *vs* 47%), with the latter likely accounted for by the greater sensitive of EUS for the detection of GV.

A novel endoscopic method that is gaining popularity globally is EUS-guided coiling of GV, which involves injection of metal embolization micro-coils coated with synthetic stainless steel-fibres, leading to turbulent blood flow and intravariceal clot formation to obliterate the varix[34]. This can be combined with injection of cyanoacrylate glue (Figure 5), in a procedure that aims to prevent systemic embolization of the cyanoacrylate, as the coils may provide a scaffold for polymerization, as well as requiring less volume of glue injection as a result of precise delivery into the target tributary[35]. The additional benefit of EUS over standard endoscopy is the ability for immediate post-treatment Doppler evaluation of the variceal bed and its afferent tributaries, to ensure complete obliteration[34,35]. In a retrospective, multicentre study by Romero-Castro *et al*[36] comparing outcomes of 30 patients who underwent EUS-guided coil ($n = 11$) with those who underwent EUS-guided cyanoacrylate injection ($n = 19$) into GVs that had previously bled ($n = 23$) or never bled ($n = 7$), both methods were highly effective in obliterating the varices (96.7% cumulatively) without a difference in re-bleeding rate. The cyanoacrylate group required more sessions to achieve obliteration (29 sessions *vs* 14 sessions, $P = 0.29$) and had lesser proportion of patients achieving variceal obliteration after a single endoscopic session (18% *vs* 82%), whilst also having a higher reported adverse event rate (58% *vs* 9%, $P < 0.01$). However, the majority of adverse events were asymptomatic pulmonary emboli detected on routine computed tomography (CT) of the chest of patients post-procedure, with no difference noted in the symptomatic adverse event rate between

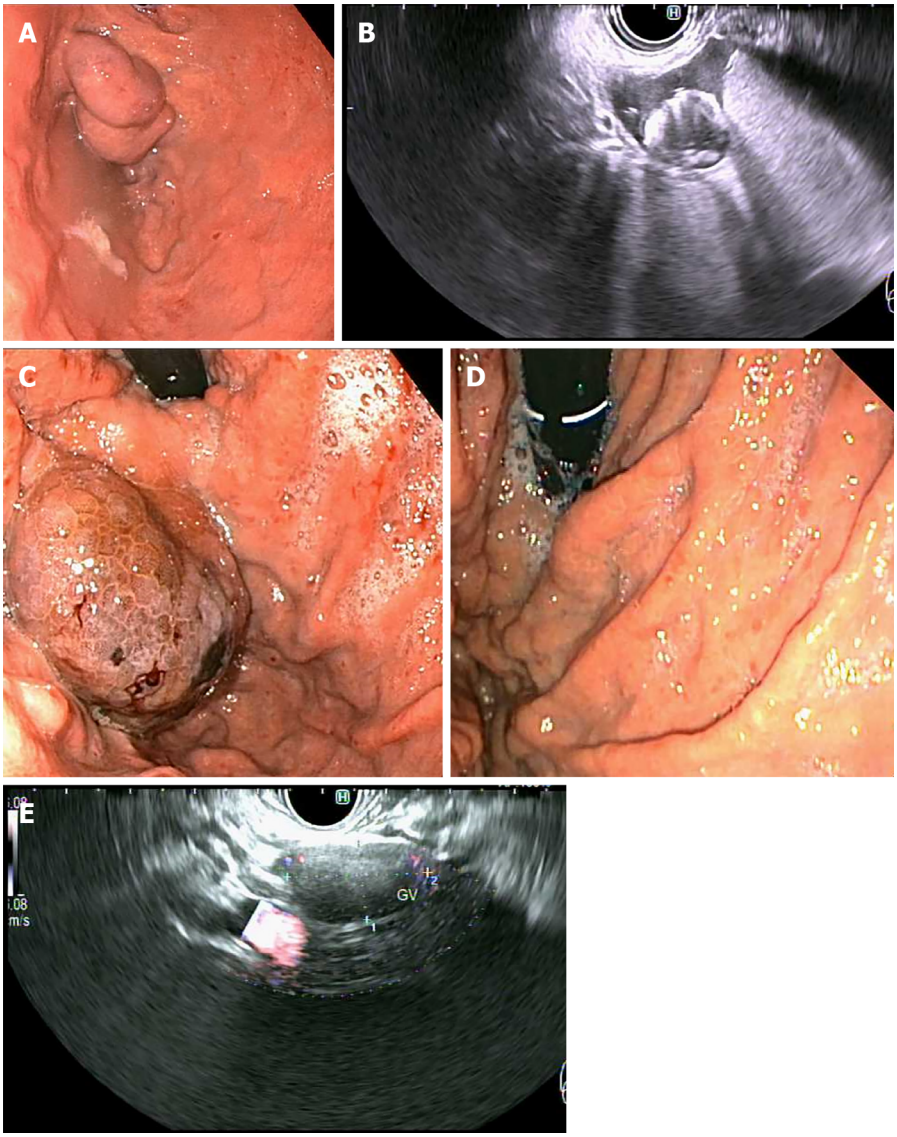

Figure 5 Primary prophylaxis of isolated gastric varices 1 with five 0.18 10 mm micronester coils in combination 1 mL cyanoacrylate glue. A: Pre-treatment endoscopic appearance; B: Endoscopic ultrasound (EUS) appearance immediately post-deployment of coils; C: Endoscopic appearance 1-mo post-treatment; D: Complete obliteration 4-mo post-treatment; E: EUS appearance of varix with no Doppler flow 4-mo post-treatment.

groups. It is also noteworthy that a statistically significant higher proportion of patients with bleeding varices and Child-Pugh C status cirrhosis were represented within the cyanoacrylate group in this study.

Binmoeller *et al*[37] were the first to publish on the efficacy and safety of combined EUS-guided coil and cyanoacrylate injection for GV, predominantly in patients who had recovered from an acute gastric variceal haemorrhage (n = 28/30). The same group reported on a more extensive patient cohort (n = 152) some years later, with high obliteration rate (93%) at follow-up endoscopy, low re-bleeding rate (16%, with 50% re-bleeding events non-variceal in origin), and few procedure-related adverse events (7%; 4/9 patients with abdominal pain, 1/9 patients with pulmonary embolus) [38]. Of note, 26% of patients in this study underwent treatment as primary prophylaxis, somewhat unique in the GV treatment evidence base. A single randomized trial has been performed evaluating combination coiling and cyanoacrylate injection with coiling alone, reporting a higher variceal disappearance rate on immediate post-procedure endoscopy (87% *vs* 13%, P < 0.001), and lower re-bleeding rate (3.3% *vs* 20%, P = 0.04), variceal reappearance rate on follow-up endoscopy at 3-mo (13% *vs* 47%, P < 0.001), and re-intervention rate (17% *vs* 40%, P = 0.045) in the arm allocated to combination therapy[39]. The cumulative mortality rate of 28% from this study despite relatively preserved liver function in participants (90% Child Pugh A, median MELD 9.5 at enrolment) is of concern and somewhat unexplained, particularly given 10/17 patients died from uncontrolled haemorrhage and 9/10 of these were variceal in

nature.

Interventional radiology

Only a single RCT has evaluated EVO with up-front TIPS for secondary prophylaxis of gastric variceal haemorrhage[40], and revealed a 71% relative risk reduction over 3 years in gastric variceal re-bleeding rate in the TIPS arm, albeit with 26% of TIPS patients suffering from hepatic encephalopathy. This study pre-dates the era of EUS-guided coils and very few patients in this study had IGV1 varices. TIPS may be an attractive option in patients with concurrent ascites and/or presence of other non-GV, but less so in those with a history of prior encephalopathy or advanced synthetic liver dysfunction.

A single prospective, non-randomized study[41] and two retrospective, observational studies[42,43] have demonstrated a lower re-bleeding rate in patients treated with BRTO rather than EVO for secondary prophylaxis of variceal haemorrhage (3%-15% *vs* 22%-71%). They each had differing inclusion criteria, with the prospective study including patients with GOV1, GOV2 and IGV1 varices, whilst the two retrospective studies both excluded patients with GOV1 varices, and one only included patients with IGV1 varices[42].

Contemporary case series have begun exploring the feasibility and safety of combined interventional radiological procedures, namely TIPS with balloon-occluded transvenous obliteration (whether in an antegrade or retrograde fashion)[44-46]. Purported benefits from retrospective audits of combined procedures are reduced re-bleeding and post-procedure encephalopathy rates, stable or improved liver function, and prevention or improvement of ascites. Finally, percutaneous transhepatic obliteration is an alternate route to obliteration of a gastric varix in those without a gastro-renal shunt and who may have contraindication to TIPS[47].

Given the wide array of therapeutic options available are reliant on specific anatomical features, such as feeding vessels into the variceal bed or presence of a gastro-renal shunt, appropriate imaging of the portomesenteric circulation with CT should be attained in patients with GV to allow the most anatomically suitable intervention to be chosen.

PRIMARY PROPHYLAXIS

Whilst there is a modest evidence-base for secondary prophylactic measures for bleeding GV, there is a paucity of data examining the role of primary prophylaxis. Few trials have recruited patients with the intention to treat GV prior to bleeding, and those that have[36,38,39] have done so in low numbers which prevents any meaningful subgroup analysis.

One RCT by Mishra *et al*[48] randomized patients with never-bled GOV2 or IGV1 ≥ 10 mm in size to cyanoacrylate injection, propranolol or no therapy in a 1:1:1 ratio. This demonstrated a significant reduction in GV bleeding in those treated with cyanoacrylate compared to those with propranolol or no therapy (10% *vs* 38% *vs* 53%, $P = 0.003$), as well as a significant reduction in bleed-related mortality between those receiving endoscopic therapy and those who received no specific therapy (0% *vs* 20%, $P = 0.025$). There was no statistical difference in overall mortality between the groups (7% *vs* 17% *vs* 26%, $P = 0.113$), nor therapy-related complications (3% *vs* 3% *vs* 7%, $P = 1.0$). This suggests endoscopic cyanoacrylate therapy could be recommended in patients with GV larger than 10mm in size, and NSBB therapy considered in those with contraindication to, or declining, cyanoacrylate injection.

In subgroup analysis of another study by Bhat *et al*[38], 93% of patients undergoing combined EUS-guided coiling with cyanoacrylate for primary prophylaxis had no GV bleeding over a mean follow-up time of 449 d.

To date, there are no head-to-head trials comparing endoscopic therapy with radiologic interventions for primary prophylaxis, nor any specific trials to compare various endoscopic therapies (coil *vs* cyanoacrylate or combination therapy *vs* monotherapy).

CONCLUSION

Future directions

The majority of evidence for the treatment of GV stems from retrospective studies, and

so there is a need for further prospective and randomized trials to better guide management. In particular, there is a paucity of data on primary prophylaxis of GV, the risk of treating small (< 10 mm) or low-risk GV, and on the optimal approach to secondary prophylaxis (endoscopic, radiologic or combined) since the advent of EUS-based combination therapy. Furthermore, little is known regarding the ideal timeframe for surveillance of GV, whether treated or untreated.

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MINIREVIEWS

Pathogenesis of autoimmune hepatitis

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Abstract

Autoimmune hepatitis (AIH) is a chronic progressive liver disease whose etiology and pathogenesis are not yet clear. It is currently believed that the occurrence of AIH is closely related to genetic susceptibility and immune abnormalities, and other factors such as environment, viral infection and drugs that may cause immune dysfunction. This article reviews the pathogenesis of AIH and describes the latest research results in the past 5 years.

Key Words: Autoimmune hepatitis; Genetic susceptibility; environmental factors; Immunomodulation; Drug-induced liver injury; Intestinal microbes

Core Tip: Autoimmune hepatitis (AIH) has no specific clinical manifestations. AIH patients often require steroid hormones plus immunosuppressive maintenance therapy. Long-term medication may cause various adverse reactions, complications, and relapse after drug withdrawal, which imposes a heavy burden on patient's health and quality of life. Although the exact pathogenesis of AIH is still unclear, there are multiple theories, and continuous in-depth research on its pathogenesis has led to development in treatment of AIH. Genetic susceptibility, environmental factors (viruses, parasites, pets, *etc.*), immune system, drugs and biological agents, pregnancy and liver transplantation have been reported to be associated with AIH.

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INTRODUCTION

Autoimmune hepatitis (AIH) is more common in female patients. There are no specific clinical symptoms in the early stage. Serology mainly manifests as hypergammaglobulinemia and multiple autoantibodies. Histologically, a large number of plasma cells infiltrate the portal area and involve the surrounding liver parenchyma to form interface hepatitis. AIH was first proposed in 1950. Because of the similar clinical manifestations and autoantibodies between this disease and systemic lupus erythematosus, it was originally called lupus-like hepatitis. After 10 years, it was discovered that this disease had obvious differences in clinical manifestations and autoantibodies from systemic lupus erythematosus, and autoimmune liver disease and autoimmune chronic active hepatitis are collectively referred to as AIH[1]. AIH has a global distribution and can occur in men or women of any age and race. The age of onset is bimodal. The peak onset is in adolescence and middle age, especially menopausal women. At present, the clinical treatment of AIH is unsatisfactory, and with the increase of morbidity, it imposes a heavy burden on health and quality of life. Although the exact pathogenesis of AIH is still unclear, there are many theories, and the continuous in-depth research on its pathogenesis has led to development in treatment of AIH. This article summarizes recent progress of research into the pathogenesis of AIH.

GENETIC PREDISPOSITION

AIH is a polygenic disease. HLA class II DRB1 alleles are associated with AIH in different populations (Table 1). HLA-DRB1*13:01 and *03:01 alleles are related to AIH type I. In South America, AIH is mainly related to HLA-DRB1*1301 alleles, while HLA-DRB1*0301-negative type I AIH is mostly related to HLA-DRB1*0401[1,2], and in Japan it is related to HLA-DRB1*0405, *0401, *0802 and *0803. It may be that the amino acid sequence in the binding region of HLA-II molecules of different races differs slightly[3]. The high frequency of HLA-DRB3*0101 and HLA- DQB1*0201 haploid is also related to type I AIH. In South America, HLA-DQ2 is a risk factor for AIH, and HLA-DR5 and DQ3 are protective factors for this population[4]. HLA-DRB1 *0405, HLA-DRB1*1301, HLA-DQB1*02 and HLA-DQB1*0603 are the main risk factors for the onset of AIH, while HLA-DRB1*1302 and DQB1*0301 are protective factors. These HLA molecules have P1, P4, and P6 pockets. The physicochemical acquaintances and differences of the key amino acids encoded by the peptide-binding grooves illustrate their influence on the development of disease. In Europe and Japan, HLA-DRB1*1501 is also a protective factor[3]. HLA-DRB1*0701, *0301, and *0201 alleles are associated with AIH type II. Patients with HLA-DRB1*0701 have rapid disease progression and poor prognosis. The genetic susceptibility and severity of disease in British and Brazilian type II AIH patients are related to HLA-DRB1*0301 alleles[1-3]. Gene mutations other than HLA are also related to AIH susceptibility or progression: Fas-670a/g and Fas-1377g/a polymorphisms[5], VDR[6], and GATA-2[7] are closely related to the onset of AIH. The high-affinity combination of y1 and -1993 c alleles inhibits expression of tbx21, which may inhibit the occurrence of AIH I by inhibiting the type 1 immune response[8]. The haplotypes of the rs7582694-c and rs7574865-t alleles in the stat4 allele are related to the increased risk of AIH I, while the rs2476601 in the ptpn22 allele is related to reduced risk of AIH I[9]. The CTLA-4 molecule is a key regulator of lymphocyte response, and ctla4a/a is a protective genotype of Tunisian patients, and the Ctla4 gene +49 polymorphism is related to AIH susceptibility. Ctla4 gene mutations may lead to changes in the structure of CTLA-4 protein, leading to onset of AIH[10]. a20 encoded by Tnfaip3 is an inhibitor of the nuclear factor (NF)-kB signaling pathway and a susceptibility gene for autoimmune diseases. The harmful mutations of tnfaip3 and drb1 alleles may be independently related to type I AIH, and are related to AIH and liver cirrhosis in Japan[1]. GATA2 encodes a transcription factor for hematopoietic cells, and mutations may be manifested as a reduction in monocytes, lack of dendritic cells and B cells, bone

Table 1 Susceptibility genes of autoimmune hepatitis

Type of AIH	Susceptibility genes or alleles (protective alleles are in bold)	Country	Ref.
AIH I	DRB1*03:01, DRB1*04:01, **DRB1*15:01**	European, North American	Higuchi *et al*[1], 2021
			Higuchi *et al*[2], 2019
	DRB1*04:01, DRB1*04:05, **DRB1*13:02**, **DRB1*15:01**, DRB1*0802, DRB1*0803	Japanese	Higuchi *et al*[1], 2021
			Higuchi *et al*[2], 2019
	DRB1*0404, DRB1*0405, DRB1*1301, **DRB1*1302**	Latin American	Duarte-Rey *et al*[4], 2009
	DQB1*02, DQB1*0603, **DQB1*0301**, **DR5**, **DQ3**, DQ2	Latin American	Duarte-Rey *et al*[4], 2009
	Fas-670a/g	New Zealand, China, United States, Japan	Yan *et al*[5], 2020
	GATA-2	European, Caucasian ancestry	Webb *et al*[7], 2016
	TBX21-1993C	China	Sun *et al*[8], 2017
	STAT4 (rs7582694-c, rs7574865-t), **Ptpn22-rs2476601**	China, Japan	Li *et al*[9], 2017
	CTLA4	European, Japanese	Chaouali *et al*[10], 2018
	SH2B3, VDR, FAS-1377g/a, TNFAIP3	Japanese	Ngu *et al*[3], 2017
			Yan *et al*[5], 2020
			Kempinska-Podhorodecka *et al*[6], 2020
			McReynolds *et al*[11], 2018
	SH2B3, CARD10	Netherlands	Motawi *et al*[13], 2019
	MIF-173gc	United States, Japan	Alsayed *et al*[14], 2020
AIH II	DRB1*0701, DRB1*0201	European	Ngu *et al*[3], 2017
			Duarte-Rey *et al*[4], 2009
	DRB1*0301	British and Brazilian	Ngu *et al*[3], 2017
	DQB1*0201	Latin American	Duarte-Rey *et al*[4], 2009

AIH: Autoimmune hepatitis.

marrow dysplasia and immunodeficiency, which are related to the pathogenesis of AIH[7,11]. HLA-DRB15 is significantly correlated with increased levels of interleukin (IL)-8. IL-6, IL-8 and tumor necrosis factor (TNF)-α may be biomarkers of AIH activity. *HLA* gene expression may play a role in the production of cytokines, and enable earlier diagnosis and better treatment[12]. Recent studies have reported that AIH I in Dutch adults is associated with mutations in the MHC region, and identified sh2b3 and card10 mutations as possible risk factors. These findings support the complex genetic basis of AIH pathogenesis and indicate partial inheritance. Susceptibility overlaps with other immune-mediated liver diseases. However, in the Japanese population, there is no connection between the card10 rs6000782 variant and AIH[13]. The Mif-173 gc polymorphism is associated with the severity of AIH in children, and may help predict the increase in serum alanine aminotransferase (ALT) levels in the early stage of onset and necrotizing inflammation/fibrosis after immunosuppressive treatment[14]. TIPE2 has a protective effect on AIH. The expression of TIPE2 in mice with AIH is significantly reduced, while the serum ALT and aspartate aminotransferase (AST) levels of TIPE2-deficient mice are significantly increased, the release of pro-inflammatory cytokines is increased, and hepatitis is more serious. It is suggested that TIPE2 alleviates liver dysfunction after AIH and inhibits harmful inflammatory immune responses, so it can be used as a new drug for the treatment of AIH[15]. Immunogenetic factors can affect the clinical manifestations of AIH in ethnic groups[3]. The prognosis of AIH patients in Asians is poor. The indigenous Alaskan population has acute jaundice hepatitis, while the Spanish ethnic group is prone to cirrhosis. HLA-DRB1*0301/*0401 also has a significant impact on the clinical manifestations of AIH. DRB1*0301-positive patients are younger and more ill. They have a poor response to

glucocorticoid treatment and are prone to relapse. It is more common to die of liver failure, and the probability of liver transplantation is high. Patients who are positive for HLA-DRB1*0401 are generally elderly women, who are relatively mildly ill, often accompanied by other autoimmune diseases, and hormone therapy is effective.

ENVIRONMENTAL FACTORS

Peptides of some viruses and hepatocyte antigens can cross-react

Since immune cross-reaction is not seen until a long time after virus infection, it is difficult to find the basis for viral infection. Common viruses include hepatitis viruses, measles virus, cytomegalovirus, Epstein–Barr virus, and varicella–zoster virus, and the most evidence is related to hepatitis viruses[16,17]. There is no difference between hepatitis E virus (HEV) seroprevalence rate in AIH patients in Catalonia and the general population. In patients with acute AIH, higher gammaglobulin levels and antibody titers, and higher HEV seropositivity indicate that there is a cross-reaction between HEV and liver antigens[17]. HEV infection may induce onset of AIH and affect its therapeutic response[16,17]. During acute HEV infection, AIH needs to be ruled out. Similarly, before diagnosis of AIH, acute HEV infection should be excluded. Immunization may also cause AIH, and influenza vaccination may trigger the development of AIH[18].

Vitamin D

Vitamin D has immunoregulatory, anti-inflammatory, antioxidative and antifibrotic effects, which may affect the occurrence and outcome of immune-mediated diseases. Macrophages and dendritic cells produce 1,25-dihydroxyvitamin D in the microenvironment, which can inhibit proliferation of immune cells, promote distribution of anti-inflammatory cytokines, expand regulatory T cells (Tregs), enhance the effect of glucocorticoids, increase production of glutathione, and inhibit hepatic stellate cells. Vitamin D deficiency usually exists in patients with immune-mediated liver disease and non-liver disease, and is related to the histological severity of AIH, advanced liver fibrosis, the ineffectiveness of conventional glucocorticoid therapy, and the need for liver transplantation[19]. Another study found that genetic variants of VDR genes (TaqI-rs731236, BsmI-rs1544410 and ApaI-rs7975232) can affect the susceptibility of individuals to chronic autoimmune liver diseases (such as AIH and primary biliary cholangitis, and affect quality of life[6].

Intestinal microenvironment and intestinal barrier

Intestinal barrier dysfunction and bacterial translocation can initiate autoimmune responses in AIH. Intestinal leakage in AIH patients is related to abnormal intestinal microbes. Damage to the intestinal barrier can cause pathogenic bacteria and their products such as lipopolysaccharide and DNA-containing unmethylated CpG to enter the liver. These gut-derived toxins may promote the signaling pathways related to liver inflammation through the abnormal activation of the innate immune system, such as activating NF-kB, inducing activation of macrophages and releasing various pathogenic inflammatory cytokines, leading to occurrence of AIH[20-22]. Because AIH patients have impaired integrity of intestinal tight junctions, they also have intestinal flora imbalance, characterized by decrease of bifidobacteria, and changes in fecal microbes of specific diseases have been found. AIH patients may have bacterial flora migration, and intestinal barrier dysfunction and bacterial translocation are related to disease severity/increased activity[21]. Study of the changes in the composition and function of the intestinal microbiome in AIH, using the intestinal microbiota as a non-invasive biomarker, can be used to assess disease activity[22]. These results indicate that the intestinal flora provides new diagnostic methods and therapeutic targets in AIH.

Alcohol, pets and parasites

Alcohol exposure can affect the function of dendritic cells, reduce antigen presentation, and thereby inhibit the immune response. Studies have pointed out that antibiotics are an independent risk factor for the occurrence of AIH. Wood heating of households is an independent protective factor for prevention of AIH[23]. Close contact with pets (especially cats) is a risk factor for autoimmune liver disease. This finding indicates that an unknown substance (*i.e.*, toxin/microorganism) is involved in the triggering of these diseases[24]. Parasite studies have shown that soluble liver

antigen/liver pancreas (SLA/LP) protein is a highly specific diagnostic marker for AIH. The immunodominant regions of SLA/LP and rickettsial surface antigen ps120 are structurally similar, and may drive the autoimmune response mediated by CD4+ T lymphocytes[25].

DRUG OR BIOLOGICAL AGENT INDUCTION OF AIH

Drug-induced AIH (DIAIH) occurs in patients who have not previously been diagnosed with AIH or are susceptible to AIH. Many drugs can induce AIH, including nitrofurantoin, minocycline, hydralazine, methyldopa, indomethacin, diclofenac, atorvastatin, Tienilic acid, interferon, TNF-α, and some Chinese herbal medicines. The occurrence of DIAIH is related to gender, age, drug dose, genetic polymorphism, and drugs. Its pathogenesis is related to autoantibodies against proteins expressed in liver cells, and results from the reaction of unstable drug metabolites with cellular components. In particular, proteins in the P450 cytochrome system are considered neoantigens[26,27]. DIAIH is different from other forms of hepatotoxicity in which autoantibodies are usually negative. DIAIH has antinuclear antibodies, elevated anti-smooth muscle antibodies or gammaglobulin, and/or a specific HLA haplotype[26-28]. The difference in the incidence of DIAIH among countries may be due to population differences and the heterogeneity of the drug supply. Nitrofurantoin and minocycline are the main causes of DIAIH. Among cases of hepatotoxicity with nitrofurantoin and minocycline, DIAIH accounts for 82% and 73%, respectively. The incidence of AIH induced by methyldopa is 55%, and 43% for hydralazine. A prospective study of the Drug-induced Liver Injury Network (DILIN) showed that nitrofurantoin and nonsteroidal anti-inflammatory drugs accounted for 84% of DIAIH cases, and nitrofurantoin cases were as high as 67%[28,29]. Biological agents (*e.g.*, infliximab/adalimumab) have recently begun to constitute a cause of DIAIH, appearing in the early stage of drug withdrawal (as early as 2 mo), accompanied by short-term immunity inhibition, but there are no records of recurrence[30,31]. Diagnosis of DIAIH and AIH is difficult to distinguish. The response of DIAIH to hormone therapy is similar to that of AIH, but DIAIH has good prognosis. After discontinuation of immunosuppressive therapy, no patients have relapsed or progressed to cirrhosis or required liver transplantation. AIH has a higher degree of fibrosis than DIAIH has, and relapse can occur after drug discontinuation, with later progress to liver cirrhosis or even liver transplantation. More importantly, compared with AIH, patients with DIAIH have higher serum ALT and AST levels, more severe lobular inflammation, and higher frequency of necrosis, the number of CD4 + Foxp3 + CD25 +/- Tregs in hepatic lobules is higher, but there is no significant difference in the frequency of peripheral blood CD4+ Foxp3+ CD25+/- Tregs between DIAIH and AIH [30]. An increasing number of studies have shown that drugs have some effect on AIH, but the specific pathogenesis needs further research.

ABNORMAL AUTOIMMUNE REGULATORY MECHANISM

It is currently believed that the immune response of AIH is likely initiated by the presentation of autoantigens to uncommitted naive CD4+ helper T (Th0) cells. CD4+ Th0 cells are activated in the antigen presentation process in the presence of appropriate co-stimulatory signals, and differentiate into different helper T cell populations according to the cytokine environment to which they are exposed. Th0 cells in the presence of IL-12 differentiate into Th1 cells, differentiate into Th2 in the presence of IL-4, and differentiate into Tregs or Th17 cells in the presence of TGF-β[32,33]. Tregs and Th17 cells play an important role in the occurrence and development of immune-mediated hepatitis. Tregs include two subgroups, CD4+ CD25+ and CD8+. The former is the main factor in maintaining immune tolerance. The surface of Tregs can express IL-2 receptor, glucocorticoid-induced TNF receptor, Foxp3, CTLA-4, and chemokine receptors 4, 6, 7, 8 and 10. CD4+ CD25+ Foxp3+ Tregs inhibitory effector cells play an important role in maintaining cell homeostasis[34-36]. AIH patients have low expression of F0xp3 in peripheral blood, decreased Tregs, and decreased ability to regulate CD4+ and CD8+ effector T cell proliferation. Th17/Th22 cells in AIH peripheral circulation and liver are increased; interferon-γ, IL-17 and IL-22 levels increase; IL-17 increases release of inflammatory factors such as TNF-α and IL-6, and induces an immune inflammatory response. The imbalance between Tregs and Th1 and Th17/Th22 cells, activated macrophages, complement and natural killer cell

activation may all participate in the pathogenesis of AIH[33-36]. The IL-1 family has a proinflammatory function, and IL-33 is a ligand for receptors of IL-1 receptor-related protein ST2 (IL1RL1/ST2) and IL-1 receptor accessory protein (IL-1RaP). The interaction of IL-33 with these receptors triggers the signaling pathways related to MyD88 and NF-κB. The interaction between IL-33 and IL1RL1/ST2 receptors regulates Th2 response, and serves as an important part of the Th1/Th17-mediated response and inflammation induced by innate immunity[37]. IL-33 and its soluble receptor ST2 play a vital role in the pathogenesis and severity of type I AIH, and may be a new target for the treatment of AIH[37,38].

PREGNANCY AND LIVER TRANSPLANTION

Patients with a past history of AIH during pregnancy have an increased risk of recurrence of AIH. The maternal immune system expands through Foxp3+ Tregs during pregnancy and guides Th2 transformation to maintain immune tolerance and immune response in the fetus to protect against invasive organisms. However, this immunotolerant state returns to Th1 dominance, leading to AIH[39,40]. Therefore, patients with elevated transaminase or immunoglobulin G (IgG) levels during pregnancy or postpartum should be alert to the possibility of secondary AIH. AIH can appear or recur after liver transplantation, and is called *de novo* AIH or recurrent AIH. AIH may occur in patients undergoing liver transplantation due to different diseases. *De novo* AIH after transplantation may be caused by an immune response to an allogeneic antigen that triggers an autoimmune response[41,42]. Recurrent AIH is associated with elevated liver enzymes and IgG before liver transplantation, lymphoplasmacytic infiltration and steroid deficiency after liver transplantation[43,44]. Although the prognosis after liver transplantation is good, AIH may still occur/relapse after transplantation, with an estimated 1-year recurrence rate of 8%–12% and 5-year recurrence rate of 36%–68%[40]. The pathogenesis of recurrent or *de novo* AIH after liver transplantation is unclear, and may be related to factors such as transplanted organs and immunosuppressive drug treatment. Early rapid diagnosis can avoid strong rejection and possible secondary liver transplantation[41-43].

SUMMARY AND OUTLOOK

AIH has no specific clinical manifestations. AIH patients often require steroid hormones plus immunosuppressive maintenance therapy. Long-term medication may lead to various adverse reactions, complications, and relapse after drug withdrawal, which imposes a heavy burden on patient's health and quality of life. The etiology of AIH has not yet been fully clarified.

CONCLUSION

Genetic susceptibility, environmental factors (viruses, parasites, pets, *etc.*), immune system, drugs and biological agents, pregnancy and liver transplantation have been reported to be associated with AIH. The pathogenesis of AIH still needs further research.

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MINIREVIEWS

Current state of endohepatology: Diagnosis and treatment of portal hypertension and its complications with endoscopic ultrasound

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Abstract

The diagnosis and management of cirrhosis and portal hypertension (PH) with its complications including variceal hemorrhage, ascites, and hepatic encephalopathy continues to evolve. Although there are established "standards of care" in liver biopsy and measurement of PH, gastric varices remain an area without a universally accepted therapeutic approach. The concept of "Endo Hepatology" has been used to describe of the applications of endoscopic ultrasound (EUS) to these challenges. EUS-liver biopsy (EUS-LB) offers an alternative to percutaneous and transjuglar liver biopsy without compromising safety or efficacy, and with added advantages including the potential to reduce sampling error by allowing biopsies in both hepatic lobes. Furthermore, EUS-LB can be performed during the same procedure as EUS-guided portal pressure gradient (PPG) measurements, allowing for the collection of valuable diagnostic and prognostic data. EUS-guided PPG measurements provide an appealing alternative to the transjugular approach, with proposed advantages including the ability to directly measure portal vein pressure. In addition, EUS-guided treatment of gastric varices (GV) offers several possible advantages to current therapies. EUS-guided treatment of GV allows detailed assessment of the vascular anatomy, similar efficacy and safety to current therapies, and allows the evaluation of treatment effect through doppler ultrasound visualization. The appropriate selection of patients for these procedures is paramount to ensuring generation of useful clinical data and patient safety.

Key Words: Portal hypertension; Endoscopic ultrasound; Liver biopsy; Gastric varices

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Core Tip: In this review we familiarize the reader to salient aspects of endoscopic ultrasound (EUS)-guided hepatic interventions including liver biopsy, portal pressure measurements, and treatment of gastric varices, and outline the data supporting their use. We highlight the potential advantages and disadvantages of EUS guided interventions compared to the current standards of care, and propose clinical scenarios in which EUS guided interventions may be favored over the current standard of care.

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INTRODUCTION

Chronic liver disease (CLD) continues to represent a substantial healthcare burden, with an estimated 1.5 billion persons affected worldwide. Since 2000 there has been a 13% increase in incidence of CLD and cirrhosis, in addition to increasing prevalence and mortality of cirrhosis in the United States. Moreover, the epidemiology of CLD is shifting from viral hepatitis to an increasing prevalence of liver disease caused by metabolic syndrome and alcohol misuse[1].

Accompanying the increase in cirrhosis is the development of portal hypertension (PH); resulting in the majority of its complications including ascites, variceal hemorrhage, and encephalopathy. Clinically, cirrhosis is often dichotomized into compensated (absence of portal hypertensive complications) and decompensated (presence of portal hypertensive complications), with decompensated cirrhosis portending a poor prognosis[2].

A diagnosis of PH typically requires invasive testing to measure the gradient between the hepatic sinusoids and the hepatic vein (which is the outflow tract of the liver), termed the hepatic venous pressure gradient (HVPG) (Figure 1). PH is present if HVPG > 5 mmHg, with clinically significant PH (CSPH) defined as > 10 mmHg associated with the development of clinical complications (hence its designation) including variceal hemorrhage and ascites. HVPG is an independent prognostic variable, with a 3% increase in mortality risk for each 1 mmHg gradient increase[3].

Accompanying the increasing burden of CLD has been the need for safe, accurate, and cost-effective diagnostic modalities to appropriately classify patients requiring additional therapeutic interventions. Classically liver biopsy; percutaneous liver biopsy (PC-LB) and transjugular liver biopsy (TJ-LB) was utilized to assess the etiology and severity (fibrosis stage) of liver disease by histology. Additionally, invasive measurement of the HVPG *via* the transjugular venous route in interventional radiology (IR) could be utilized to obtain additional prognostic data in appropriate circumstances. Noninvasive modalities, such as elastography or serologic markers, have been developed as alternatives to liver biopsy[4].

The concept of "Endo-hepatology" was introduced in 2012 as an area of integration or overlap of endoscopic procedures within the practice of Hepatology[5]. In this review we focus on two diagnostic modalities including endoscopic ultrasound (EUS) guided liver biopsy (EUS-LB) and EUS-guided measurement of PH, and one therapeutic application; EUS-guided management of gastric varices (GV).

Hepatologists should have a fundamental understanding of the similarities and differences in techniques between current clinical standards of practice and EUS-guided modalities, while also recognizing opportunities to appropriately implement EUS-guided diagnostics and therapeutics into their practice. An in depth review of EUS anatomy, devices, and techniques is outside the purview of this review.

LIVER BIOPSY

Once considered the cornerstone in the evaluation and management of liver disease, the role and modalities of liver biopsy has evolved substantially over the past decade.

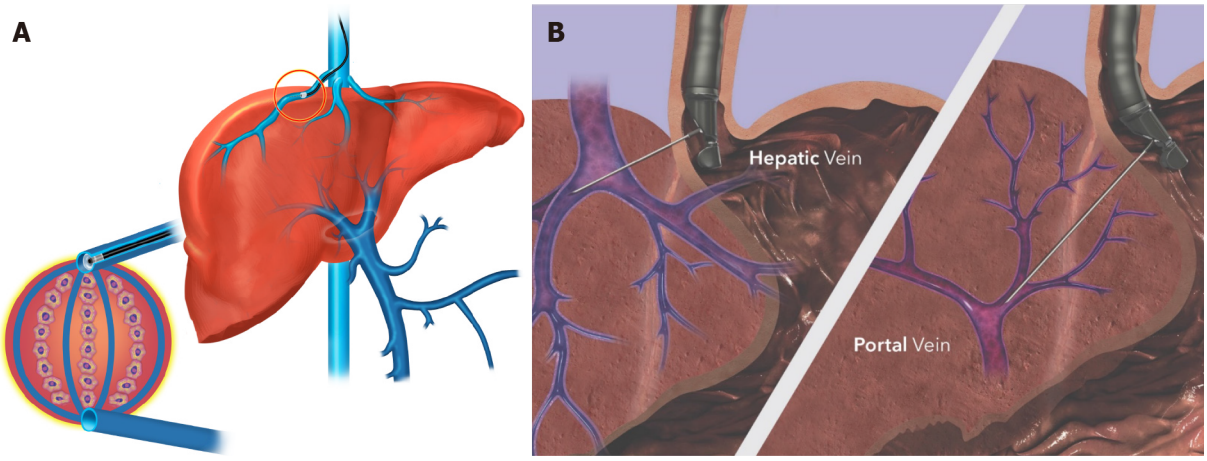

Figure 1 Comparison of modalities for measuring portal hypertension. A: Methods for obtaining hepatic venous pressure gradient measurement *via* the transjugular approach. Placement of catheter into right hepatic vein for measurement of free hepatic venous pressure, followed by balloon or "wedged" occlusion (inset) to measure wedged hepatic venous pressure, indirectly measuring the portal vein pressure *via* the sinusoids; B: Portal pressure gradient measurement *via* endoscopic ultrasound. The hepatic vein (left panel) and portal vein (right panel) are both directly accessed with transgastric needle puncture. Permission for use granted by Cook Medical, Bloomington, Indiana.

The evolution of noninvasive testing coupled with concerns regarding the cost and risk of liver biopsy has brought into question the exact role of liver biopsy in the early 21st century[4]. At present, liver biopsy is still considered appropriate for establishing diagnosis, evaluating stage of liver disease (fibrosis), and directing management decisions[6].

Traditionally, liver biopsy has been performed through percutaneous, transjugular, or surgical approaches. At present, image-guided liver biopsy ("real time" or marking) has become the de facto standard of care in most centers, replacing the palpation/percussion guided technique[7]. Because the diagnosis, grading, and staging of liver disease is dependent upon adequate sample size, it is recommended that the length of the sample is at least 2-3 cm and 16-gauge in caliber (or wider), ideally with ≥ 11 portal tracts for evaluation[6]. Complications related to liver biopsy include pain (30%-50% patients)[8], serious bleeding (0.6%)[9], injury to other organs (0.08%)[10], and rarely death (0.1%)[6].

Since its first description in 2007, publications describing experience with EUS-LB have continued proliferate[11]. Proposed advantages to EUS-LB include more precise localization and characterization of the target tissue, ability to biopsy both lobes of the liver, decreased invasiveness, improved patient tolerance, decreased recovery time, and decreased complications[12]. Acknowledged disadvantages include increased technical difficulty and higher cost compared to other available methods (Table 1).

A single center retrospective study compared the safety and efficacy of "standard of care" [PC-LB (n = 287) & TJ-LB (n = 91)] to EUS-LB (n = 135). There were no statistically significant differences between modalities in regards to rates of adverse events, technical success rate, and diagnostic adequacy. Notably, the number of complete portal tracts for analysis and mean specimen length (two metrics for assessing specimen adequacy) were higher in the EUS-LB group compared to PC-LB and TJ-LB[13]. These results support comparable safety profile and diagnostic adequacy (*i.e.*, non-inferiority) of EUS-LB to current standard of care liver biopsy modalities.

In 2019 a systematic review and meta-analysis that included eight studies with a total of 437 patients reported the efficacy and safety of EUS-LB biopsy[14]. The primary analysis focused on diagnostic yield; specifically addressing successful histologic diagnosis and frequency of insufficient histologic sample size. A second analysis described pooled rates of all adverse events. A subgroup analysis was performed regarding needle type used for biopsy [core needle *vs* fine-needle aspiration (FNA) needle]. A 19-gauge needle was used in all included studies. Indications for liver biopsy included abnormal liver tests, non-alcoholic steatohepatitis, cholestasis, primary sclerosing cholangitis, cirrhosis, and congestive heart failure.

The pooled rate of successful histologic diagnosis was 93.9% and the pooled insufficient specimen rate was 10.1%. The pooled rates of adverse events and bleeding were 2.3%, and 1.2%, respectively. In the subgroup analysis, the only statistically significant difference between core needle and FNA needle was obtaining insufficient specimen, which occurred in 20% of patients biopsied with core needle compared to

Table 1 Relative advantages and disadvantages of liver biopsy modalities

Modality	EUS-LB	PC-LB	TJ-LB
Advantages	Ability to obtain simultaneous bi-lobar biopsies	Familiarity	Circumvent challenging body habitus
	Circumvent challenging body habitus	Less technical expertise	Ability to perform other diagnostics simultaneously (*i.e.*, HVPG measurement)
	Improved patient tolerance	Lower cost	Fewer contraindications (*i.e.*, ascites and coagulopathy)
	Decreased recovery time		
	Ability to perform other diagnostics simultaneously (*i.e.*, PPG measurement)		
Disadvantages	Higher cost	Poorer patient tolerance	Higher cost
	Need for technical expertise	May be limited by patient body habitus	Need for technical expertise
		More prone to sampling error	More prone to sampling error

PH: Portal hypertension; TJ-LB: Transjugular liver biopsy; EUS-LB: Endoscopic ultrasound-guided liver biopsy; PPG: Portal pressure gradient; HVPG: Hepatic venous pressure gradient; PC-LB: Percutaneous liver biopsy.

4% of patients biopsied with FNA needle (P = 0.03). The authors concluded that FNA needles provide better specimens and have improved diagnostic outcomes compared to other core needle biopsies, though they acknowledged significant heterogeneity in the overall analysis.

Despites its limitations, the study by Mohan *et al*[14] provides robust data describing the performance characteristics and technical considerations (needle device choice) of EUS-LB. The safety profile of "standard of care"; (PC-LB or TJ-LB) was compared head-to-head in a propensity score matched analysis of 978 patients who underwent PC-LB compared to 489 undergoing TJ-LB. Hematomas developed in 1.2% of patients undergoing PC-LB compared to 0.2% with TJ-LB (P = 0.049). Cardiac complications occurred more frequently in TJ-LB compared to PC-LB (0.4% *vs* 0%; P = 0.045). There were no significant differences in other adverse events or complications [15].

Ultimately, multiple factors influence the choice of liver biopsy modality, and the decision should be made on a case-by-case basis (Figure 2). A seemingly pertinent use of EUS-LB, is in patients with discordant noninvasive testing in whom the goal is to exclude cirrhosis and/or PH, as direct measurements of portal pressures can also be performed simultaneously and biopsies from both lobes can be obtained. With discordant noninvasive testing, accurate fibrosis staging by liver biopsy is paramount. Indeed, it has been demonstrated in patients with NAFLD, biopsies performed on the same day characterized 35% of patients with advanced fibrosis on one sample, while the other sample from the same day did not suggests significant fibrosis[16]. This discordance is of profound significance and directly influences clinical decision-making. As PC-LB and TJ-LB typically sample one hepatic lobe, obtaining "bilobar" biopsies by EUS-LB provides a potential advantage to minimize the risk of misclassifying fibrosis stage.

MEASUREMENT OF PH

Although invasive and considered the gold standard in assessment of PH, HVPG is in fact an indirect method of measurement[17]. Calculation of the HVPG includes measuring the free hepatic venous pressure (FHVP) and wedged hepatic venous pressure (WHVP; typically wedged pressure in the right hepatic vein). The transduced wedged hepatic venous pressure estimates sinusoidal pressure. The difference between the WHVP and FHVP is the estimated portosystemic gradient[18]. Conceptually, this is analogous to Swan-Ganz catheterization in the pulmonary artery.

In the absence of fibrosis/nodules (*i.e.* cirrhosis), the pressure equalizes throughout the interconnected sinusoidal network, and results in minimal gradient (*i.e.*, normal; up to 4 mmHg). Thus, it does not provide useful information regarding prehepatic or presinusoidal PH (*i.e.*, non-cirrhotic causes of PH). In the presence of cirrhosis, the

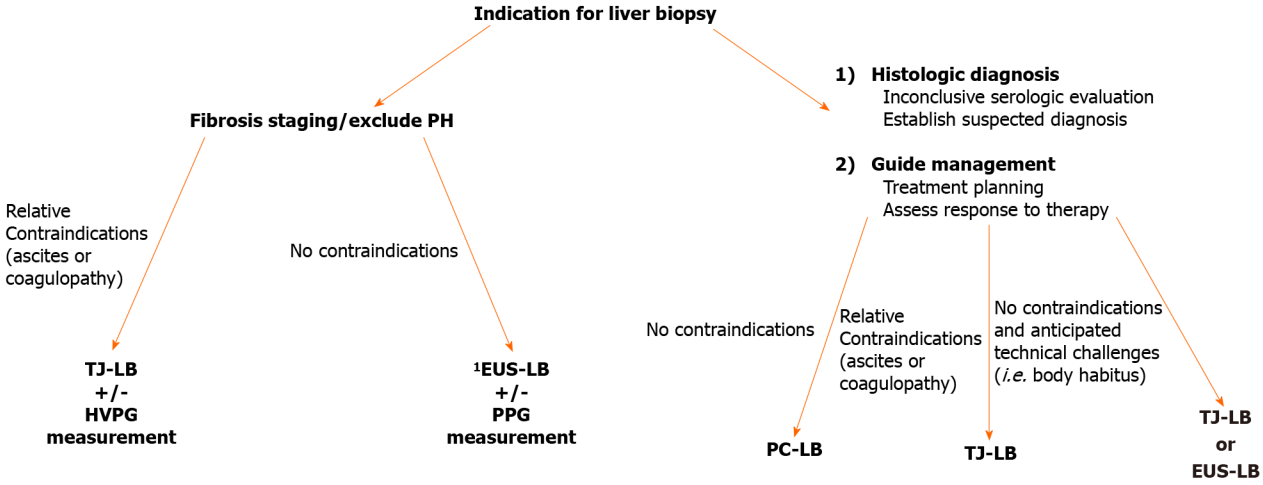

Figure 2 Proposed algorithm for choosing suitable modality for liver biopsy. [1]Allows endoscopic exam for evidence of portal hypertension (*i.e.*, varices/PHG), high-resolution endoscopic ultrasound images of liver contours/parenchyma, endoscopic "palpation" of the liver, bi-lobar biopsies, and direct measure of portal pressure gradient. PH: Portal hypertension; TJ-LB: Transjugular liver biopsy; EUS-LB: endoscopic ultrasound-guided liver biopsy; PPG: Portal pressure gradient; HVPG: Hepatic venous pressure gradient; PC-LB: Percutaneous liver biopsy.

WHVP is an accurate surrogate for portal vein pressure, allowing calculation of the gradient by the equation: WHVP-FHVP = HVPG. As previously outlined, HVPG has significant prognostic value in predicting poor outcomes in patients with PH[3].

In comparison, EUS-guided portal pressure gradient (PPG) measurements employ a direct sampling technique. Thus, the direct measurement of the portal vein pressure could be considered the gold standard because it is not an estimate of sinusoidal pressure as is WHVP. The difference in the mean measurement of these pressures is termed the PPG which is analogous to the HVPG, with the caveat that direct portal vein measurement also allows for the assessment of prehepatic/presinusoidal PH; a limitation of the transjugular approach.

In 2016, Huang *et al*[19] published their experience in a porcine animal model with a novel EUS-guided system which included a manometer attached to a 25-gauge FNA needle for directly measuring pressures in the hepatic and portal veins. The purpose of this animal study was to assess clinical feasibility and assess correlation with the standard of care; HVPG measurement through transjugular approach[19].

In a pilot study, 28 patients between the age of 18-75 years with a history of liver disease or suspected cirrhosis underwent EUS-PPG measurements utilizing the technique and equipment in the animal study. The portal vein and hepatic vein were targeted *via* a transgastric–transduodenal approach (IVC was substituted for hepatic vein when not technically feasible). Feasibility was defined as the technical success of obtaining pertinent measurements. Safety was assessed by postprocedural interview and telephone call 48 h following procedure. As correlation to the standard of care (transjugular HVPG) was obtained in animal studies, clinical parameters of PH were evaluated in each patient. Exclusion criteria included pregnancy, international normalized ratio (INR) > 1.5, platelet count < 50000, active GI bleeding, and post sinusoidal PH[20].

Technical success rate of EUS-PPG measurement was 100% without any adverse events. PPG measurements had excellent correlation with clinical parameters of PH. Mean PPG in patients with varices was 14.37 mmHg, compared to 4.26 mmHg in patients without varices (P = 0.0002); which is consistent with criteria that gradients ≥ 10 mmHg (*i.e.*, CSPH) are associated with the development of varices. The authors concluded that EUS-PPG measurement was a safe and feasible alternative to currently available diagnostics[20].

There are obvious limitations of this pilot study which may limit widespread generalizability of this technique. The exclusion of patients with INR > 1.5 and inclusion of only 4 patients with INR > 1.2 (especially with the knowledge that INR is a poor predictor of procedural bleeding risk in patients with cirrhosis) is a major limitation of this small pilot study[21].

Results of this pilot study ultimately led to the Food and Drug Administration approval of the EchoTip Insight portosystemic pressure gradient measurement system (Cook Medical, Winston-Salem, NC, United States) in 2019 (Figure 1). Following approval, multiple centers have begun utilizing this method. Registry data are eagerly

anticipated to assess the feasibility, utility, and safety profile of this method outside the realm of small pilot study/clinical trials.

One of the challenges facing any new technology, including EUS-PPG measurement is identifying the appropriate clinical application. Despite the useful prognostic information it provides, in current clinical practice, obtaining the HVPG is not considered standard of care in many areas due to its invasiveness, cost, and limited availability[2]. With the exception of Transjugular intrahepatic portosystemic shunt (TIPS) and TJ-LB in the authors' experience, HVPG measurements are not routinely obtained.

A potential role of EUS–PPG measurements in current practice would be to supplant the transjugular approach for HVPG/biopsy, and reserve the latter approach for patients undergoing TIPS and in those with more severe coagulopathy. Furthermore, the additional evidence gleaned during the endoscopic evaluation (*i.e.*, presence/absence of varices or portal hypertensive gastropathy) would have treatment implications. Whether the combination of EUS-PPG measurements (with or without simultaneous liver biopsy) can be routinely incorporated during evaluation of patients with cirrhosis remains to be seen.

TREATMENT OF GV

There is significant heterogeneity in the location, vascular anatomy, bleeding risk, and response to treatment of GV. The Sarin classification has been the most commonly used for risk stratification and management, however it is limited to describing endoscopic anatomy, and does not necessarily reflect the underlying vascular anatomy of GV; which has significant treatment implications[22,23].

A proposed algorithm for the treatment of acute GV bleeding suggests utilizing variceal band ligation for treatment of gastroesophageal varices (GOV) 1 (*i.e.*, treat as esophageal varices), while utilizing injection therapies (*i.e.*, tissue adhesives such as cyanoacrylate) in the management of GOV2 and isolated gastric varices 1 (IGV1) (together known as "cardiofundal varics")[24]. At present, therapeutic options for treatment of GV hemorrhage include endoscopic injection of tissue adhesives (*via* EGD or EUS), TIPS, and balloon-occluded retrograde transvenous obliteration) (BRTO). It has been suggested that EUS-guided therapy of GV is superior to endoscopic injection as it decreases the rate of rebleeding[25].

In 2000, Lee *et al*[26] published their results of a prospective study utilizing cyanoacrylate and lipiodol injection in the management of bleeding GV[26]. In this study 38% of patients had GOV2 and 27% patients had IGV1. After initial bleeding was controlled, 47 patients received "on demand" therapy if bleeding recurred, while 54 patients underwent biweekly EUS with injection until obliteration of varices was confirmed. Although early rebleeding rates (defined ≤ 48 h) were similar between both groups, the recurrence of late bleeding (> 48 h) was significantly reduced in the repeat injection group (18.5% *vs* 44.7%, $P = 0.0053$).

A randomized trial evaluated prevention of first GV bleed (primary prophylaxis) [27]. In a study of 89 patients with large (≥ 10 mm) GOV2 and IGV1, patients were randomized to endoscopic cyanoacrylate glue injection, nonselective beta blocker (NSBB), and observation. Overall, cyanoacrylate injection was associated with lower bleeding rates (10%) than NSBB (38%), and observation (53%). Survival was similar in the cyanoacrylate (93%), and NSBB group (83%), but higher compared to the observation group (74%). Of note, only 15% of patients in the study had IGV1. This study formed the basis for recommendation of NSBB for primary prophylaxis of GV hemorrhage in GOV2 and IGV1.

The management of active hemorrhage from GV remains a significant clinical challenge. A meta-analysis comparing cyanoacrylate glue injection to endoscopic band ligation demonstrated similar results for initial hemostasis, but favored cyanoacrylate injection for prevention of rebleeding[28]. Limitations of this meta-analysis included variable quality of evidence, and heterogeneity in type of varices treated.

The addition of endovascular coils to cyanoacrylate glue injection has been proposed to reduce the risk of systemic embolization, a rare but potentially fatal complication[29,30]. A single center retrospective study of 152 patients specifically addressed the use of coil injection and cyanoacrylate glue in patients with cardiofundal varices; 94% of whom had IGV1. Over a 6-year period, 5% of patients treated had active hemorrhage, while 69% had evidence of recent bleeding (*i.e.*, treatment constituted secondary prophylaxis). Technical success rate was 99%. Follow-up EUS examinations were available for 100/152 patients. Complete obliteration of varices

Table 2 Comparison of endoscopic ultrasound-guided treatment modalities for gastric varices; combination therapy *vs* monotherapy [31]

Treatment	CYA+ coil (combination therapy)	CYA alone	Coil alone	*P* value (combination *vs* CYA alone/combination *vs* coil alone)
Outcome rate (%)				
Technical success	100	97	99	< 0.001/< 0.001
Clinical success	98	96	90	< 0.001/< 0.001
Adverse event	10	21	3	< 0.001/0.057
Adverse event	14	30	17	< 0.001/1.00
Re-intervention	15	26	25	< 0.001/0.047

CYA: Cyanoacrylate.

based on Doppler was confirmed in 93%, and bleeding from obliterated varices occurred in 3% of patients. The authors concluded that combination of therapy with cyanoacrylate and coil embolization is highly effective for hemostasis and active bleeding, and for primary and secondary prophylaxis with minimal adverse effects.

A systematic review and meta-analysis compared combination therapy (cyanoacrylate + coils) to monotherapy with (cyanoacrylate alone *vs* coil alone or non-cyanoacrylate treatment)[31]. Eleven studies were included (n = 536) which included 2 randomized control trials, one prospective study, and 8 retrospective studies. Measured outcomes included technical success, clinical success, adverse events, and rate of rebleeding/or intervention. Subgroup analysis compared 3 treatment cohorts; EUS- guided cyanoacrylate injection/EUS-guided coil embolization + cyanoacrylate injection/EUS-guided coil injection alone) (Table 2).

Overall technical success of EUS-guided therapies was 100%, clinical success was 97%, and adverse events were 14%. In the subgroup analysis, combination therapy resulted in better technical success (100%) and clinical success (98%) compared to monotherapy with cyanoacrylate alone (97% and 96%, respectively) or coil embolization alone (99% and 90%, respectively). Combination therapy also resulted in lower adverse event rates (10%) compared to monotherapy with cyanoacrylate alone (21%), and coil embolization alone (3%). The authors concluded that EUS-guided treatment is safe and effective, and that combination therapies should be the preferred strategy for management of GV.

Based upon current treatment algorithms, and understanding the limitations of currently available data, EUS-guided treatment for GV should be reserved for cardiofundal varices. The main advantages of this approach include acute hemostasis and prevention of rebleeding. Furthermore, the use of EUS allows delineation of the vascular anatomy of the variceal complex, which can enable precise delivery of therapy into the varix lumen or afferent vessel (potentially decreasing the risk of embolization) and allow confirmation of vessel obliteration *via* Doppler examination [32-34]. Cyanoacrylate is off-label for the treatment of GV hemorrhage in the United States, so its use should be limited to centers with appropriately trained endoscopists and experience[2,35].

CONCLUSION

EUS-guided interventions for the diagnosis and management of PH and its complications have evolved from a novel innovation into a useful clinical tool with a growing evidence-base supporting its role.

Available data suggests that EUS-LB results in comparable diagnostic adequacy (*i.e.*, tissue specimen) to currently available options with similar low rates of adverse events [14]. Measurements of PPG correlate with HVPG measurements and have a similar safety profile[19,20]. An additional benefit is the direct measurement of the portal vein pressure, allowing diagnosis of prehepatic/presinusoidal PH that is not obtained during HVPG measurements as well as the ability to perform liver biopsy. EUS-treatment for GV bleeding may be more effective than current endoscopic therapies, and offers several potential advantages[25,31].

EUS-guided interventions have demonstrated similar efficacy and safety to current standards of care, and should be viewed as a complement (not a replacement) to current diagnostic and therapeutic modalities. A multidisciplinary approach between Hepatologists and EUS-trained endoscopists is vital to ensure appropriate patient selection, ensure accurate and useful data are generated from diagnostic procedures, and that maximal therapeutic benefit is derived from EUS-guided treatments.

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MINIREVIEWS

Solid pseudopapillary neoplasm of the pancreas

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Abstract

Solid pseudopapillary neoplasms are rare. This article reviews the clinical and pathologic features of solid pseudopapillary neoplasm of the pancreas, including the epidemiology, cytology, molecular pathology, differential diagnosis, treatment, and prognosis. Solid pseudopapillary neoplasms are low-grade malignant tumours of the pancreas characterized by poorly cohesive epithelial cells with solid and pseudopapillary patterns. Solid pseudopapillary neoplasms occur predominantly in young women. Although solid pseudopapillary neoplasms can occur throughout the pancreas, they arise slightly more frequently in the tail of the pancreas. The aetiology is unknown. Extremely rare cases have been reported in the setting of familial adenomatous polyposis. There are no symptoms unique to solid pseudopapillary neoplasms, however, the most common symptom is abdominal pain or discomfort. The features of solid pseudopapillary neoplasms on computed tomography imaging are indicative of the pathologic changes within the tumour. Typically, well-demarcated masses with variably solid and cystic appearances. Microscopically, these tumours are composed of epithelial cells forming solid and pseudopapillary structures, frequently undergoing haemorrhagic cystic degeneration. Typically, these tumours express nuclear and/or cytoplasmic β-catenin. Almost all solid pseudopapillary neoplasms harbour mutations in exon 3 of *CTNNB1*, the gene encoding β-catenin. The overall prognosis is excellent, and most patients are cured by complete surgical resection.

Key Words: Cancer of pancreas; Pancreatic neoplasms; Solid pseudopapillary neoplasm of the pancreas; Non-ductal pancreatic tumours; Pancreas

Core Tip: Solid pseudopapillary neoplasms are low-grade malignant tumours that mimic other solid cellular neoplasms of the pancreas. This article summarizes the

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INTRODUCTION

First described by Frantz in 1959[1], solid pseudopapillary neoplasms are low-grade malignant tumours composed of poorly cohesive uniform epithelial cells forming solid and pseudopapillary structures[2]. Several names have been used to describe these tumours including solid cystic tumour, papillary cystic tumour, solid and papillary epithelial neoplasm, papillary cystic carcinoma, Hamoudi's tumour, and Frantz's tumour[3-5]. Solid pseudopapillary neoplasms are rare tumours of uncertain histogenesis. In certain cases, distinguishing between solid pseudopapillary neoplasms and other solid cellular neoplasms of the pancreas may pose a diagnostic dilemma.

This article reviews state-of-the-art knowledge on the clinical and pathologic features of solid pseudopapillary neoplasm of the pancreas, including the epidemiology, cytology, and molecular pathology, and also provides the differential diagnosis, treatment, and prognosis.

EPIDEMIOLOGY

Solid pseudopapillary neoplasms are exceptionally rare. They account for approximately 0.9%-2.7% of all exocrine pancreatic neoplasms and 5% of cystic pancreatic neoplasms[2,3]. Although these tumours occur in a wide age range from 2 to 85 years [3], the mean age at presentation is 28.5 years[5]. They occur predominantly in young women with a female-male ratio of 9.8:1[3]. There is no known ethnic predilection. An increased number of cases have been reported in the literature since 2000, most likely because of rising awareness of these tumours and advances in imaging and other diagnostic techniques[5].

AETIOLOGY

The aetiology is currently unknown. Although rare cases have been reported in the setting of familial adenomatous polyposis[6,7], there are no well-established risk factors for solid pseudopapillary neoplasms. There is no association with functional endocrine syndromes[2].

CLINICAL FEATURES

The clinical symptoms are non-specific. A large number of patients are asymptomatic (38.1%)[5], however most patients are symptomatic, with the most common presenting symptom being abdominal pain or discomfort[3,5,8]. Other symptoms include abdominal mass, weight loss, jaundice, anorexia, fever, fatigue, abdominal discomfort, nausea, and vomiting[3,5,8]. Rarely, patients may present with spontaneous[9,10] or traumatic[11] rupture of the tumour leading to haemoperitoneum.

These tumours may involve any portion of the pancreas but are slightly more common in the tail of the pancreas[2,3,5,12]. Rarely, these tumours can arise in extra pancreatic sites including the omentum[13], mesentery[14], retroperitoneum[15], ovary [16], stomach, and duodenum[17].

Distant metastases occur in 7.7% of cases and lymph node metastases occur in approximately 1.6% of cases[5]. Other sites of metastases include the lung[18], small and large bowel mesentery, liver, and peritoneum[3,5,12,19]. Occasionally, these tumours directly infiltrate adjacent structures including the portal vein, duodenum, and spleen[2,3,5].

IMAGING

Solid pseudopapillary neoplasms on computed tomography (CT) imaging show features reflective of the pathologic changes within the tumour. Usually, well-demarcated large heterogeneous masses with variably solid and cystic appearances on CT. Enhancing solid areas are mostly peripheral, with cystic areas tending to be centrally located. Peripheral or central stippled calcifications may be identified in the tumour[20,21].

MRI shows a well-defined mass with heterogeneous signal intensity on T1- and T2-weighted images indicative of the variably solid and cystic nature of the tumour. High signal intensity on T1-weighted images correspond to areas of haemorrhagic necrosis or debris[21,22]. The signal intensity of these areas is variable on T2-weighted images because of the presence of multiple degradation products of haemoglobin. The solid component of the tumour may show iso- to low signal intensity on T1-weighted images and slightly high signal intensity on T2-weighted images[21,22].

CYTOLOGY

Although endoscopic ultrasound-guided fine needle aspiration is operator dependent, it is a well-tolerated minimally invasive procedure that has become the method of choice for the diagnosis of solid and cystic pancreatic neoplasms. The sensitivity for malignant cytology is 85% and the specificity is about 98%[23].

Typically, these smears are very cellular, with neoplastic cells forming loose papillary clusters with central fibrovascular cores. The neoplastic cells are uniform with nuclear indentations. There are multinucleated giant cells, foamy macrophages, and haemorrhagic debris in the background[24,25].

PATHOLOGY

Grossly, solid pseudopapillary neoplasms are round solitary masses with fibrous pseudocapsule. Multicentric tumours are exceptionally rare[26]. They are large tumours ranging from 0.5 cm to 25 cm (mean, 10 cm)[2]. These tumours are typically solid with varying proportion of cystic degeneration. They have a well-demarcated fleshy cut surface with haemorrhagic and necrotic areas[27]. In rare cases, extension into adjacent structures may occur[2].

Microscopically, solid pseudopapillary neoplasms are composed of poorly cohesive epithelial cells forming solid and pseudopapillary structures (Figure 1A and B). The pseudopapillae are formed by epithelial cells loosely arranged around hyalinised stroma that contains thin-walled blood vessels (Figure 1A and B). The neoplastic cells are small and monomorphic. The cytoplasm of the neoplastic cells is eosinophilic or clear, and usually lacks mucin. The nuclei are round to oval and may show grooves, indentations, and clefts. The nuclei have fine chromatin pattern and absent or inconspicuous nucleoli. Mitotic figures are infrequent.

Although not specific, the presence of hyaline globules is a characteristic feature of solid pseudopapillary neoplasms. These globules are diastase-resistant, periodic acid-Schiff (PASD)-positive eosinophilic cytoplasmic inclusions (Figure 1C), corresponding to α-1-antitrypsin granules[2,27]. Most tumours contain foamy histiocytes (Figure 1D), cholesterol clefts, and foreign body giant cells (Figure 1E). Calcifications may be present. Perineural infiltration and vascular invasion is uncommon[28]. Rarely, undifferentiated carcinoma component may be seen[19].

Solid pseudopapillary neoplasms usually express nuclear and/or cytoplasmic β-catenin (Figure 1F). They are also positive for a wide range of antibodies including CD56 (Figure 1G), vimentin (Figure 1H), CD10 (Figure 1I), α-1-antitrypsin, α-1-antichymotrypsin, cyclin D1 (Figure 1J), CD99, claudin 5, claudin 7, and progesterone receptors[2,12,27]. Immunoreactivity for E-cadherin depends on the antibodies used.

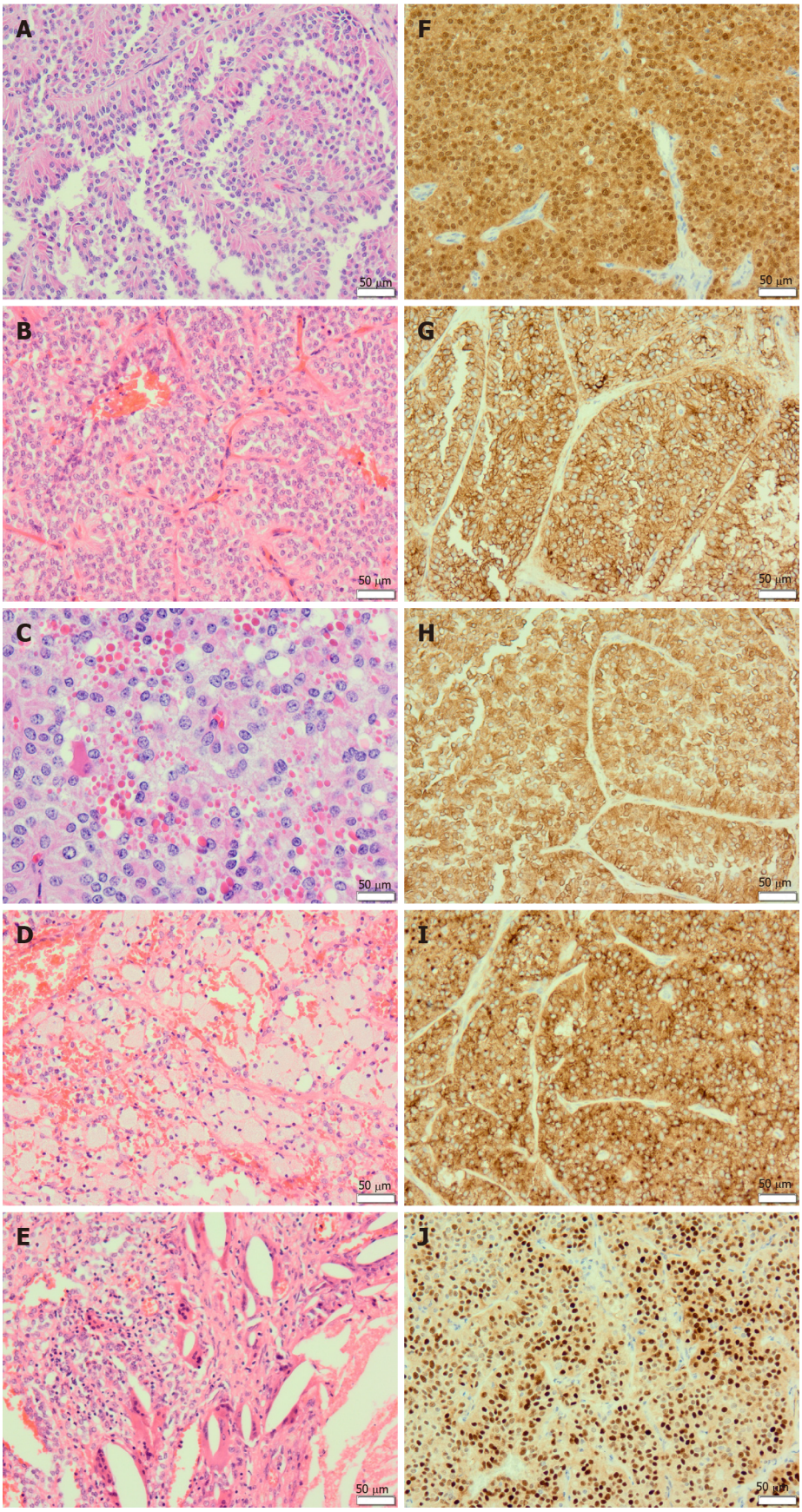

Figure 1 Solid pseudopapillary neoplasm of the pancreas. A: The tumour shows pseudopapillae formed by poorly cohesive cells arranged around hyalinized fibrovascular stalks (200 ×); B: The tumour consists of cells forming solid and pseudopapillary structures (200 ×); C: Solid pseudopapillary neoplasm of the pancreas shows characteristic eosinophilic cytoplasmic hyaline globules (400 ×); D: An example showing foamy histiocytes (200 ×); E: These are cholesterol clefts surrounded by foreign body giant cells (200 ×); F: The tumour shows nuclear and cytoplasmic expression of β-catenin (200 ×); G: The tumour shows immunolabelling

for CD56 (200 ×); H: The tumour is positive for vimentin (200 ×); I: The tumour shows immunolabelling for CD10 (200 ×); J: Solid pseudopapillary neoplasm of the pancreas shows nuclear positivity for cyclin D1 (200 ×).

Antibodies to the intracellular domain of E-cadherin shows an abnormal cytoplasmic/nuclear expression while antibodies to the extracellular domain of the protein shows complete loss of expression[28].

Solid pseudopapillary neoplasms may be focally positive for synaptophysin and neurone-specific enolase. However, these tumours are negative for chromogranin A, trypsin, chymotrypsin, lipase, oestrogen receptors, and BCL10[2,27,28].

MOLECULAR PATHOLOGY

Solid pseudopapillary neoplasms harbour mutations in exon 3 of *CTNNB1*, the gene encoding β-catenin[2,27,28]. They lack the molecular alterations that have been described in pancreatic ductal adenocarcinoma such as KRAS, TP53, SMAD4/DPC4, and CDKN2A[27,28].

B-catenin maintains cell-cell adhesion and regulates gene transcription in the canonical Wnt (β-catenin dependent) signalling pathway[29,30]. β-catenin is regulated by the β-catenin destruction complex composed of proteins including adenomatous polyposis coli, axin, protein phosphatase 2A, glycogen synthase kinase 3, and casein kinase-1[29,30]. In the absence of Wnt signalling, the β-catenin destruction complex targets β-catenin for ubiquitin-mediated proteasomal degradation. However, in the presence of Wnt signalling, the β-catenin destruction complex is inactivated, preventing β-catenin degradation. This leads to β-catenin accumulation in the cytoplasm and eventual translocation into the nucleus, where it acts as a co-transcriptional activator of lymphoid enhancer binding factor/T cell factor (LEF/TCF) family of transcription factors. Activated LEF/TCF family of transcription factors upregulates the expression of a variety of target genes involved in diverse cell functions such as cell proliferation, differentiation, and epithelial-mesenchymal transition. The *CTNNB1* mutations observed in solid pseudopapillary neoplasms and other cancers lead to constitutive activation of the Wnt/β-catenin pathway and abnormal stabilization of cytoplasmic β-catenin[29,30].

Gene expression studies have identified solid pseudopapillary neoplasm-specific mRNA and microRNA expression profiles distinct from other pancreatic tumours[31]. Pathway enrichment analysis of differentially expressed genes in solid pseudopapillary neoplasms has shown that in addition to Wnt/β-catenin signalling pathway, Hedgehog and androgen receptor signaling pathways are also activated in these tumours[31].

Proteomic analyses of solid pseudopapillary neoplasms have confirmed that proteins involved in Wnt/β-catenin signaling (CTNNB1 and DKK4) and proteins that bind directly to β-catenin (FUS, hnRNPM, BGN, NONO, YWHAZ, DDX5, SELENBP1, and FN1) are upregulated in these tumours[32]. Furthermore, 9 proteins involved in metabolism including SLC25A13, GPI, PGK1, HK1, ENO2, PDHB, ALDH7A1, PKM2, and DLD are overexpressed in solid pseudopapillary neoplasms[32].

DIFFERENTIAL DIAGNOSIS

Distinguishing solid pseudopapillary neoplasm of the pancreas from the more common pancreatic ductal adenocarcinoma is not diagnostically challenging. The differential diagnosis of solid pseudopapillary neoplasms include pancreatoblastoma, acinar cell carcinoma and pancreatic neuroendocrine tumour.

Pancreatoblastoma is a malignant epithelial tumour composed of neoplastic cells showing predominantly acinar differentiation with characteristic squamoid nests. Although pancreatoblastomas frequently occur in childhood, they can be seen in adults[33,34,35]. Pancreatoblastomas exhibit malignant behaviour with local infiltration of adjacent structures and distant metastasis at the time of diagnosis or afterwards in the course of the disease[33,34,35]. Both pancreatoblastomas and solid pseudopapillary neoplasms are solid cellular tumours of the pancreas. The features that favour a diagnosis of pancreatoblastomas include predominant acinar units, squamoid nests, prominent central nucleoli, granular eosinophilic cytoplasm containing DPAS-positive zymogen granules, and immunolabelling for trypsin,

chymotrypsin, BCL10, and lipase[2,33,35].

Acinar cell carcinomas are malignant neoplasms of the pancreas characterized by acinar differentiation but without squamoid nests. Unlike solid pseudopapillary neoplasms, acinar cell carcinomas frequently occur in men, lack pseudopapillary structures, and express trypsin, chymotrypsin, lipase, and BCL10. Acinar cell carcinomas are negative for β-catenin, CD56, and CD10. The prognosis of acinar cell carcinoma is poor with a 5-year survival rate of 25%[2].

Solid pseudopapillary neoplasms with a predominant solid pattern can be confused with pancreatic neuroendocrine tumours. In addition, both tumours express synaptophysin and CD56. Typically, pancreatic neuroendocrine tumours are composed of uniform cells with round to oval nuclei. The nuclei are centrally located with characteristic salt and pepper chromatin[2,33]. Features that favour a diagnosis of solid pseudopapillary neoplasms include the presence of solid and pseudopapillary structures, foamy histiocytes, cholesterol clefts, foreign body giant cells, scattered PASD-positive hyaline globules, nuclei with indentations, and expression of nuclear and/or cytoplasmic β-catenin, CD56, CD10, and vimentin.

TREATMENT

Surgical resection is the treatment of choice for solid pseudopapillary neoplasms[3,5]. The type of operation will depend on the site and size of tumour. Common surgical procedures include distal pancreatectomy and splenectomy, spleen preserving distal pancreatectomy, central pancreatectomy, total pancreatectomy, pancreaticoduodenectomy, and pylorus-preserving pancreaticoduodenectomy[3,5]. A Cochrane systematic review comparing the effectiveness of classic Whipple procedure *vs* pylorus-preserving pancreaticoduodenectomy, showed no significant differences in overall survival, post-operative mortality, and morbidity between both procedures except for delayed gastric emptying, which significantly favoured classic Whipple procedure[36].

Although most of these tumours are treated by open surgery[5], a recent systematic review suggested that compared to a traditional open approach, minimally invasive pancreatectomy is associated with decreased intraoperative blood loss, lower blood transfusion requirements, and a shorter post-operative time to diet and hospital stay [37]. However, there were no significant differences in operating time, margin positivity, post-operative morbidity, and post-operative pancreatic fistula rates[37].

PROGNOSIS

The overall prognosis is excellent with a cure rate of > 95% following complete surgical resection[2,3,5]. Of the 2158 patients with solid pseudopapillary neoplasm reported in a systematic review, outcome data were available in 1952 patients with a mean follow-up of 36.1 mo. Eighty-six patients (4.4%) had recurrent disease and twenty-nine patients (1.5%) died of the disease[5]. Long-term survival has been reported for patients with locally advanced, recurrent, and metastatic disease[3,12]. It is worth emphasizing that malignant behaviour cannot be predicted by vascular invasion, perineural invasion, and invasion of adjacent structures in these tumours[2]. However, it has been suggested that tumours with an undifferentiated carcinoma component have a dismal outcome[19], and large tumour size, high proliferation index, and lymph node metastasis may be risk factors for a poor prognosis[38].

CONCLUSION

In summary, solid pseudopapillary neoplasms are rare low-grade malignant tumours occurring predominantly in adolescent girls and young women with an excellent prognosis. It is therefore important to distinguish solid pseudopapillary neoplasms from morphological mimics.

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MINIREVIEWS

Therapeutic plasma exchange in liver failure

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Abstract

The multi-organ failure syndrome associated with acute and acute-on-chronic liver failure (ACLF) is thought to be mediated by overwhelming systemic inflammation triggered by both microbial and non-microbial factors. Therapeutic plasma exchange (TPE) has been proven to be an efficacious therapy in autoimmune conditions and altered immunity, with more recent data supporting its use in the management of liver failure. Few therapies have been shown to improve survival in critically ill patients with liver failure who are not expected to survive until liver transplantation (LT), who are ineligible for LT or who have no access to LT. TPE has been shown to reduce the levels of inflammatory cytokines, modulate adaptive immunity with the potential to lessen the susceptibility to infections, and reduce the levels of albumin-bound and water-bound toxins in liver failure. In patients with acute liver failure, high volume TPE has been shown to reduce the vasopressor requirement and improve survival, particularly in patients not eligible for LT. Standard volume TPE has also been shown to reduce mortality in certain sub-populations of patients with ACLF. TPE may be most favorably employed as a bridge to LT in patients with ACLF. In this review, we discuss the efficacy and technical considerations of TPE in both acute and acute-on-chronic liver failure.

Key Words: Therapeutic plasma exchange; High volume plasma exchange; Acute liver failure; Acute-on-chronic liver failure; Cirrhosis; Liver transplantation; Cytokines

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Core Tip: Multi-organ failure accompanying liver failure is mediated by overwhelming systemic inflammation and altered host immunity. Therapeutic plasma exchange has been proven to be an efficacious therapy in autoimmune conditions and altered immunity. We review the efficacy and technical considerations of therapeutic plasma exchange in both acute and acute-on-chronic liver failure.

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INTRODUCTION

Acute liver failure (ALF) and acute-on-chronic liver failure (ACLF) are two distinct classifications of severe hepatic dysfunction associated with secondary multi-organ failures (MOFs), both of which effect significant morbidity and mortality[1-4]. The exact mechanisms by which MOFs are mediated have not been definitively established but are thought to be driven by excessive systemic inflammation and dysregulated immune activation triggered by both microbial and non-microbial factors, and less so by the primary insult to the liver[3,5-7].

The pathogenesis of MOFs in ALF has been attributed to the release of damage-associated molecular patterns (DAMPs) from injured hepatic cells and microbial pathogen-associated molecular patterns (PAMPs) in the presence of superimposed infection or bacterial translocation[7]. The innate immune cells activated by PAMPs and DAMPs produce proinflammatory cytokines [interleukin (IL)-6, IL-1ß, IL-8, tumor necrosis factor-alpha (TNF-α)] that mediate systemic inflammation and further hepatocyte injury[7,8]. In support of this hypothesis, levels of TNF-α and IL-6 have been shown to be significantly higher in patients with fulminant hepatitis when compared to patients with acute liver injury[9].

Similarly, the hallmark of the ACLF clinical syndrome is excessive systemic inflammation and bacterial translocation mediated by PAMPs and DAMPs[1,6,10]. ACLF patients have been shown to manifest elevated levels of pro- and anti-inflammatory cytokines, as well as white blood cell count and C reactive protein. Moreover, there is a proven correlation between cytokine levels and number of organ failures in ACLF[6,11].

Despite advances in the supportive medical management of patients with liver failure, significant morbidity and mortality persist[12,13]. Urgent liver transplantation (LT) remains the definitive treatment in patients with high likelihood of death; however, access to transplant remains limited. In addition, eligibility for transplant can be hampered by psychosocial factors, active substance use, and progressive MOFs that may preclude safe LT or contribute to mortality while awaiting LT[14,15]. Expanded treatment options are needed to bridge critically ill patients to LT or to preserve liver function when LT is either contra-indicated or unavailable. Therapeutic plasma exchange (TPE) has been proposed as a beneficial treatment modality in these patients. The practice of exchange transfusion in patients with cirrhosis dates back to the 1960s when exchange blood transfusion was employed for the treatment of hepatic coma [16]. Therapies were later modified to TPE as apheresis equipment became more widely available and as a means to reduce the risks associated with whole blood transfusion[17,18]. Historically, TPE in liver failure has been primarily described in case series and cohort studies. The first randomized control trial (RCT) describing the utility of TPE in ALF patients was reported in 2016 by Larsen *et al*[19].

TPE in liver failure requires the extracorporeal removal of large compounds from the blood, including albumin-bound and water-soluble toxins and replacement with plasma and/or albumin. As shown in Figure 1, these toxins include cytokines, endotoxins, bilirubin, bile acids, ammonia, and aromatic amino acids[20,21]. These substances have been proposed as important mediators of both hepatic encephalopathy (HE) and MOFs in ALF and ACLF[8,22-24]. By comparison, extracorporeal albumin dialysis (ECAD) systems remove albumin-bound and water-soluble toxins *via* hemodialysis augmented by an albumin-infused dialysate with or without the addition of adsorption columns (charcoal filter and anion exchange resins). These

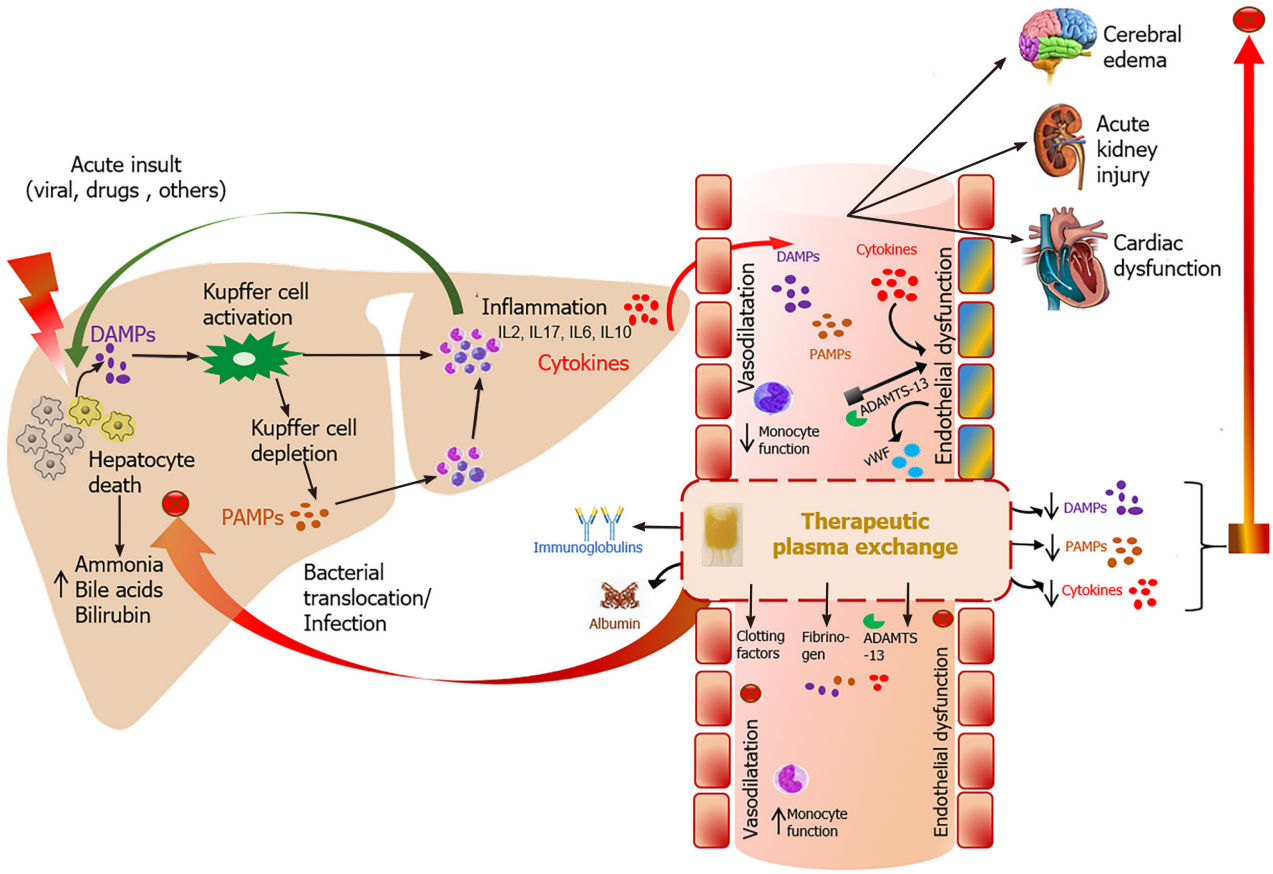

Figure 1 Theoretical model depicting the therapeutic effects of therapeutic plasma exchange in liver failure.

ECAD systems include the molecular adsorbent recirculation system (MARS), single pass albumin dialysis, and fractionated plasma separation and adsorption[25-27].

When considering the therapeutic differences between TPE and ECAD, MARS in particular has been recognized to be more costly than TPE and can entail a more logistically complex initiation. Furthermore, the MARS filter-membrane dictates a size selection threshold of approximately 50 KDa[28], whereas TPE is capable of removing larger molecular proteins, including antibodies, immune complexes, and lipoproteins [29]. To date, no head-to-head adult clinical trial has directly compared TPE with MARS or any of the ECAD systems. However, in a retrospective single center pediatric study comparing MARS with the combination of TPE and hemodialysis, TPE and hemodialysis effected a greater reduction in bilirubin, ammonia, and international normalized ratio[30]. Another theoretical advantage of TPE over ECAD hinges on the exchange of plasma, which replaces plasma proteins, including clotting factors, that may be decreased as a result of impaired hepatic synthetic function in both ALF and ACLF.

EFFECT ON BIOCHEMICAL PARAMETERS AND CLINICAL OUTCOMES

Acute liver failure

TPE has been shown to reduce levels of circulating inflammatory cytokines, improve hemodynamics, and improve transplant-free survival in ALF[9,19,31-33]. While encouraging, head-to-head comparisons between the studies supporting these findings have been challenging due to the broad variation in treatment protocols. Often the volume of exchange, treatment frequency and duration of therapy vary between studies.

Specifically, TPE has been shown to moderate TNF-α, histone-associated DNA (member of the DAMP family), IL-6, IL-8, endotoxins, bilirubin, ammonia, and to improve coagulopathy[9,19,34]. In addition, TPE modulates adaptive immunity in ALF through the reduction of soluble B7 molecules, particularly sCD86[35]. Soluble B7 molecules are produced by injured hepatocytes and increase the expression of

cytotoxic T-lymphocyte-associated protein 4 on CD4+ T cells, resulting in impaired antimicrobial responses and increased susceptibility to infections[35].

In the only RCT designed to study outcomes associated with high volume TPE (HV-TPE) in ALF, patients who received HV-TPE manifested significantly improved mean arterial blood pressure (MAP) with associated reduction in vasopressor requirement when compared to patients who received standard medical therapy (SMT) only[19]. In the same study, plasma creatinine remained stable in the HV-TPE group but increased significantly in the SMT group. Accordingly, fewer HV-TPE patients required renal replacement therapy when compared to those who received SMT. In contrast, Wiersema *et al*[31] reported no significant reduction in vasopressor requirement in ALF patients receiving TPE, despite reporting significantly improved MAP on therapy. Notably, this single arm, single centered study employed standard volume TPE as opposed to HV-TPE.

In addition to hemodynamic benefits, TPE has been shown to reduce ammonia level, improve HE grades and cerebral hemodynamics independent of simultaneous filtration or dialysis[33,36]. However, TPE has not been shown to effect significant differences in intracranial pressure (ICP) in ALF, though few patients in Larsen's study underwent invasive ICP assessment (32 of the randomized 182 patients)[19]. On the contrary, a retrospective review of 43 patients with Wilsonian-ALF who received HV-TPE manifested no improvement in ammonia or creatinine levels, but did demonstrate improved transplant-free survival at 90 d[37].

Finally, Larsen's RCT in ALF demonstrated a significant improvement in transplant-free survival in patients who received HV-TPE when compared to SMT [hazard ratio (HR) 0.56, 95% confidence interval (CI) 0.36-0.86, P = 0.0083], with no difference in outcomes between paracetamol and non-paracetamol etiology of liver failure[19]. In subgroup analysis of the same study, HV-TPE was shown to specifically improve survival among patients not listed for LT due to contraindications. By contrast, no survival benefit was identified in patients who received HV-TPE as a bridge to LT. Other non-randomized studies in ALF have reported improvement in survival days with TPE in non-transplanted patients[38,39]. There have been no studies to date that have examined the combination of TPE with any of the ECAD systems in ALF patients.

Acute on chronic liver failure

Patients with ACLF have been shown to manifest significantly higher levels of cytokines (TNF- alpha, IL-10, IL-2, IL-4, and IFN-Y) compared to healthy controls. These same cytokines are also effectively reduced after TPE[40]. In the same study by Mao *et al*[40], higher cytokine levels predicted poor prognosis irrespective of the treatment received. Moreover, bilirubin levels, coagulopathy, and ammonia levels have been shown to improve after TPE-based therapy[41-43]. The effect of TPE on blood pressure and vasopressor requirement in ACLF patients has not been reported. In their single center and small sample size study, Stahl et. al. reported no difference in vasopressor requirement between patients who underwent TPE *vs* SMT[44].

TPE has been shown in limited series to improve survival in ACLF; however, this data is limited by protocol variation. Many of these studies have been performed in Asia among patients with hepatitis B virus- (HBV) related ACLF, used different definitions for ACLF, combined TPE with other liver support systems, and were single center retrospective studies[42,45-47]. Tan *et al*[48] reported improved survival with TPE-based therapies (combined with other extracorporeal therapy) compared to SMT in non-transplanted patients at 30 d and 90 d with a pooled odds ratio (OR) of 0.60 [95%CI: 0.46-0.77]. In the only RCT of TPE in ACLF, patients with HBV ineligible for LT who received TPE-based therapies manifested significantly improved survival rates when compared to patients who received SMT (60% *vs* 47%, P < 0.05) at 90 d[47]. In addition, Mao *et al*[45] demonstrated improved survival with TPE among patients with HBV-ACLF and model for end-stage liver disease (MELD) scores between 20-30 (50%) when compared to patients with MELD scores above 30 (31.7%)[45]. Whether the results of these studies can be extrapolated and generalized to the ACLF patient population at large remains uncertain. Stahl *et al*[44] retrospectively studied the differences in outcomes between ACLF patients bridged to LT *vs* patients bridged to spontaneous recovery. In this study, the risk of 30-d mortality was significantly lower in LT candidates (bridge to transplant group) than in non-transplant candidates (recovery strategy group) treated with TPE (HR 0.35, 95%CI 0.14-0.87, P = 0.024).

As described above, TPE is commonly combined with another dialysis modality depending on the individual patient profile (coagulopathy, renal function, HE, or water and/or electrolyte imbalance). Although continuous renal replacement therapy (CRRT), without TPE, is commonly employed in liver failure-induced severe hyperam-

monemia to reduce the risk of cerebral edema and intracranial hypertension (ICH)[49,50], no head-to-head comparison study has yet been done to compare ammonia clearance in TPE *vs* CRRT. Among patients with HBV-ACLF, Yao *et al*[43] compared TPE with double plasma molecular adsorption (DPMAS) therapy, a special broad-spectrum adsorption column that binds inflammatory mediators and bilirubin. Their group found a significantly higher rate of 28-d survival in the TPE with DPMAS group compared with TPE alone (57.4% *vs* 41.7%, $P = 0.043$) only among patients with intermediate and advanced stage ACLF (defined as prothrombin activity less than 30%)[43]. Separate studies have shown that DPMAS alone or in combination with TPE in ACLF does not confer survival benefits despite increasing the clearance of bilirubin [42,43].

Severe acute alcohol-associated hepatitis (SAH) is recognized to be a common precipitant of ACLF[5]; however, TPE has not been specifically studied in this important patient population. Moreover, sub-group analysis of the limited number of patients with alcohol-associated liver disease included in the available trials has not been described. Case reports suggest that TPE with standard medical therapy may lead to clinical improvement in patients with SAH[51,52]. Randomized, controlled trials in patients with SAH are needed to better define the therapeutic effect of TPE for this indication.

TECHNICAL ASPECTS

TPE can be performed by either centrifugation or filtration-based mechanisms. Centrifugation separates the blood into its components using density, whereas filtration uses a hollow fiber design to separate the plasma from the cellular components. Both centrifugation and filtration-based systems are similar in safety, efficiency, therapeutic effects[53,54], and are approved by the Food and Drug Administration for use in the United States. TPE is usually provided in collaboration with nephrologists or hematologists depending on the center's preference.

REPLACEMENT FLUID, VOLUME, AND DURATION

Acute liver failure

Typical TPE treatments exchange 1 to 1.5 times the patient's estimated plasma volume, approximately 3 L in an average sized adult. For reference, a plasma volume is an estimate of the total volume of plasma in an individual and is a common unit of measurement in therapeutic apheresis procedures. Plasma volume can be calculated from estimated total blood volume using common physiological variables, including an individual's sex, height, weight, body muscle composition, and hematocrit[55]. The removal of substances using TPE follows the formula: $y/y_o = e^{-x}$, where y and y_o are the concentration of the removed substance after and before plasma exchange and x is the number of plasma volumes processed[56]. A 1 to 1.5 plasma volume exchange will remove approximately 70% of the substances in the intravascular space[56].

The only RCT comparing TPE and SMT in ALF patients studied HV-TPE, defined as plasma replacement at 15% of ideal body weight or 8 to 12 L per session[19]. HV-TPE should remove approximately 90%-98% of the toxins in the intravascular space. The majority of studies on TPE in ALF patients before this RCT treated one plasma volume (2 L to 4 L) during each exchange[38,57-59]. Recently, Stahl *et al*[60] in their single center study compared 20 patients with ALF who received low volume TPE and SMT with 20 matched historical controls who received SMT only. TPE volume exchange was employed using 3 L to 4L per session daily until clinical improvement or LT. No head-to-head comparison of standard volume and HV-TPE in ALF has been performed, but the current evidence favors HV-TPE for ALF[61,62].

There is also no consensus or evidence-based strategy for the frequency and duration of treatment. A small single center study showed that one treatment session of TPE is associated with improvement in biochemical parameters and survival in patients with Wilsonian ALF[37]. The RCT by Larsen et. al performed HV-TPE for 3 consecutive days[19]. Other studies employed either the same regimen or every other day treatments, and continued until the patient improved clinically, died, or underwent LT[63-65]. The most commonly used replacement fluid is plasma, although albumin or plasma substitute is sometimes used in conjunction with plasma[66-69]. However, no studies have used albumin alone as a replacement fluid. Plasma is typically chosen as a replacement fluid as it contains coagulation factors and is

thought to replenish those missing as a consequence of the underlying liver dysfunction.

Acute on chronic liver failure

All studies in the ACLF population have used standard volume replacement ranging from 2 L to 4.5L exchange per session. Most studies utilized plasma as replacement fluid and performed TPE sessions 2 to 3 times per week and continued until clinical improvement, transplant, or death[41,70-72]. Only one study reported daily plasma exchange, but the proportion of the study population that received daily exchanges was not described[41].

ANTICOAGULATION

Sodium citrate and heparin are the two common anticoagulants employed to prevent clotting of the extracorporeal circuits. The patient's clinical condition and physician's preferences guide selection; both agents can be used if a single agent is inadequate for anticoagulation. Citrate is preferred because of its shorter half-life of 30-60 min, favorable safety profile, rapid reversibility with intravenous calcium, and its minimal systemic anticoagulation effect[73]. Sodium citrate undergoes hepatic and renal metabolism. Patients with liver failure are particularly susceptible to citrate toxicity as a consequence of impaired hepatic metabolism, often exacerbated by concomitant renal impairment. Citrated plasma replacement fluid can further worsen the risk of procedural hypocalcemia. Citrate is partly cleared by the kidney and can be safely utilized in acute kidney injury as long as the acid-base balance is closely monitored[74,75]. In a single study, tandem procedure with dialysis reduced the risk of citrate toxicity in ACLF patients undergoing TPE[76].

Common adverse effects of citrate include hypocalcemia (with or without symptoms) and metabolic alkalosis. Symptomatic hypocalcemia is not uncommon and occurs in 1.5% to 9% of all patients undergoing TPE[74]. Notably patients receiving TPE for liver failure are at increased risk of hypocalcemia due to the associated metabolic impairment. Prophylactic calcium replacement based on citrate load and continuous ionized calcium monitoring is recommended[29]. Supplementation with Calcium gluconate or Calcium chloride can reduce the risk of symptomatic hypocalcemia[77].

Some physicians favor heparin because of the associated risks with citrate as described above. The application of both unfractionated and low molecular weight heparin have been reported[78,79]. Nevertheless, most patients can undergo filtration-based TPE without the need for anticoagulation similar to anticoagulation-free hemodialysis and hemofiltration[80-82].

COMBINATION WITH OTHER EXTRACORPOREAL THERAPY

Acute kidney injury requiring CRRT is a common manifestation of MOF in both ALF and ACLF[83-85]. In addition, CRRT is commonly utilized in patients with severe hyperammonemia to reduce the risk of ICH and cerebral edema[49,50,86]. CRRT is usually delivered over 24 h and the interruption of CRRT for TPE may compromise the duration of CRRT. Moreover, additional vascular access for TPE exposes the patient to the otherwise avoidable risk of catheter related complications. Simultaneous dialysis and TPE was first introduced in 1999; descriptions of the safety and feasibility of the combined therapies are limited to case reports and case series[21,87-90]. There are no defined standards for connection; tandem procedures connected in series or parallel have been reported in the literature[21,80,87,75,91]. These tandem connections have the advantage of minimizing vascular access procedures.

The combination of TPE with other extracorporeal therapies aside from CRRT in adults is not well described. In a randomized controlled study from Huang *et al*[92], MARS in combination with TPE was shown to reduce serum total bilirubin more effectively when compared with MARS monotherapy. There was no significant difference in survival between the two groups. However, the theoretical benefit of MARS therapy combined with TPE is unclear, as both therapies rely on the removal of albumin-bound toxins. TPE employed simultaneously with extracorporeal membrane oxygenation (ECMO) in adults with liver failure has not been reported. However, tandem ECMO, TPE, and CRRT combination therapy has been described in the

pediatric population with sepsis-induced multiorgan failure[93].

COMPLICATIONS

The common complications associated with TPE are related to the choice of anticoagulation, replacement fluid, and vascular access. This includes citrate-induced hypocalcemia, hemodynamic instability, and transfusion reactions. In their RCT of HV-TPE in ALF patients, Larsen et. al found no significant differences in cardiac arrhythmias, pancreatitis, transfusion related acute lung injury, acute respiratory distress syndrome, hemorrhage, and infection between patients who received HV-TPE *vs* SMT[19]. A prospective study comparing HV-TPE with SMT in Wilsonian ALF similarly demonstrated no significant difference in the incidence of complications[37]. In addition, TPE has been shown to be safe and tolerable in ACLF patients; severe procedure-related adverse effects have not been reported[44,47]. An open label RCT in ACLF patients reported a higher rate of hypotension in patients who received TPE-based therapy compared to SMT (20.2% *vs* 9.2%, $P = 0.02$)[46]. Moreover, there were no significant differences in the rates of bleeding, infection, and respiratory failure between groups[47].

CURRENT GUIDELINES

The 2019 American Society for Apheresis (ASFA) has recommended HV-TPE as a first line therapy for ALF and fulminant Wilson disease. In ALF, ASFA recommends performing at least 3 HV-TPE procedures daily and to consider performing daily treatments until LT or liver recovery. In fulminant Wilson disease, daily standard volume plasma exchange treatments until LT or liver recovery is recommended[61]. The 2016 European Association for the study of liver disease recommended HV-TPE as a level I, grade I evidence in ALF, but no recommendation has been made for ACLF [62]. The 2011 American Association for the Study of Liver Disease guidelines suggested plasma exchange as a means to acutely lower serum copper and limit copper-mediated kidney damage in Wilsonian ALF while waiting LT. However, no recommendation was made for the general use of TPE in ALF and ACLF patients[94].

CONCLUSION

Advanced therapies aimed at improving survival in liver failure rely on the removal of toxins and inflammatory mediators while simultaneously supporting the synthetic and metabolic function of the liver while awaiting either LT or spontaneous hepatic regeneration. Although no ideal extracorporeal liver replacement therapy yet exists, TPE remains a safe, reliable, and feasible treatment. Future studies should replicate the survival benefit demonstrated by Larsen *et al*[19], examine the role of combination therapies with ECADs, identify which etiologies of ALF and ACLF are best served by TPE, and confirm the optimal exchange volume, frequency, and duration of treatment.

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MINIREVIEWS

Association of non-alcoholic fatty liver disease and COVID-19: A literature review of current evidence

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has swept through nations, crippled economies and caused millions of deaths worldwide. Many people diagnosed with COVID-19 infections are often found to develop liver injury, which, in a small portion of patients, progresses to severe liver disease. Liver injury in the form of elevated transaminases, hyperbilirubinemia and alterations in serum albumin has been observed to be higher in patients with severe forms of the disease. Those who already have insult to the liver from chronic disease, such as nonalcoholic fatty liver disease (NAFLD) may be at the greatest disadvantage. The severity of COVID-19 also seems to be driven by the presence of NAFLD and other co-morbidities. About 25% of the global population has NAFLD. With such a widespread prevalence of NAFLD, understanding the disease progression of COVID-19 and the occurrence of liver injury in this vulnerable population assumes great significance. In this review, we present an overview of COVID-19 infection in patients with NAFLD.

Key Words: SARS-CoV-2; Fatty liver; Mitochondria; Nitrosative stress; Oxidative stress; COVID-19; Metabolic associated fatty liver disease; Nonalcoholic fatty liver disease; Progressive liver disease; Nonalcoholic steatohepatitis

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Core Tip: Liver injury in the form of elevated transaminases and hyperbilirubinemia in coronavirus disease 2019 (COVID-19) may be attributed to multiple factors, including the presence of pre-existing liver disease. The presence of nonalcoholic fatty liver disease (NAFLD) in patients with COVID-19 is likely to make them susceptible to severe forms of liver injury. Given the high prevalence of NAFLD worldwide, it is important to understand the implications of COVID-19 in such patients including role of comorbidities, disease progression, and the severity of COVID-19.

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INTRODUCTION

The worldwide figures of coronavirus disease 2019 (COVID-19) presently stand at 154640649 confirmed cases with 3232285 deaths[1]. Although primarily a respiratory syndrome, COVID-19 has been reported to cause liver injury in multiple studies, including metanalyses[2-4]. The incidence of liver injury as assessed by several indicators like transaminases, bilirubin and albumin has been found to be higher in patients with severe COVID-19 infection[3,5]. Increasing severity of liver chemistry abnormalities on hospital admission predicts early in-hospital mortality in COVID-19 patients[4].

There is a high global burden of pre-existing liver disease[6], including chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD) and alcohol-associated liver disease (ALD). For example, in China, where the pandemic originated, liver cirrhosis affects around 7 million people[7]. Similarly, in the United States which has the highest number of recorded COVID 19 cases, about 4.5 million of adults are diagnosed with chronic liver disease[8]. In a cross-sectional analysis based on data from National Health and Nutrition Examination Surveys (NHANES), it was observed that the prevalence of NAFLD (by US-Fatty Liver Index) spiked from 20.0% (1988-1994) to 28.3% (1999-2004) to 33.2% (2009-2012) and 31.9% (2013-2016)[9]. This increasing trend is in concurrence with increases in obesity, diabetes mellitus, hypertension and insulin resistance[9]. It is also to be noted that many patients with fatty liver disease remain undiagnosed and are incidentally detected. Therefore, the actual prevalence of NAFLD may be much higher. In such a background of widespread prevalence of chronic liver disease especially NAFLD, the incidence of liver injury in COVID-19 and its impact on disease progression assumes greater significance. In a recent study, we found that mortality associated with the known risk factors of COVID19 (hypertension, diabetes, male sex, and old age) was accentuated in the presence of liver chemistry abnormalities in those diagnosed with COVID-19[4].

PATHOGENESIS AND PATTERN OF LIVER INJURY IN COVID-19

The pathogenesis of liver injury in COVID-19 is multifactorial. A number of factors have been identified for perpetuating and potentiating liver injury in COVID-19. Direct viral-mediated hepatocyte injury, liver injury ensuing from cytokine release syndrome, drug-induced liver injury and ischemic hepatitis are just some of the mechanisms responsible for hepatic dysfunction in COVID-19[10]. The pattern of liver injury in COVID-19, as evidence from multiple studies, is a rise in liver enzymes [primarily aspartate aminotransferase and alanine aminotransferase (ALT)] with mild increases in bilirubin[10]. In a study by Cai *et al*[11] from China, among 417 patients, 20.75% had hepatocellular pattern of liver injury, 29.25% had a cholestatic pattern, while 43.4% had a mixed type of liver injury. Liver injury is transient in most cases and liver enzymes usually return to normal with recovery from COVID-19[2]. The rampant use of multiple medications-antibiotics, antivirals, nonsteroidal anti-inflammatory drugs, herbal medications, interferon and other immunomodulators has been as-

sociated with increased liver test abnormalities[11]. To add to this is the presence of pre-existing liver disease in patients with COVID-19 which makes the pathogenesis of hepatic dysfunction even more complex. In the largest reported cohort of 745 chronic liver disease and cirrhotic patients with COVID-19, it was observed that baseline liver disease stage and ALD were independent risk factors for death from COVID-19[12]. The APASL COVID19 Liver Injury Spectrum (APCOLIS) Study has shown that pre-existing liver disease is associated with poor outcome in patients with SARS CoV2 infection. Additionally, if these patients also have chronic liver disease, diabetes and/or obesity, they are more vulnerable and should be closely monitored[13]. In a study on 12 COVID-19 patients with pulmonary embolism on autopsy, hepatic steatosis involving 50-60 percent of hepatocytes was found in all patients. This data supports the fact that pre-existing liver diseases like ALD and NAFLD could play significant roles in COVID-19 progression[14].

COVID-19 IN THE SETTING OF NAFLD

Whether NAFLD is an independent or dependent determinant for worse outcomes in COVID-19 has been a hot topic of debate in recent times. A look at the figures and the results of several studies done in the midst of this pandemic opens up conflicting and debatable viewpoints in this regard. Interestingly, in this above-mentioned cohort of 745 patients, 43% of patients had NAFLD, while hypertension, diabetes and obesity — established risk factors for developing severe COVID-19 — constituted the major comorbidities[12]. While one can argue that it is ALD and not NAFLD which has been observed to be a significant predictor of mortality in COVID-19, it would be worthwhile to take note of the fact that patients with ALD had more severe underlying liver disease compared to those with NAFLD. In a retrospective study on 202 patients with confirmed COVID-19, it was observed that patients with NAFLD had a higher risk of disease progression, greater likelihood of abnormal liver function from admission to discharge and longer viral shedding time[15]. An association between the presence of metabolic associated fatty liver disease (MAFLD) and COVID-19 severity was observed in younger patients[16]. In another study on 589 patients from the eastern Mediterranean region, NAFLD has been found to be a predictor of liver injury in COVID-19. However, quite contrary to the results of other studies, NAFLD did not seem to be an independent predictor of mortality, disease severity, or markers of disease progression[17]. Similarly, in another study by Huang *et al*[18], although more patients with NAFLD developed abnormal liver function tests, concurrent NAFLD was not found to be associated with adverse clinical outcomes in patients with COVID-19. Table 1 shows a summary of the various studies describing the association between NAFLD and COVID-19.

MECHANISM OF COVID-19 PROGRESSION IN PATIENTS WITH NAFLD

The role of inflammation in the pathophysiology of NAFLD has been well recognized [19]. It has been hypothesized that hepatic inflammation resulting from pro-inflammatory cytokines released by adipose tissue is even furthered by COVID-19[15]. The liver is a major site of lipid metabolism and the generation of lipid species plays an important role in regulating metabolic inflammation. The complex pathways in lipid metabolism drive innate immunity and have been found to affect the progression to steatohepatitis and fibrosis in NAFLD[20]. Additionally, NAFLD patients are found to have elevated plasma levels of von Willebrand factor and circulating plasminogen activator inhibitor type 1[21]. This has been hypothesized to predispose such patients to higher risks of adverse cardiovascular events. It has also been postulated that hepatic and systemic immune responses due to underlying NAFLD could contribute to the cytokine storm in younger patients with COVID-19[16,22]. While severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin-converting enzyme 2 (ACE2) receptors and attaches to the cell, cellular entry is made possible by cleavage of the SARS-CoV-2 spike protein by transmembrane serine protease 2 (TMPRSS2)[23]. Interestingly, it has been seen that while there were no differences in liver mRNA expression of both ACE2 and TMPRSS2 between subjects without liver injury and patients with only steatosis, upregulation of these genes occurred in obese patients with nonalcoholic steatohepatitis (NASH). Additionally, there was positive correlation of ACE2 and TMPRSS2 with NAFLD activity score and TMPRSS2 positively correlated with weight, body mass index (BMI) and cholesterol[24]. However,

Table 1 Summary of various studies describing the association between nonalcoholic fatty liver disease and coronavirus disease 2019

Ref.	Type of study	Study origin	Number of COVID patients/number of NAFLD/NASH patients	Overall impact of occurrence of concomitant NAFLD and COVID-19	Impact of NAFLD on COVID-19 liver injury
Marjot *et al*[12]	Retrospective	Multinational Cohort	No. of COVID patients with CLD: 745; No. of NAFLD patients: 322	Baseline liver disease stage and ALD are independent risk factor for death from COVID-19	NA
Sarin *et al*[13]	Retrospective	Multinational Cohort	No. of COVID patients with CLD: 228; No. of fatty liver disease patients: 113	CLD patients with diabetes and obesity are more vulnerable and should be closely monitored	Comorbidities like MAFLD, obesity and diabetes were present in 80% of the patients. MAFLD was the commonest cause for CLD without cirrhosis. Obese cirrhotics had more acute liver injury than normal weight patients [OR 8.9 (95%CI: 1.9-38.8) P = 0.02]. Patients of CLD with diabetes had higher risk [57.7% *vs* 39.7%, P = 0.01, OR = 2.061.14-3.73)] of liver injury
Ji *et al*[15]	Retrospective	China	No. of COVID patients: 202; No. of NAFLD patients: 76	Patients with NAFLD also had a higher risk of progression to severe COVID-19 and longer viral shedding time	Patients with NAFLD had a higher likelihood of abnormal liver function from admission to discharge [70% (53/76) *vs* 11.1% (14/126); P < 0.0001] compared to patients without NAFLD
Zhou *et al*[16]	Retrospective	Wenzhou, China	No. of COVID patients: 327; No. of patients with fatty liver disease: 93	In patients younger than 60 yr, a more than 2-fold higher prevalence of severe COVID-19 was observed in those with MAFLD compared to those without. MAFLD was not associated with disease severity in multivariable analysis in elderly patients	NA
Mushtaq *et al*[17]	Retrospective	Qatar	No. of COVID patients: 589; No. of NAFLD patients: 320	NAFLD was not an independent predictor of mortality, disease severity on presentation, or disease progression in patients with COVID-19	Presence of NAFLD was a predictor of the development of mild liver injury (OR 2.99; 95%CI: 1.62-4.37; P = 0.000) and moderate liver injury (OR 5.104; 95%CI: 3.21-6.99; P = 0.000)
Huang *et al*[18]	Retrospective	Jiangsu, China	No. of COVID patients: 280; No. of NAFLD patients: 86	No patient developed severe liver-related complications during hospitalization	Concurrent NAFLD was identified as a risk factor of elevated ALT (OR, 2.962; 95%CI: 1.745-5.028; P < 0.001) on univariate analysis. Concurrent NAFLD (OR, 2.956; 95%CI: 1.526-5.726; P = 0.001) was an independent risk factor of ALT elevation on multivariate analysis
Fondevila *et al*[24]	Retrospective	Spain	No. of patients without NAFLD: 17; No. of patients with NAFLD: 77	Obese patients with NASH show markedly higher expression of *ACE2* and *TMPRSS2*, suggesting that advanced stages of NAFLD might predispose individuals to COVID-19	NA
Biquard *et al*[25]	Retrospective	France	No. of patients without fatty liver disease: 28; No. of patients with fatty liver disease: 26	MAFLD is not associated with changes in liver expression of genes implicated in SARS-CoV-2 infection	NA
Zheng *et al*[28]	Retrospective	Wenzhou, China	No. of COVID patients: 214; No. of NAFLD patients: 66	Risk of obesity to COVID-19 severity is greater in those with compared to those without MAFLD	NA
Ghoneim *et al*[29]	Retrospective	Multination electronic health records	No. of COVID patients: 8885; No. of NAFLD patients: 102	The adjusted odds ratio of having COVID-19 were higher in patients if they were diagnosed with NASH	NA
Targher *et al*[33]	Retrospective	Zhejiang Province, China	No. of COVID patients: 310; No. of NAFLD patients: 94	Patients with MAFLD with increased FIB-4 or NFS are at higher likelihood of having severe COVID-19 illness, irrespective of metabolic	COVID-19 patients with MAFLD with intermediate or high FIB-4 scores were more likely to have higher liver enzymes [AST > 40 IU/L (%) -27.8/57.1, ALT > 40 IU/L (%) -30.6/42.9],

				comorbidities	compared with their counterparts with low FIB-4 score or those without MAFLD [AST > 40 IU/L (%) 7.9/9.1, ALT > 40 IU/L (%) -13/29.6], $P < 0.001$
Forlano *et al*[34]	Retrospective	Imperial College Healthcare NHS Trust (London, United Kingdom)	No. of COVID patients: 193; No. of NAFLD patients: 61	Presence of NAFLD *per se* was not associated with worse outcomes in hospitalised patients. Mortality was associated with pronounced inflammatory response in NAFLD group	NA
Gao *et al* [35]	Retrospective	3 Chinese hospitals: (the First Affiliated Hospital of Wenzhou Medical University, the Ningbo No. 2 Hospital, and the Ruian People's Hospital)	No. of COVID-19 patients: 167; No. of MAFLD patients: 46	MAFLD patients with elevated serum IL-6 levels at admission are at higher risk for severe illness from COVID-19	NA
Sachdeva *et al*[37]	Pooled analysis	-	No. of COVID patients: 8142; No. of NAFLD patients: 833	NAFLD is a predictor of severe COVID-19, even after adjusting for the presence of obesity	NA

NAFLD: Nonalcoholic fatty liver disease; COVID-19: Coronavirus disease 2019; CLD: Chronic liver disease; ALD: Alcohol-associated liver disease; NA: Not available; MAFLD: Metabolic associated fatty liver disease; CI: Confidence interval; OR: Odds ratio; ALT: Alanine aminotransferase; NASH: Nonalcoholic steatohepatitis; ACE2: Angiotensin-converting enzyme 2; TMPRSS2: Transmembrane serine protease 2; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; AST: Aspartate aminotransferase; IL-6: Interleukin-6.

to complicate matters, in another study by Biquard *et al*[25], none of the genes necessary for SARS-CoV-2 infection-TMPRSS2 and ACE2 included- were differentially expressed between lean or obese controls and patients with simple steatosis or with NASH. Hence the role of underlying NAFLD on the outcomes of COVID-19 infection is still up for debate.

ROLE OF COMORBIDITIES

In such a background of conflicting data, it is worthwhile to analyze the role of comorbidities that are present in patients with NAFLD which might lead to disease progression in COVID-19. It needs no reiteration that NAFLD is usually accompanied by a cluster of several other conditions such as obesity, insulin resistance, dyslipidemia and hypertension, collectively reflecting underlying metabolic syndrome (MS). According to the ATP III criteria, the prevalence of the MS in patients with NAFLD is 22.8%[26]. The strong association between MS and NAFLD has led investigators to term NAFLD the hepatic component of MS[27]. Thus, it is entirely understandable that the presence of these components would potentially cause increased severity of COVID-19. This has been validated by a multicentric study by Zheng *et al*[28] which showed that obesity conferred a nearly sixfold higher risk of severe COVID-19 in patients with NAFLD. A strong positive association between the different components of MS and COVID-19 has also been reported in a population-based study[29]. Obesity and a state of insulin resistance impairs the ability to mount an effective immune response and predisposes to viral infections and respiratory diseases[30,31]. The questions that naturally arise from these observations are: (1) Do the different components of MS drive outcomes in COVID-19 infection and is NAFLD merely a bystander? and (2) Does NAFLD independently drive inflammation and disease progression in COVID-19? The latter is supported by the finding that NAFLD is associated with 30-d all-cause mortality in patients with community-acquired pneumonia with a significant higher degree of association in patients with advanced hepatic fibrosis[32].

IS NAFLD INDEPENDENTLY ASSOCIATED WITH COVID-19 SEVERITY?

In the population-based study by Ghoneim *et al*[29], among different components of MS, NASH was found to be associated with the highest risk of COVID-19 after calculating the adjusted odds ratio. A study by Targher *et al*[33] sheds some light on

this conundrum. In this study on 310 COVID-19 patients, subjects with MAFLD with increased fibrosis-4 (FIB-4) or NAFLD fibrosis score were more likely to have severe COVID-19 illness, irrespective of metabolic comorbidities like obesity and diabetes. Forlano *et al*[34] showed that although NAFLD patients have higher levels of inflammatory markers like CRP compared to the non-NAFLD group, the presence of NAFLD *per se* was not associated with adverse outcomes in the whole study population. Additionally, the presence of intermediate/high-risk FIB-4 scores as well as the presence of liver cirrhosis did not demonstrate any association with adverse outcomes in the NAFLD cohort[34]. Furthermore, a study by Gao *et al*[35] showed that patients with MAFLD and elevated serum interleukin-6 levels at admission are at higher risk for severe illness from COVID-19. However, mortality in the NAFLD cohort was associated with a pronounced inflammatory response. Therefore, what could be inferred from these results is that rather than attributing the severity of COVID-19 to underlying liver disease, it might possibly be a result of the general state of host inflammation in NAFLD patients. Increased liver fat has been independently associated with a higher likelihood of testing positive for COVID-19 in a United Kingdom based study[36]. In a pooled analysis on the association of fatty liver and COVID-19, it was found that NAFLD was associated with an increased risk of severe COVID-19, even after adjusting for obesity as a possible confounding factor[37]. From these results, one is led to believe that NAFLD is indeed independently associated with increased severity in COVID-19. Whether it is the liver disease that is responsible for this increasing severity, the general state of inflammation that accompanies NAFLD or the associated comorbidities that drives the outcome is a matter of debate. Interestingly, a recent study showed that the presence of fibrosis rather than the presence of MAFLD is associated with increased risk for mechanical ventilation, development of acute kidney injury, and higher mortality in COVID-19 patients[38].

LEAN *VS* OBESE NAFLD IN COVID-19

While a BMI greater than 23 kg/sq. metres increases the risk of developing fatty liver disease[39], many people with normal BMI's are capable of developing NAFLD. Additionally, significant proportion of NAFLD patients do not have insulin resistance either[40,41]. Termed 'lean' NAFLD, this so-called 'entity' indicates that there is more to NAFLD than just the mere presence of MS. Zheng *et al*[28] showed that compared to MAFLD patients without obesity those with obesity were at a 6-fold increased risk of severe COVID-19 illness and this association was significant even after adjusting for various parameters like diabetes, hypertension and dyslipidemia. This raises an important question as to whether the worse outcome in NAFLD patients is related to underlying liver disease or related to associated obesity? However, the small sample size of this study makes it difficult to arrive at such sweeping conclusions. Also, the cut-off for obesity in this study has been taken as 25 kg/m^2.

INFLAMMATION IN NAFLD

The bidirectional relationship between hepatic steatosis and insulin resistance is well established[42]. Hepatic steatosis can itself be a driver of insulin resistance and MS has opened avenues for further investigation in the pathophysiology of inflammation in NAFLD. There has been increasing evidence of the presence of significant cross-talk between the liver and other extrahepatic tissues and organs mediated by cytokines, hepatokines. It also involves nuclear factor-κB and c-Jun N-terminal kinase pathways which implies that hepatic inflammation could be a potential driver of cellular dysfunction, cell death and deleterious remodelling in various body tissues and organs [43]. This state of chronic inflammation may directly impact disease severity by adding up to the dysregulated immune response in COVID-19. In a peripheral blood genome-wide gene expression analysis among 1650 participants, it was observed that after adjustment for known risk factors, fatty liver was associated with blood gene sets of extracellular matrix turnover, inflammatory response, immune system activation and a prothrombotic state[44]. This could lead to morbidities in multiple organs including the cardiovascular system, and may, in our opinion, exacerbate disease processes in COVID-19.

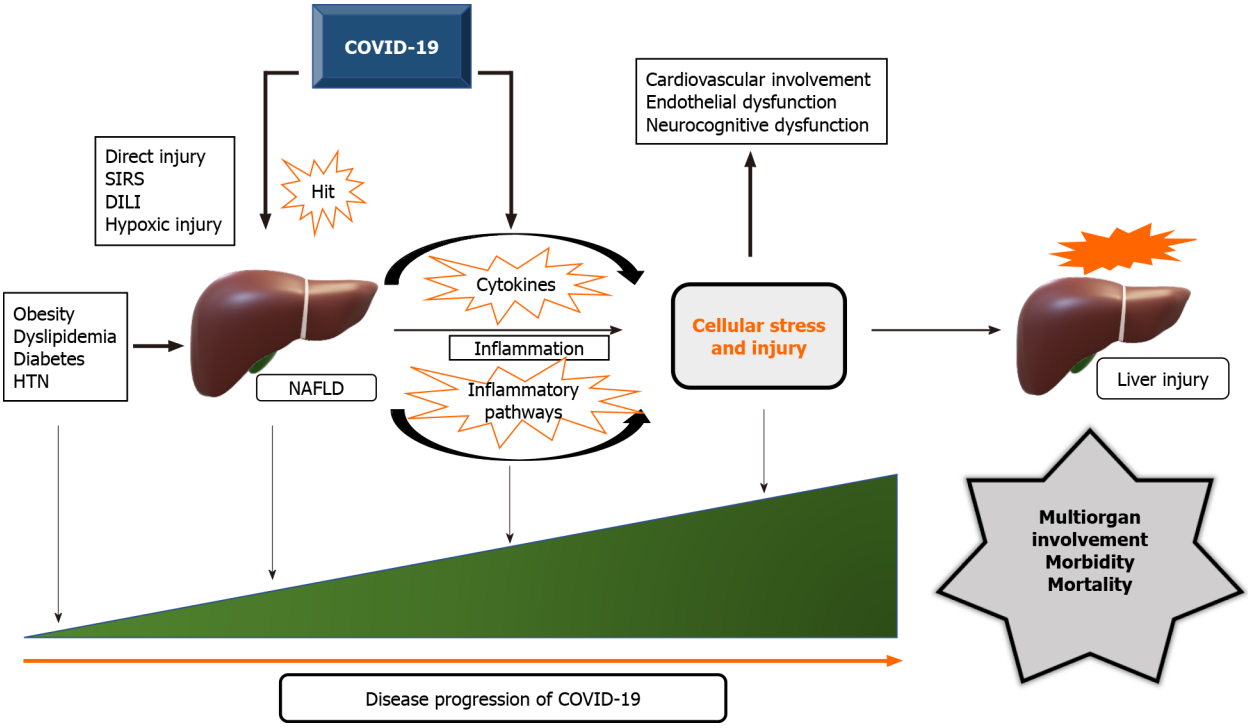

Figure 1 Pathophysiological processes driving disease progression in patients of nonalcoholic fatty liver disease with coronavirus disease 2019 and the impact on hepatic status. NAFLD: Nonalcoholic fatty liver disease; COVID-19: Coronavirus disease 2019.

LIVER INJURY IN NAFLD PATIENTS WITH COVID-19

NAFLD patients have been reported to be more likely to develop liver injury when infected by COVID-19[18]. Median ALT levels and the proportion of elevated ALT were found to be significantly greater in patients with NAFLD than in patients without NAFLD on admission. In addition, the proportion of elevated ALT in patients with NAFLD was significantly higher than patients without NAFLD during hospitalization. However, severe liver-related complications during hospitalization were not observed in any of the patients. Mushtaq *et al*[17] found that NAFLD is an independent predictor of the development of mild to moderate liver injury in hospitalized patients with COVID-19. Moreover, COVID-19 patients with persistent liver injury have been found to have NAFLD and high BMI in one particular study[15]. The APCOLIS study also found that the presence of MAFLD aggravates the risk of liver injury in COVID-19[13]. In the study by Targher *et al*[33], COVID-19 patients with MAFLD with intermediate or high FIB-4 scores were more likely to have higher liver enzymes, compared with their counterparts with low FIB-4 score or those without MAFLD. The reasons for this increased likelihood of liver injury in NAFLD patients affected by COVID-19 could be multifactorial- pre-existing steatohepatitis, systemic inflammation, the severity of COVID-19 itself and a combination of any of these. The 'cocktail' of medications used in this pandemic deserves special attention while evaluating the relationship between NAFLD and COVID-19. Antivirals, antibiotics and glucocorticoids have been the most rampantly used medications in the quest to control COVID-19 and may contribute to liver injury, especially in those with NAFLD.

A summary of the pathophysiological processes that could presumably drive disease progression in patients of NAFLD with COVID-19 and the resulting impact on hepatic status is illustrated in Figure 1.

CONCLUSION

The bulk of the evidence-based on pooled analysis so far shows NAFLD patients are at increased risk of severe COVID-19 infection. However, judging by the results based on few studies that have been carried out to date, it seems the disease severity is determined more by the presence of co-morbidities like obesity, insulin resistance and dyslipidemia which are frequent accompaniments of NAFLD. The studies showing the

association of NAFLD/MAFLD with severity of COVID-19 independent of associated comorbidities have shown conflicting results. The presence of fibrosis rather than the presence of MAFLD/NAFLD is associated with worse clinical outcomes and higher mortality in COVID-19 patients. Additionally, there seems to be an increased likelihood of liver injury in NAFLD patients with COVID-19. Further studies are required to delineate these pathophysiological details.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Clostridioides difficile infection in liver cirrhosis patients: A population-based study in United States

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Abstract

BACKGROUND

Clostridioides (formerly *Clostridium*) *difficile* infection (CDI) is an increasingly frequent cause of morbidity and mortality in hospitalized patients. Multiple risk factors are documented in the literature that includes, but are not limited to, antibiotics use, advanced age, and gastric acid suppression. Several epidemiological studies have reported an increased incidence of CDI in advanced liver disease patients. Some have also demonstrated a higher prevalence of nosocomial infections in cirrhotic patients.

AIM

To use a large nationwide database, we sought to determine CDI's risk among liver cirrhosis patients in the United States.

METHODS

We queried a commercial database (Explorys Inc™, Cleveland, OH, United States), and obtained an aggregate of electronic health record data from 26 major integrated United States healthcare systems comprising 360 hospitals in the United States from 2018 to 2021. Diagnoses were organized into the Systematized Nomenclature of Medicine Clinical Terms (SNOMED–CT) hierarchy. Statistical analysis for the multivariable model was performed using Statistical Package for Social Sciences (SPSS version 25, IBM Corp™). For all analyses, a two-sided *P* value of < 0.05 was considered statistically significant.

interests (personal or financial).

Data sharing statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

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RESULTS

There were a total of 19387760 patients in the database who were above 20 years of age between the years 2018-2021. Of those, 133400 were diagnosed with liver cirrhosis. The prevalence of CDI amongst the liver cirrhosis population was 134.93 per 100.000 *vs* 19.06 per 100.000 in non-cirrhotic patients ($P < 0.0001$). The multivariate analysis model uncovered that cirrhotic patients were more likely to develop CDI (OR: 1.857; 95%CI: 1.665-2.113, $P < 0.0001$) compared to those without any prior history of liver cirrhosis.

CONCLUSION

In this large database study, we uncovered that cirrhotic patients have a significantly higher CDI prevalence than those without cirrhosis. Liver cirrhosis may be an independent risk factor for CDI. Further prospective studies are needed to clarify this possible risk association that may lead to the implementation of screening methods in this high-risk population.

Key Words: *Clostridioides difficile*; Chronic liver disease; Liver cirrhosis; Liver transplant

Core Tip: *Clostridium difficile* infections (CDI) are a leading cause of hospital morbidity and mortality. The risk factors for CDI in liver cirrhosis patients are studied in the national data base. CDIs in liver transplantation is a life-threatening situation as these patients are malnourished and immunocompromised. Therefore, special emphasis was given to the cohort with history of liver transplantation and relevant literature was reviewed.

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INTRODUCTION

Clostridiodes difficile is a gram-positive anaerobic bacillus. It is widespread in the surrounding environment and a significant contributor to inpatient mortality in vulnerable subgroups[1]. Risk factors for being predisposed to CDI include advanced age, enteral feeding, smoking, alcohol abuse, and use of antibiotics and acid-suppressive therapy. It is particularly predominant in elderly patients who reside in nursing homes and long-term acute care facilities and have a history of recurrent hospitalizations. CDI carries a significant economic burden on the USA health care system. A recent study by Desai *et al*[2] uncovered that CDI's economic cost was roughly $5.4 billion, with $4.7 billion in the healthcare settings and $725 million in the community.

CDI has a spectrum of clinical symptoms, including nausea, vomiting, abdominal pain, watery diarrhea with the formation of pseudomembranous, progression to fulminant colitis, and even toxic mega colon[3-8]. CDI can culminate in the possible rupture of the large colon, septic shock, and death. Reactive arthritis is also seen as one of the complications of CDI[9].

Broad-spectrum antibiotic use (penicillin, cephalosporins, clindamycin, fluoroquinolones) predispose individuals to selective elimination of healthy gut microbiota and overgrowth of *Clostridium difficile* (*C. difficile)* in the gastrointestinal flora[10,11] with the highest risk of CDI within the first three months of antibiotic exposure[12]. As the environment and normal human gastrointestinal tract are heavily colonized with C. *difficile*[5,13-15], it is just a matter of loss of balance where C. *difficile* invades the protective gastrointestinal barriers through the production of toxins (enterotoxin A, cytotoxin B, binary toxin/CDT) and enzymes (collagenase, chondroitin sulfatase, hyaluronidase) which promote inflammation[16-18]. The virulence and

pathogenicity are compounded by new hypervirulent strains and the potential ability of C. *difficile* to create biofilms *in vivo* (after an *in vitro* demonstration)[19,20]. For instance, *C. difficile*, especially the new hypervirulent strain, NAP1/BI/027 that was uncovered in the year 2000, was responsible for a significant CDI-related mortality increase 5.7 deaths per million in 1999 to 23.7 deaths per million in 2004[21]. CDI is currently considered the most common cause of nosocomial diarrhea in the western world.

CDI's have been classified based on the severity of infection, utilizing the markers of inflammation and organ function, including white blood cell count (WBC), creatinine and albumin levels. Prognostic markers in patients with *C. difficile* colitis included low serum albumin (< 2.5 mg/dL) or a 1.1 mg/dL reduction in serum albumin from baseline, use of multiple antibiotics, and a positive CD cytotoxin in stool after completion of treatment (after seven or more days of treatment)[22].

The poor outcomes with CDI are not uncommon. They are particularly pronounced in patients with underlying chronic comorbidities (congestive heart failure, chronic obstructive pulmonary disease, and chronic kidney disease), history of solid organ transplants and immunosuppressive therapy, and chronic inflammatory diseases, including Crohn's disease and Ulcerative colitis[23-29]. The morbidity and mortality from liver cirrhosis is on the rise[30]. A prospective study by Bouza *et al*[31] that focused on the recent outbreak of *C. difficile* PCR ribotype 027 in Spain uncovered that this strain was most evident in patients with age > 75 years, the male gender, and comorbidities such hypertension, chronic cardiovascular disease, type 2 diabetes, and liver cirrhosis. Interestingly, liver cirrhosis was associated with an increased CDI recurrence risk of 44.4% *vs* 14.8%[31]. The increased prevalence of CDI in patients with advanced liver disease is being investigated as they are already immunocompromised [32,33].

Poor outcomes in cirrhotic patients who acquired CDI are reported in a recent study by Abdalla *et al*[34]. Liver cirrhosis itself can predispose the individuals to nosocomial infections, the deadliest of them being CDI. For instance, several studies have reported that CLD patients with CDI have a higher mortality rate, prolonged length of stay, and higher hospital cost[35-37]. We performed this large database study to re-evaluate the risk and severity of CDI in patients with cirrhosis. Prevalence of *C. difficile* associated disease (CDAD) was determined in the subgroups with established risk factors and comorbidities and prior history of liver disease and liver transplant.

MATERIALS AND METHODS

Database

Our study is a retrospective cohort analysis of a large, multicenter database (Explorys, Cleveland, OH, United States). Explorys aggregates healthcare data of more than 50 million unique patient records. Diagnoses, findings, and procedures are arranged into the Systematized Nomenclature of Medicine–Clinical Terms (SNOMED-CT) hierarchy, whereas prescription drug orders are mapped into RxNorm. Explorys provides an interactive search engine to generate multiple cohorts based on medical diagnoses. Medical data are de-identified, and therefore, it is a Health Insurance Portability and Accountability Act-compliant platform.

Patient selection

Using the Explorys platform, we identified cohorts of patients diagnosed with Liver cirrhosis between the period of March 2018 and March 2021. The study cohorts (liver cirrhosis) were identified by searching the database for a SNOMED-CT diagnosis of "Cirrhosis of Liver" after excluding patients younger than 20 years old. The control group was then identified for those who have no liver cirrhosis. Subsequently, a cohort of patients with "clostridioides difficile infection" diagnosis was identified between the period of March 2018 to March 2021 to calculate the prevalence of CDI in both study groups. Risk factors and predisposing medical conditions associated with CDI, in addition to demographic information, were collected. Possible risk factors included comorbid medical conditions, antibiotics, acid-suppressive therapy, liver transplant, and inpatient/skilled nursing facility settings were investigated using SNOMED-CT diagnostic codes.

Statistical analysis

The prevalence was calculated by dividing the total number of individuals with CDI in each cohort (liver cirrhosis and non-cirrhotics) by the total number of individuals in

each cohort as identified by Explorys [2018-2021], thus making sure that all patients in the denominator had an equal opportunity of being diagnosed with CDI. We calculated the prevalence in subgroups based on sex, race, and age by dividing the number of individuals with CDI in each subgroup by a total number of patients in the same subgroup. A multivariate regression model was constructed using binary logistic regression, with CDI being the outcome to adjust for possible confounding from the covariates listed previously. We used SPSS version 25 (IBM Corp) to perform the multivariate regression analysis. A 2-sided *P* value of < 0.05 was considered statistically significant.

RESULTS

Descriptive epidemiology

There were a total of 19387760 patients in the database who were above 20 years of age. Of those, 133400 were diagnosed with liver cirrhosis. The baseline characteristics of the study population are presented in Table 1. The prevalence of CDI amongst the liver cirrhosis population was 134.93 per 100.000 *vs* 19.06 per 100.000 in non-cirrhotic patients ($P < 0.0001$). Figure 1 represents the prevalence of CDI in different age groups among cirrhotics. Females and Caucasian patients had a higher CDI prevalence than males and non-caucasian among both study groups (Table 2). Patients with nonalcoholic liver disease (NAFLD) as well as an alcoholic liver disease were found to have a higher prevalence of CDI when compared to cirrhotic patients with viral hepatitis (184.9/100.000 in NAFLD *vs* 174.0/100.000 in alcoholic liver disease *vs* 117.9/100.000 in hepatitis C *vs* 81.7/100.000 in hepatitis B) (Figure 2).

Multivariate analysis

The multivariate analysis model uncovered that cirrhotic patients were more likely to develop CDI (OR 1.857; 95%CI: 1.665-2.113, $P < 0.0001$) compared to those without any prior history of liver cirrhosis. The characteristics of the liver cirrhosis patients who developed CDI revealed that they were more likely to be of advanced age (age > 65) as opposed to being young (age < 65) with an OR 2.307, 95%CI: 2.179-2.442 ($P < 0.0001$); had prior use of antibiotics (OR 19.749, 95%CI: 17.3-22.545, $P < 0.0001$); had used acid-suppressive therapy (OR 2.243, 95%CI: 2.122-2.371, $P < 0.0001$); and were mostly inpatients/skilled nursing facility occupants *vs* the community (OR 2.02, 95%CI: 1.911-2.134, $P < 0.0001$). Among cirrhotic patients, those with a history of liver transplant (OR 2.737, 95%CI: 2.087-3.589) were highly likely to develop CDI. The multivariate analysis model with CDI being the outcome is presented in Table 3.

DISCUSSION

The multivariate analysis of this database study holds true for the high prevalence of CDI in cirrhotic patients with all the established risk factors (advanced age, use of antibiotics and acid suppression therapy, enteral feeding, residence at long term care facilities, and frequent hospitalizations) and comorbidities (obesity, hypertension, Diabetes Mellitus, chronic obstructive pulmonary disease, congestive heart failure)[38-45]. The highest prevalence of CDI was reported in patients with a history of antibiotic use. CDI's were also encountered in traditionally low-risk population groups in hospitalized patients with recent antibiotic exposure[46].

The colonization of *C. difficile* has been higher in cirrhotic patients with simultaneous hepatic encephalopathy and advanced stage (Child-Pugh C)[47]. Risk factors of CDI in cirrhotic patients have been determined by Yan *et al*[48] in their latest study (advanced age, antibiotics, and proton pump inhibitors, prolonged and recurrent hospitalizations, hyponatremia, *C. difficile* colonization, hepatic encephalopathy).

The bacterial infections, which generally would have been countered with immunoregulatory mechanisms (chemotaxis, phagocytosis, oxidation, interferon cascade, complement system, inflammatory response) in an immunocompetent individual, go rampant[49-52]. High ammonia levels alter these neutrophilic responses [53]. The inflammation response is also dampened from poor nutrition status and alcoholism, which come with cirrhosis. The mechanisms responsible include reticuloendothelial system dysfunction, portosystemic shunting, hyperdynamic circulation, increased permeability of gut, and bacterial translocation. The systemic inflammatory response syndrome (SIRS) is amplified by the increased nitric oxide (NO) and the

Table 1 Baseline characteristics of study population, *n* (%)

	Patients with history of liver cirrhosis (*n* = 133400)	Patients with no history of liver cirrhosis (*n* = 19254360)
Age groups (yr)		
20-64	73620 (55)	14934590 (78)
> 65	59780 (45)	4319770 (22)
Gender		
Male	71230 (53)	8702330 (45)
Female	62170 (47)	10552030 (55)
Race		
Caucasian	101680 (76)	11165060 (58)
Non-Caucasian	31720 (24)	8089300 (42)
Comorbidities		
Smoking	54400 (41)	2985460 (16)
Alcohol abuse	30040 (23)	359900 (2)
HTN	100830 (76)	5307920 (28)
DM	63770 (48)	2211420 (11)
Obesity	24400 (18)	1148460 (6)
Chronic kidney disease	33640 (25)	853630 (4)
Coronary artery disease	6280 (5)	229410 (1)
Heart failure	39960 (30)	761710 (4)
Chronic obstructive pulmonary disease	42740 (32)	986400 (5)

DM: Diabetes mellitus.

Table 2 Prevalence of *Clostridioides difficile* infection in different age and race groups in patients with liver cirrhosis *vs* no cirrhosis (per 100000)

	Liver cirrhosis	No cirrhosis
Female	176.93	23.31
Male	112.31	14.34
Caucasian	147.52	26.87
African American	54.59	7.34
Hispanic/Latino	257.73	10.96

cytokine storm.

The rate of CDI was significantly high in patients who underwent hepatic transplantation. CDI risk is increased in immunocompromising health conditions involving any solid organ transplant[54,55], including liver transplant recipients[56]. The timeline of CDI in post-transplant patients has been established based on the underlying severity of cirrhosis dictated by model for end-stage liver disease (MELD) scoring, concurrent intra-abdominal hemorrhage, repeat grafting and transplant, vascular complications, infections, and the need for endoscopy with sicker patients developing CDI earlier with higher mortality[57,58]. Musa *et al*[59] researched CDI and chronic liver disease with an additional focus on liver transplant patients. Male sex and high pre-op creatinine levels (> 1 g/L) are considered predisposing risk factors for CDI in the subgroup who received a living donor hepatic transplant[60]. Advanced cirrhosis (High MELD score), impaired renal function in the donor, and postoperative complications (infection, bleeding, wound) leading to prolonged hospital stay were

Table 3 Multivariable model with *Clostridioides difficile* infection being the outcome

Multivariable model	Odds ratio	95%CI	*P* value
Age (> 65 yr *vs* < 65 yr)	2.307	2.179-2.442	< 0.0001
Gender (female *vs* male)	1.29	1.221-1.363	< 0.0001
Race (non-Caucasian *vs* Caucasian)	1.16	1.088-1.237	< 0.0001
Antibiotics	19.749	17.3-22.545	< 0.0001
Skilled nursing facility or inpatients	2.02	1.911-2.134	< 0.0001
Acid suppressive therapy (Proton pump inhibitors or H2 blockers)	2.243	2.122-2.371	< 0.0001
Comorbidities[1]	1.258	1.192-1.328	< 0.0001
Liver transplant	2.737	2.087-3.589	< 0.0001
Liver cirrhosis	1.875	1.665-2.113	< 0.0001

[1]Comorbidities: One or more of the following (heart failure, coronary artery disease, chronic kidney disease, inflammatory bowel disease, chronic obstructive pulmonary disease, diabetes mellitus, obesity, hypertension or metabolic syndrome).

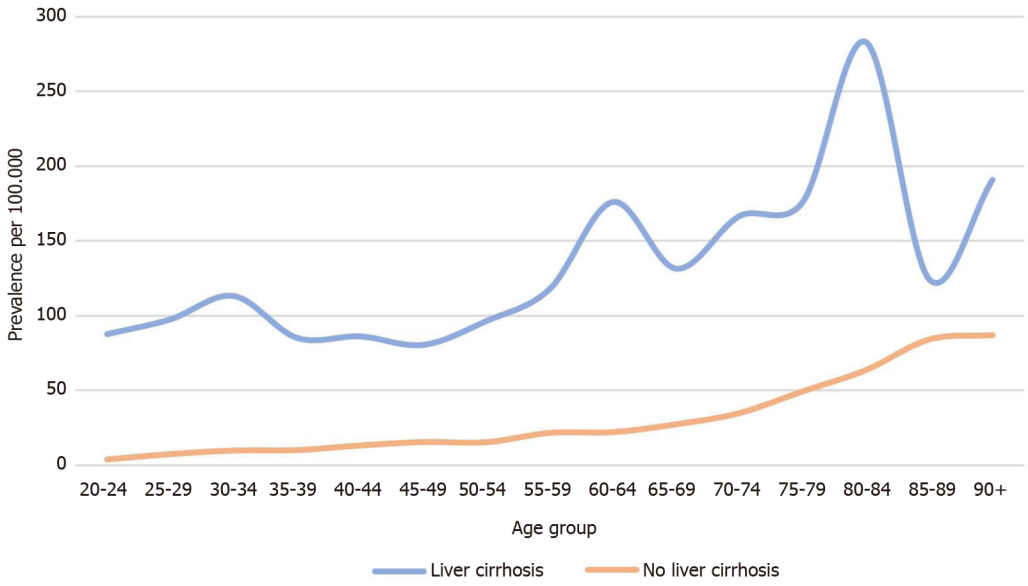

Figure 1 Prevalence of *Clostridioides difficile* infection in patients with liver cirrhosis *vs* no cirrhosis.

concluded predisposing factors for CDI after a deceased liver transplant[57]. Recurrence of pseudomembranous colitis up to five times after living donor liver transplantation has been reported in the literature[61].

The CDI rate was higher in patients with autoimmune hepatitis, prolonged hospital stay, and antibiotic exposure in a study performed by Vanjak *et al*[62]. Hepatitis C is increasingly identified as an underlying viral infection responsible for cirrhosis in patients who developed CDI later in life[63]. Comparing CDI incidence in cirrhosis due to hepatitis B and hepatitis C has not been explored yet. Our study explores this comparison and demonstrates that the prevalence of CDI is higher in inpatient subgroups with hepatitis C than hepatitis B. Sundaram *et al*[36] reported higher inpatient mortality secondary to CDI in patient subgroups with alcohol abuse-related hepatic cirrhosis. Additionally, NAFLD has been identified as a risk factor for CDI by Papić *et al*[64]; after adjusting for other comorbidities, hospitalization rates, and antibiotic exposure (Sundaram *et al*[36]).

Acid suppressive therapy has been implicated with CDI in the general population. A study reported increased 30-d mortality in cirrhotic patients with proton pump inhibitor (PPI) use[35]. The association is being attributed to their excessive unindicated use. The majority of people presenting with variceal bleed get discharged with PPIs renewed on each visit[33,65]. Chronic use of PPIs causes altered gut flora

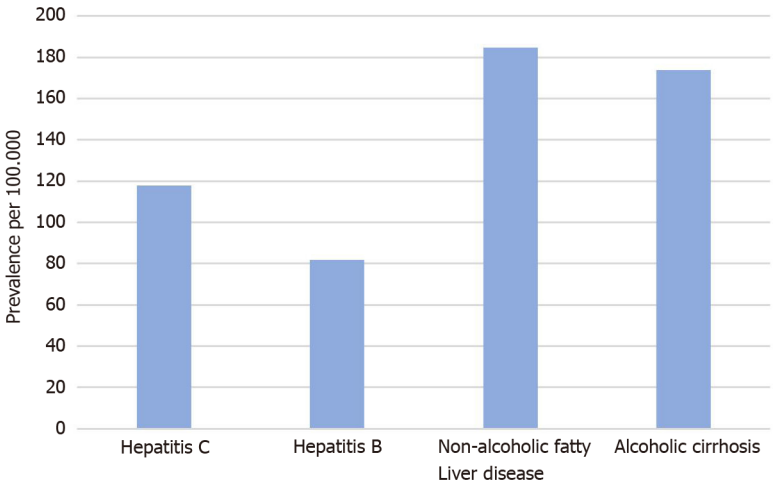

Figure 2 Prevalence of *Clostridioides difficile* infection in cirrhotic patients based on the etiology of cirrhosis.

and motility and decreased neutrophilic function[66]. Long-term PPIs use has been attributed to CDI's by suppressing gastric acid, although the evidence[67-71]. PPIs are said to have worse outcomes in cirrhotic patients than H2 blockers in one study[8]. Hence, the proper need for PPIs should be assessed at each visit and discharge.

Generally, women are more likely to get CDI regardless of their liver function, which was also reflected in our study[72]. The incidence rate of CDI is higher in Caucasians with cirrhosis. A higher incidence and mortality rate from CDI in the caucasian population has been reported in the literature[73-75]. An even higher prevalence of CDI was seen in the African American and Hispanic/Latin subgroups, which could be due to regional data differences[76]. The hospitalization patterns have been fluctuating, and long-term mortality from CDI has been counterintuitively low, as concluded by recent studies[59,76,77].

Vancomycin and metronidazole have been used historically in the treatment of initial and recurrent CDI. Several meta-analyses have been performed, emphasizing the non-inferiority of metronidazole, and thereby, guidelines have been revised. Vancomycin and fidaxomicin are considered the mainstay of antibiotic treatment now, along with fecal microbiota transplantation (FMT). Surgery is pursued when there is a suspicion of toxic megacolon or colon perforation[5,17,78-82]. Lactulose was also evaluated in liver cirrhosis patients carrying *C. difficile* in a study done by Ito *et al*[83] with promising results. Lactulose may increase fecal acidity by decreasing short-chain fatty acids and increasing lactate and acetate, leading to possible suppression of *C. difficile* growth. FMT has been used in patient subgroups with cirrhosis to help with recurrent CDI colonization[78,84]. Additionally, lactulose as a prebiotic may play a prominent role in restoring the hosts' indigenous microbiota and conferring resistance against CDI[85]. Recently, the benefit of preventing CDI by using maintenance rifaximin[86].

The benefit of screening hospitalized cirrhotic patients for *C. difficile* might be purely theoretical, as screening in the absence of symptoms would lead to over-reporting[87, 88]. Meltzer *et al*[89] did a 10-wk surveillance study after screening asymptomatic patients on admission. They demonstrated a higher incidence of CDI during hospitalization in patients who tested positive for *C. difficile* on admission rectal swabs. Whether clinicians should treat a prior CDI carrier state still remains unclear, as most of the positive patients in that particular study had the classical risk factors for CDI (prolonged hospital and rehabilitation stays, exposure to infections, and antibiotics). Third-generation cephalosporins are the treatment of choice for subacute bacterial peritonitis (SBP), which are counterintuitively associated with increased risk of CDI. Both SBP and CDI translate into poor outcomes for the patient[21]. Bactrim and fluoroquinolones (ciprofloxacin, norfloxacin) are recommended as SBP prophylaxis in high-risk patients, but their long-term benefit is questionable for now[90].

C. difficile toxins in stool sample or visualization of pseudomembrane formation on endoscopic or histological examination are diagnostic for CDI. Due to its ability to spread by spore formation[91,92], poor hygiene contributes to its rapid spread *via* the fecal-oral route and can result in outbreaks in health care facilities. Hand hygiene, therefore, has been the cornerstone in the control of CDI spread along with isolation of symptomatic patients and implementation of environmental sanitation protocols[93-

97].

The results obtained from this database are significant due to the large sample size, appropriate gender and racial representation, and inclusion of patients above the age of twenty years. Recent studies have confirmed poor outcomes with concurrent CDI and CLD[37]. All data prior to 2018 has been excluded to determine the persistence of historically established risk factors for CDI based on point prevalence. Relevant comorbidities have been included along with a subgroup of patients with liver transplants. The underlying cause of cirrhosis has also been delineated (Table 2).

The study is at a disadvantage as it is retrospective. The sample size is subjected to selection bias which was attempted to be minimized by relevant inclusion and exclusion criterion. The prevalence of liver cirrhosis in the population database is lower than the general population (0.69 %)[98]. While this may reduce the effective sample size, it has no bearing on the conclusions drawn regarding the risk factors associated with CDI in cirrhotic patients. The inclusion of patient classification based on their MELD score would have indicated the severity of CDI at different cirrhosis stages. The multivariate analysis by Hong *et al*[99] had suggested that the patients with higher MELD scoring are at increased risk of mortality from CDI (1.06 ± 0.02, *P*-value < 0.022 with an increase of 21.5% mortality rate with every five-unit increase of MELD score), and MELD scoring should be used to triage them and monitor their outcomes. However, the application of MELD score in SNOMED-CT would be scrupulous as the routine discharge diagnoses are not updated based on the patient's current MELD scores. Results from future perspective studies with patient cohorts stratified into liver, solid organ transplants and MELD classes can vindicate the yield of *C. difficile* screening in asymptomatic patients.

CONCLUSION

The prevalence of CDI is seven times higher in cirrhotic patients than those without liver cirrhosis. In the multivariate analysis, cirrhotic patients with advanced age, frequent hospitalizations, residence in a nursing home and long-term facilities, along with the use of antibiotics, acid-suppressive therapy, chronic comorbidities, and history of hepatic transplantation, were more likely to develop CDI. Further studies are needed to explore this risk, and precautionary measures are needed to be implemented to prevent CDI in this group of patients.

ARTICLE HIGHLIGHTS

Research background

Clostridium difficile (*C. difficile*) is one of the major causes of nosocomial diarrhea and associated morbidity and mortality. The risk factors of *C. difficile* are historically established. Cirrhosis is a major disease burden in the United States health care system. The risk of morbidity and mortality is higher in cirrhotic patients who acquire *C. difficile* infection.

Research motivation

This research was motivated by the lack of recent large population study describing the risk factors of *C. difficile* in liver cirrhotic patients. We also wanted to study the association in patient cohorts who underwent liver transplant as it was not done previously with such higher sample size.

Research objectives

To determine the prevalence of *C. difficile* infection in patients with liver cirrhosis and to establish the risk factors of *C. difficile* infection in patients with liver cirrhosis with special emphasis on liver transplantation cohort.

Research methods

The authors used the Explorys database to obtain data that was classified using SNOMED diagnostic codes. Prevalence and association were calculated using multivariate regression and SPS Software. Details are in the main manuscript.

Research results

The prevalence of *C. difficile* infection (CDI) amongst the liver cirrhosis population was 134.93 per 100.000 *vs* 19.06 per 100.000 in non-cirrhotic patients. The multivariate analysis model showed that cirrhotic patients were more likely to develop CDI.

Research conclusions

This research study concluded that cirrhotic patients have a significantly higher CDI prevalence, and liver cirrhosis may be an independent risk factor for CDI.

Research perspectives

There is a possibility of reducing the CDI mortality in cirrhotic patients by screening them for CDI. Future prospective studies are needed in this regard.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Hepatocellular injury and the mortality risk among patients with COVID-19: A retrospective cohort study

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Abstract

BACKGROUND

Clearly, infection with severe acute respiratory syndrome coronavirus 2 is not limited to the lung but also affects other organs. We need predictive models to determine patients' prognoses and to improve health care resource allocation during the coronavirus disease 2019 (COVID-19) pandemic. While treating COVID-19, we observed differential outcome prediction weights for markers of hepatocellular injury among hospitalized patients.

AIM

To investigate the association between hepatocellular injury and all-cause in-hospital mortality among patients with COVID-19.

METHODS

This multicentre study employed a retrospective cohort design. All adult patients admitted to Al-Azhar University Hospital, Assiut, Egypt and Abo Teeg General Hospital, Assiut, Egypt with confirmed COVID-19 from June 1, 2020, to July 30, 2020 were eligible. We categorized our cohort into three groups of (1) patients with COVID-19 presenting normal aminotransferase levels; (2) patients with COVID-19 presenting one-fold higher aminotransferase levels; and (3) patients with COVID-19 presenting two-fold higher aminotransferase levels. We analysed the association between elevated aminotransferase levels and all-cause in-hospital

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mortality. The survival analysis was performed using the Kaplan–Meier method and tested by log-rank analysis.

RESULTS

In total, 376 of 419 patients met the inclusion criteria, while 29 (8%) patients in our cohort died during the hospital stay. The median age was 40 years (range: 28-56 years), and 51% were males (n = 194). At admission, 54% of the study cohort had liver injury. The pattern of liver injury was hepatocellular injury with an aspartate aminotransferase (AST) predominance. Admission AST levels were independently associated with all-cause in-hospital mortality in the logistic regression analysis. A one-fold increase in serum AST levels among patients with COVID-19 led to an eleven-fold increase in in-hospital mortality (P < 0.001). Admission AST levels correlated with C-reactive protein (r = 0.2; P < 0.003) and serum ferritin (r = 0.2; P < 0.0002) levels. Admission alanine aminotransferase levels correlated with serum ferritin levels (r = 0.1; P < 0.04). Serum total bilirubin levels were independently associated with in-hospital mortality in the binary logistic regression analysis after adjusting for age and sex but lost its statistical significance in the fully adjusted model. Serum ferritin levels were significantly associated with in-hospital mortality (P < 0.01). The probability of survival was significantly different between the AST groups and showed the following order: a two-fold increase in AST levels > a one-fold increase in in AST levels > normal AST levels (P < 0.0001).

CONCLUSION

Liver injury with an AST-dominant pattern predicts the severity of COVID-19. Elevated serum ferritin levels are associated with fatal outcomes.

Key Words: COVID-19; Liver injury; Aspartate amino transferase; All-cause in-hospital mortality; Serum ferritin; SARS-CoV-2

Core Tip: Liver injury with an aspartate aminotransferase (AST)-dominant pattern can predict the severity of coronavirus disease 2019 (COVID-19). A one-fold and two-fold increase in serum AST levels increased the odds of in-hospital mortality by eleven-fold and thirteen-fold, respectively, compared with individuals with normal AST levels. Our study confirmed an elevated level of ferritin in patients with COVID-19 that was associated with fatal outcomes. Meticulous monitoring is highly recommended for patients with COVID-19 presenting AST-dominant hepatocellular injury, especially those older than 60 years, those with elevated ferritin levels or those with diabetes mellitus.

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INTRODUCTION

Globally, coronavirus disease 2019 (COVID-19) has a substantial impact on the healthcare system. Over time, clinicians have clearly determined that the infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is not limited to the lung but also affects the nervous system, gastrointestinal tract and hepatobiliary system[1].

The entry of SARS-CoV-2 into target cells is facilitated by angiotensin-converting enzyme 2 (ACE2) receptors. ACE2 receptors are expressed at high levels in lung alveolar epithelial cells, vascular endothelium and epithelium of the small intestine[2]. This pattern might explain the pathophysiology of gastrointestinal manifestations

associated with COVID-19, such as vomiting and diarrhoea. Moreover, ACE2 receptors are expressed at high levels on cholangiocytes (60% of cells) and to a lesser extent on hepatocytes (3% of cells)[3]. Therefore, the hepatobiliary system may be at increased risk of SARS-CoV-2 infection. The liver appears to be the second most affected organ after the lung[4].

In fact, many case series identified abnormal elevations in the levels of aminotransferases and hypoalbuminemia early and during the progression of COVID-19[5,6]. However, substantial variability in the reported prevalence of liver injury among patients with COVID-19 was noted (14% to 50%)[3]. Moreover, the clinical effect of de novo liver injury on the prognosis of patients with COVID-19 has not been investigated in detail. In addition, multiple clinical comorbidities that might confound liver injury-associated mortality should be studied[7].

Ideally, clinicians should be able to identify patient outcomes to improve health care resource allocation.

The aim of the present study was to investigate whether biomarkers of hepatocellular injury have prognostic value in predicting all-cause in-hospital mortality among patients with COVID-19.

MATERIALS AND METHODS

This multicentre study employed a retrospective cohort design. The medical records of all consecutive adult patients admitted to Al-Azhar University Hospital, Assiut, Egypt and Abo Teeg General Hospital, Assiut, Egypt with confirmed COVID-19 from June 1, 2020, to July 30, 2020 were retrieved and analysed. Both hospitals were designated to treat patients with confirmed COVID-19 by the Egyptian Ministry of Health. The inclusion criteria were hospitalization and adult patients > 18-years-old with confirmed COVID-19 based on a positive nasopharyngeal swab for SARS-CoV-2. Exclusion criteria were non-hospitalized patients, pregnant females, patients with chronic liver disease (in accordance with institutional clinical guidelines, patients with elevated aminotransferase levels were screened for markers of viral hepatitis and markers of autoimmune hepatitis), patients who refused to participate in the study and patients with incomplete data. The institutional review board granted approval for the study protocol.

Exposure measurement

The exposure of interest was the serum levels of aminotransferases at the time of hospital admission. We measured the levels of aminotransferases within 24 h of hospital admission. Elevated aminotransferase levels were defined as either an increase in the levels of aspartate aminotransferase (AST) (> 40 U/L), alanine aminotransferase (ALT) (> 40 U/L) or both proteins compared with the upper limit of normal. We classified our cohort into three groups based on serum levels of aminotransferases: (1) Patients with COVID-19 presenting normal aminotransferase levels; (2) Patients with COVID-19 presenting a one-fold increase in aminotransferase levels; and (3) Patients with COVID-19 presenting a two-fold increase in aminotransferase levels.

Covariates

In every enrolled participant, covariates analysed included baseline patient characteristics, such as age, sex and smoking status, comorbidities, such as diabetes mellitus, hypertension, ischaemic heart disease, chronic kidney disease and chronic obstructive pulmonary disease, Deyo–Charlson index, obesity and initial laboratory investigations and AST/ALT, serum total bilirubin, serum albumin, blood urea, serum creatinine, C-reactive protein (CRP), creatine kinase (CK), serum ferritin and D-dimer levels.

Outcome measurement

The predefined primary outcome of the study was all-cause in-hospital mortality. The secondary outcome was the length of the hospital stay. The vital status of the study participants was obtained from hospital records. The censoring date for follow-up of the outcome was August 15, 2020.

Statistical analysis

Continuous variables were presented as medians and interquartile ranges, and categorical variables were presented as absolute numbers and percentages. For the statistical analysis of group differences, we performed unadjusted binary logistic

regression analyses. We utilized a stepwise analysis adjusted for sex and age as well as for clinically relevant confounders listed above to investigate confounding factors. Spearman's correlation coefficients were calculated to analyse the relationships between variables. All *P* values presented were two-tailed; values less than 0.05 were considered statistically significant. We used Stata Software (Stata Statistical Software: Release 16. College Station, TX: Stata Corp LP) for data visualization and analysis.

RESULTS

In total, 376 of 419 patients met the inclusion criteria, and 8% of these patients died during the hospital stay (n = 29) (Figure 1). The median age was 40 years (range: 28-56 years), and 51% were males (n = 194) (Table 1). Patients in our study cohort were stratified according to all-cause in-hospital mortality into alive and dead groups, and their characteristics are presented in Table 1.

Predictors of the outcome

Regression analysis: (1) Unadjusted analysis. The unadjusted binary logistic regression analysis revealed that age, diabetes, hypertension, ischaemic heart disease, chronic kidney disease, obesity and Deyo-Charlson index were significant clinical factors associated with all-cause in-hospital mortality. In addition, AST, serum total bilirubin, serum albumin, serum creatinine and serum ferritin levels were the laboratory biomarkers significantly associated with all-cause in-hospital mortality. However, sex, chronic obstructive pulmonary disease and ALT, CRP, CK and D-dimer levels were not associated with all-cause in-hospital mortality; and (2) Adjusted analysis. The AST level at admission was the only biomarker of liver injury that was independently associated with all-cause in-hospital mortality in the unadjusted binary logistic regression analysis and model 1 adjusted for age and sex (Table 2). In addition, in model 2, after stepwise adjustment for several clinically relevant confounders, AST levels were still significantly associated with all-cause in-hospital mortality. Serum total bilirubin levels were independently associated with in-hospital mortality in the binary logistic regression after adjusting for age and sex but lost its statistical significance in the fully adjusted model.

Association of aminotransferase levels with CK levels

CK was studied as a marker of muscle injury. At admission, AST levels did not correlate with CK levels (r = -0.006; P = 0.9). In addition, admission ALT levels did not correlate with CK levels (r = -0.02; P = 0.6).

Association of aminotransferase levels with inflammatory markers

Serum ferritin and CRP levels were examined as markers of inflammation. At admission, AST levels correlated with CRP (r = 0.2; P < 0.003) and serum ferritin (r = 0.2; P < 0.0002) levels. Admission ALT levels correlated with serum ferritin levels (r = 0.1; P < 0.04) but not with CRP (r = 0.09; P = 0.08).

Association of serum ferritin levels with inflammatory markers

Admission serum ferritin levels correlated with CRP levels (r = 0.4; P < 0.0001).

Among our cohort, we identified 6 patients with biphasic hyperbilirubinemia. ALT levels correlated with serum total bilirubin levels (r = 0.2; P < 0.003). Therefore, hyperbilirubinemia was due to liver injury and not haemolysis.

Probability of survival

The probability of survival was significantly different between AST groups. As shown in the Kaplan-Meier curves (Figure 2), the probability of mortality progressively increased as the serum level of AST increased in the following order: two-fold increase in AST levels > a one-fold increase in AST levels > normal AST levels.

DISCUSSION

Numerous studies have reported the effect of liver injury on the outcomes of hospitalized patients with COVID-19[8]. However, a growing concern is that many demographic, clinical and laboratory markers might confound this association. These potential confounders should be recognized, and their effects on the association

Table 1 Baseline demographic, clinical and laboratory characteristics of alive and dead groups

Characteristics	Alive, *n* = 347	Dead, *n* = 29	Unadjusted odds ratio	*P* value
Age years median (IQR)			1.08 (1.05 to 1.11)	0.0001
< 40	186 (98.4)	3 (1.6)	Ref	
40-60	118 (93.6)	8 (6.3)	4.8 (1.7 to 13.4)	0.003
> 60	43 (70.5)	18 (29.5)	17.6 (6.5 to 48.0)	0.0001
Male sex, *n* (%)	179 (92.2)	15 (7.7)	1.1 (0.6 to 2.1)	0.98
Comorbidities				
Diabetes mellitus, *n* (%)	60 (77.0)	18 (23.0)	5.4 (2.9 to 10.2)	0.0001
Hypertension, *n* (%)	88 (83.8)	17 (16.2)	2.8 (1.5 to 5.2)	0.001
Ischaemic heart disease, *n* (%)	7 (50.0)	7 (50.0)	16.1 (5.2 to 50.2)	0.0001
Chronic kidney disease, *n* (%)	5 (45.5)	6 (54.5)	18.5 (5.2 to 65.5)	0.0001
Chronic respiratory disease, *n* (%)	57 (89.1)	7 (10.9)	1.7 (0.7 to 4.1)	0.25
Deyo–Charlson index, *n* (%)				
0-1	281 (96.6)	10 (3.4)	Ref	
2-3	64 (80.0)	16 (20.0)	7.8 (3.2 to 18.3)	0.0001
> 3	2 (40.0)	3 (60.0)	46.5 (6.9 to 313.5)	0.0001
Biochemical results on admission				
Serum ALT, *n* (%)				
< 40 U/L	225 (93.4)	16 (6.6)	Ref	
40-80 U/L	98 (89.9)	11 (10.1)	1.5 (0.6 to 3.3)	0.36
> 80 U/L	24 (92.3)	2 (7.7)	1.1 (0.3 to 5.4)	0.83
Serum AST, *n* (%)				
< 40 U/L	246 (96.8)	8 (3.2)	Ref	
40-80 U/L	87 (82.3)	18 (17.1)	6.1 (2.5 to 14.7)	0.0001
> 80 U/L	14 (82.3)	3 (17.6)	6.1 (1.5 to 25.5)	0.01
AST/ALT				
1.2-1.5	41 (87.2)	6 (12.7)	2.6 (1.0 to 7.2)	0.05
> 1.5	35 (81.4)	8 (18.6)	4.1 (1.6 to 10.4)	0.003
Serum albumin < 3.5 g/dL, *n* (%)	125 (85.6)	21 (14.4)	4.5 (1.9 to 10.5)	0.001
Serum total bilirubin > 1.5 mg/dL, *n* (%)	2 (33.3)	4 (66.7)	8.0 (2.2 to 29.4)	0.002
Serum creatinine > 1.1 mg/dL for males; > 0.95 mg/dL for females, *n* (%)	66 (77.7)	19 (22.3)	8.9 (3.9 to 20.6)	0.0001
C-reactive protein ≥ 1 mg/L, *n* (%)	338 (92.1)	29 (7.9)	1.0	
Serum ferritin > 400 μg/L for males; > 150 μg/L for females	183 (86.7)	28 (13.3)	24.7 (3.3 to 184.1)	0.002
D-dimer > 0.5 μg/mL	335 (92.0)	29 (8.0)	1.0	
Obesity (body mass index > 30)	106 (86.2)	17 (13.8)	3.2 (1.5 to 7.0)	0.003
Creatin kinase > 117 IU/L	11 (91.7)	1 (8.3)	1.1 (0.1 to 8.7)	0.9

Odds ratios were calculated by univariate logistic regression. Univariate logistic regression was used to calculate *P* value for the characteristics' differences between alive and dead patients. AST: Aspartate transferase; ALT: Alanine transferase; IQR: Interquartile range; Ref: Reference.

between biomarkers of hepatocellular injury and patient outcomes should be investigated. In fact, while treating COVID-19, we observed differential outcome prediction weights for markers of hepatocellular injury among hospitalized patients.

Table 2 Odds ratios of liver injury associated mortality and 95% confidence intervals by aspartate aminotransferase categories

	Unadjusted			Model 1 (adjusted for age and sex)			Model 2		
AST	**OR**	**CI**	***P* value**	**OR**	**CI**	***P* value**	**OR**	**CI**	***P* value**
< 40	Ref			Ref			Ref		
40-80	6.1	2.5-14.7	0.0001	4.8	1.9-12.1	0.001	10.8	2.5-40.9	0.001
> 80	6.1	1.5-25.5	0.01	2.9	0.6-13.7	0.18	12.8	1.5-93.4	0.02
						Covariates			
						Age			
						< 40	Ref		
						40-60	1.1	0.2-6.2	0.9
						> 60	6.3	1.2-33.1	0.03
						Sex	1.1	0.4-3.7	0.7
						DM	5.7	1.0-31.7	0.04
						HTN	0.3	0.1-2.0	0.2
						IHD	5.0	0.7-37.3	0.1
						COPD	0.7	0.1-3.6	0.6
						DCI			
						0-1			
						2-3	0.90	0.090-9.600	0.07
						> 3	0.200	0.001-25.600	0.5
						ALT			
						< 40	Ref		
						40-80	0.30	0.09-1.20	0.1
						> 80	0.10	0.02-1.20	0.07
						Albumin	0.8	0.2-2.5	0.7
						Bilirubin	17.2	0.9-312.8	0.05
						Ferritin	20.7	1.7-247.0	0.01
						Creatinine	1.8	0.6-5.8	0.3
						Obesity	3.3	0.9-11.4	0.06

Adjusted odds ratios for in-hospital mortality. Model adjusted for sex and age. Model 2 adjusted for age, sex. OR: Odds ratio; CI: Confidence interval; DCI: Deyo–Charlson index; DM: Diabetes mellites; HTN: Hypertension; IHD: Ischaemic heart disease; COPD: Chronic obstructive pulmonary disease; ALT: Alanine transferase; AST: Aspartate transferase, albumin, bilirubin, ferritin, creatinine and obesity.

At admission, 54.0% of patients in our cohort had liver injury. AST levels were elevated in 32.5% (n = 122), ALT levels were elevated in 36.0% (n = 135), and both ALT and AST levels were increased in 23.0% (n = 87). Among nonsurvivors, the pattern of liver injury was hepatocellular injury with an AST predominance. Both ALT and AST levels were elevated in 45.0% of nonsurvivors. An isolated elevation of AST levels was detected in 31.0% of nonsurvivors, while an isolated elevation of ALT levels was detected in 3.0%. Notably, 48.0% of nonsurvivors presented an AST/ALT ratio > 1.2, and serum total bilirubin levels were increased in 1.6% of patients in our study cohort.

We observed an obvious increase in mortality among patients with COVID-19 presenting elevated serum AST levels at the time of admission. A one-fold increase in the serum level of AST increased the odds of in-hospital mortality eleven-fold compared to those with normal AST levels at admission. Moreover, a two-fold increase in serum AST levels predicated a thirteen-fold increase in mortality. We can thus postulate from the findings of the present study that elevated AST levels at admission are a harbinger of a worse prognosis for patients with COVID-19. On the other hand, serum ALT, bilirubin and albumin levels did not alter mortality after

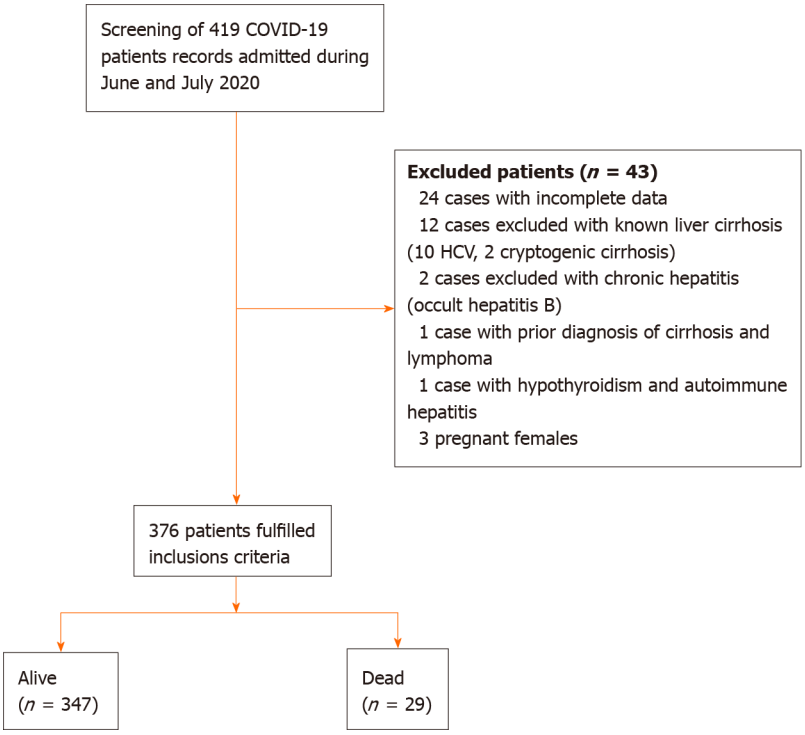

Figure 1 Flowchart of studied cohort. COVID-19: Coronavirus disease 2019; HCV: Hepatitis C virus.

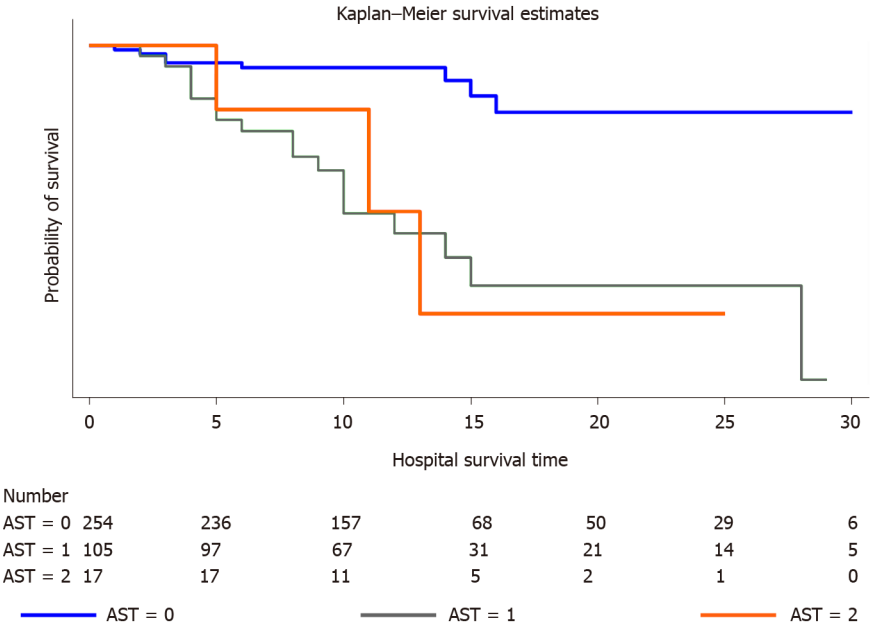

Figure 2 Kaplan–Meier curves. Survival curves show probability of survival (days) for aspartate aminotransferase (AST) groups, tested by log-rank test. AST = 0: Group with normal AST; AST = 1: Group with one-fold elevated AST; AST = 2: Group with two-fold elevated AST.

correction for age, sex and other relevant clinical factors. Our findings are consistent with recent reports investigating progressive liver injury and the risk of mortality among patients with COVID-19 where AST levels but not ALT levels at admission were a strong predictor of mortality[9-11].

In our cohort, AST levels did not correlate with the levels of CK, a marker of muscle injury, at admission. Moreover, AST levels correlated moderately with inflammatory markers at admission. Based on these findings, liver injury in patients with COVID-19 may be related to the proinflammatory state associated with cytokine release.

In healthy individuals, plasma levels of ALT and AST represent the balance between normal turnover of hepatocytes by apoptosis and the clearance rates of these

enzymes from hepatic sinusoids. Normally, ALT is present in the cytoplasm of hepatocytes, whereas AST is present in the cytoplasm and mitochondria of hepatocytes. Although the ratio of hepatic AST/ALT is 2.5:1, the serum levels of AST and ALT are similar after hepatocyte turnover because the clearance rate of AST is two times faster than the clearance rate of ALT[12,13].

In individuals with hepatocellular injury, serum levels of AST and ALT reflect the time course of hepatic injury and prognosis of hepatic insult. Early hepatocyte injury results in the release of cytosolic AST and ALT. If hepatocyte injury is severe, mitochondrial damage will result in increased release of mitochondrial AST in serum. Therefore, the predominant increase in the admission AST levels in our cohort might reflect early and severe hepatocyte injury. Furthermore, SARS-CoV-2 may induce endothelial cell injury in the hepatic microcirculation and promote portal or sinusoidal microthrombosis. In individuals with ischaemic liver injury (due to microthrombosis), serum AST levels peak before ALT levels, a pattern that was observed in our cohort[9,11,13].

In practice, an isolated and predominant elevation of AST levels indicates a nonhepatic source of AST, *e.g.*, muscle, and haemolysis.

Myositis results in increased levels of AST and, to a lesser extent, ALT; however, the increased serum levels of muscular aminotransferases should be associated with increased serum levels of CK. In our cohort, no significant correlation between AST and CK levels at admission was observed. This finding suggests true hepatic injury as the main source of elevated AST levels.

Haemolysis results in increased levels of AST and unconjugated bilirubin. In our cohort, we identified 6 patients with biphasic hyperbilirubinemia. ALT levels correlated with serum total bilirubin levels. Therefore, hyperbilirubinemia was due to liver injury and not haemolysis.

Among our cohort, 33.0% of patients were obese, perhaps with underdiagnosed nonalcoholic fatty liver disease. In addition, 20.0% of patients in our cohort were diagnosed with diabetes. Both diabetes mellitus and obesity increase serum levels of AST and ALT, but this change is more prominent for ALT than for AST[14].

The mechanism of liver injury among patients with COVID-19 is unclear and possibly multifactorial. The entry of SARS-CoV-2 into hepatocytes and cholangiocytes is mediated by ACE2 receptors. Liver biopsies obtained from deceased patients diagnosed with COVID-19 revealed focal degeneration and necrosis. In addition, SARS-CoV-2 particles were detected in hepatocytes[4]. Focal hepatic degeneration and necrosis may be due to the direct cytopathic effect of viral entry or could be an immune-mediated process. Entry of SARS-CoV-2 into hepatocytes triggers an innate and adaptive immune response that results in clearing of virus-infected cells. However, if the mounted immune response is exaggerated and uncontrolled, this aberrant immune response may contribute to the development of a cytokine storm and multisystem dysfunction[4,15]. Moreover, hyperinflammatory syndrome can induce disseminated intravascular coagulation with ischaemic hepatocellular injury by microvascular thrombosis in the hepatic microcirculation. In addition, direct endothelial cell damage in the hepatic microcirculation induced by SARS-CoV-2 may promote microvascular thrombosis and ischaemic liver injury[11].

In addition, our findings indicated that an age > 60 years, diabetes mellitus and increased serum ferritin levels were independent strong predictors of mortality among patients with COVID-19 presenting liver injury. These observations are consistent with recent studies[10].

Our study provides evidence that serum ferritin levels were associated with all-cause in-hospital mortality. Of our cohort, 56.0% of patients presented elevated serum ferritin levels. Moreover, 97.0% of nonsurvivors had elevated serum ferritin levels. In addition, logistic regression analysis showed that the serum ferritin level was an independent risk biomarker for in-hospital mortality among patients with COVID-19. Furthermore, admission serum ferritin levels correlated with CRP levels. These results suggest that elevated serum ferritin levels at admission may reflect disease severity. Our findings are consistent with a recent report confirming that increased ferritin levels are associated with in-hospital mortality in patients with COVID-19[16].

Inflammatory cytokines stimulate hepatocytes and macrophages to release ferritin, which plays a vital role in many autoimmune diseases and inflammatory disorders. A vicious loop exists between ferritin and inflammatory cytokines, *i.e.* activated hepatocytes and macrophages release ferritin, which in turn stimulates the production of various inflammatory cytokines. Serum ferritin is an inflammatory cytokine that indirectly stimulates proinflammatory pathways through the activation of the transcription factor nuclear factor kappa-B. Moreover, the heavy subunit of ferritin directly increases the mRNA expression of many inflammatory cytokines, such as

interleukin-1, interleukin-6, tumour necrosis factor and NOD-like receptor 3, indicating the proinflammatory properties of ferritin[16,17].

Patients with diabetes have elevated serum ferritin levels, and these patients are at increased risk of serious complications from COVID-19. Therefore, ferritin may be a key mediator of immune dysregulation that contributes to the cytokine storm in patients with diabetes mellitus and COVID-19[16,17].

Limitations

This retrospective study revealed an association between AST levels and mortality in patients with COVID-19 but did not reveal causality. Numerous medications and clinical and biological conditions injure hepatocytes but were only partially considered in the regression analysis. We used liver enzyme level at the time of admission for group categorization without knowing whether they were episodic or progressive changes. We did not consider concurrent medication use, such as angiotensin converting enzyme inhibitors or angiotensin receptor blockers, in our analysis. Underdiagnosed nonalcoholic fatty liver disease and occult consumption of alcohol were not considered.

CONCLUSION

Our study revealed that liver injury is highly prevalent among patients with COVID-19 at admission. Liver injury with an AST-dominant pattern can be used to predict the severity of COVID-19. This study confirmed an elevated level of ferritin in patients with COVID-19. Admission serum ferritin levels are associated with fatal outcomes. Meticulous monitoring is highly recommended for patients with COVID-19 presenting AST-dominant hepatocellular injury, especially those older than 60 years, those with elevated levels of ferritin and those with diabetes mellitus.

ARTICLE HIGHLIGHTS

Research background

Clearly, infection with severe acute respiratory syndrome coronavirus 2 is not limited to the lung but also affects other organs.

Research motivation

Predictive models are needed to determine patients' prognoses and to improve health care resource allocation during the coronavirus disease 2019 (COVID-19) pandemic.

Research objectives

To investigate whether biomarkers of hepatocellular injury at admission have prognostic value in predicting all-cause in-hospital mortality in patients with COVID-19.

Research methods

A retrospective cohort study was conducted on 376 consecutive adult patients admitted to Al-Azhar University Hospital, Assiut, Egypt and Abo Teeg General Hospital, Assiut, Egypt with confirmed COVID-19 from June 1, 2020 to July 30, 2020.

Research results

High-risk populations, especially patients aged ≥ 60 years, patients with aspartate aminotransferase (AST)-dominant liver injury or those with diabetes, should be intensively monitored. Admission serum AST and serum ferritin levels have the strongest association with the prognosis of patients with COVID-19 and can be used to monitor patients with COVID-19 at risk of liver injury.

Research conclusions

Liver injury with an AST-dominant pattern can predict the severity of COVID-19. This study confirmed an elevated level of ferritin in patients with COVID-19. Elevated serum ferritin levels are associated with in-hospital mortality.

Research perspectives

Meticulous monitoring is highly recommended for patients with COVID-19 presenting AST-dominant hepatocellular injury, especially those older than 60 years, patients with elevated serum ferritin levels or those with diabetes mellitus.

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META-ANALYSIS

Non-invasive tests for predicting liver outcomes in chronic hepatitis C patients: A systematic review and meta-analysis

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Abstract

BACKGROUND

Liver fibrosis leads to liver-related events in patients with chronic hepatitis C (CHC) infection. Although non-invasive tests (NITs) are critical to early detection of the development of liver fibrosis, the prognostic role of NITs remains unclear due to the limited types of NITs and liver outcomes explored in previous studies.

AIM

To determine the prognostic value of NITs for risk stratification in CHC patients.

METHODS

The protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews; no. CRD42019128176). The systematic review was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Search was performed using MEDLINE and EMBASE

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databases under a timeframe from the inception of the databases through February 25, 2020. We restricted our search to CHC cohort studies reporting an association between liver fibrosis assessed by NITs and the development of hepatocellular carcinoma, decompensation, or mortality. Pooled hazard ratios (HR) and area under the receiver operating characteristic (AUROC) for each NIT were estimated using a random effects model. Subgroup analyses were performed for NITs assessed at pre-treatment or post-treatment with sustained virologic response (SVR), treatment with either pegylated interferon and ribavirin or direct acting antiviral, Eastern or Western countries, and different cutoff points.

RESULTS
The present meta-analysis included 29 cohort studies, enrolling 69339 CHC patients. Fibrosis-4 (FIB-4) index, aspartate aminotransferase to platelet ratio (APRI) score, and liver stiffness measurement (LSM) were found to have hepatocellular carcinoma predictive potential with pooled adjusted HRs of 2.48 [95% confidence interval (CI): 1.91-3.23, I^2 = 96%], 4.24 (95%CI: 2.15-8.38, I^2 = 20%) and 7.90 (95%CI: 3.98-15.68, I^2 = 52%) and AUROCs of 0.81 (95%CI: 0.73-0.89, I^2 = 77%), 0.81 (95%CI: 0.75-0.87, I^2 = 68%), and 0.79 (95%CI: 0.63-0.96, I^2 = 90%), respectively. Pooled adjusted HR with a pre-treatment FIB-4 cutoff of 3.25 was 3.22 (95%CI: 2.32-4.47, I^2 = 80%). Pooled adjusted HRs for post-treatment with SVR FIB-4, APRI, and LSM were 3.01 (95%CI: 0.32-28.61, I^2 = 89%), 9.88 (95%CI: 2.21-44.17, I^2 = 24%), and 6.33 (95%CI: 2.57-15.59, I^2 = 17%), respectively. Pooled adjusted HRs for LSM in patients with SVR following direct acting antiviral therapy was 5.55 (95%CI: 1.47-21.02, I^2 = 36%). Pooled AUROCs for post-treatment with SVR FIB-4 and LSM were 0.75 (95%CI: 0.55-0.95, I^2 = 88%) and 0.84 (95%CI: 0.66-1.03, I^2 = 88%), respectively. Additionally, FIB-4 and LSM were associated with overall mortality, with pooled adjusted HRs of 2.07 (95%CI: 1.49-2.88, I^2 = 27%) and 4.04 (95%CI: 2.40-6.80, I^2 = 63%), respectively.

CONCLUSION
FIB-4, APRI, and LSM showed potential for risk stratification in CHC patients. Cutoff levels need further validation.

Key Words: Non-invasive tests; Prognosis; Hepatitis C virus; Hepatocellular carcinoma; Mortality; Liver-related outcomes

Core Tip: Previous meta-analyses have evidenced the potential of non-invasive tests (NITs) in determining prognosis. However, these syntheses included studies on chronic liver diseases from various etiologies and did not comprehensively explore all liver-related outcomes. We aimed to assess the importance of validated NITs in risk stratification, specifically in chronic hepatitis C (CHC) patients. Fibrosis-4 (FIB-4) index, aspartate aminotransferase to platelet ratio (APRI) score and liver stiffness measurement (LSM) were found to have prognostic value and can be leveraged to stratify risk for CHC patients, regardless of treatment status or regimen. Further validation of FIB-4, APRI and LSM cutoff levels are needed.

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INTRODUCTION

Chronic hepatitis C (CHC) infection can lead to the development of liver fibrosis and cirrhosis that are commonly associated with hepatocellular carcinoma (HCC), other

liver-related events (LREs), and mortality. Liver biopsy is considered the gold standard for evaluating liver fibrosis in patients with chronic liver disease. Since the introduction of non-invasive tests (NITs), biopsy use has substantially declined. Currently available NITs for liver fibrosis assessment include direct and indirect serum markers and radiologic examination such as liver stiffness measurement (LSM). According to the 2018 European Association for the Study of the Liver guidelines, the degree of liver fibrosis should be assessed by NITs in CHC patients prior to any treatment[1]. The degree of liver fibrosis determines optimal treatment regimen and whether the patient requires post-treatment monitoring of HCC development. NITs are also recommended for monitoring untreated CHC patients every 1 to 2 years[2].

Although serum markers and LSM have been shown to identify accurately patients with cirrhosis (F4) and patients without fibrosis (F0), their ability to stage intermediate degrees of fibrosis and post-treatment residual fibrosis is suboptimal[2,3]. The difficulties in the prediction of significant or advanced fibrosis without histologic confirmation has made risk stratification problematic for some CHC patients. For instance, the decision to pursue HCC surveillance following successful treatment of hepatitis C virus (HCV) infection [*i.e.* sustained virologic response (SVR)] is controversial for patients with advanced fibrosis (F3)[2,4].

Previous meta-analyses have evidenced the potential of NITs in determining prognosis. However, these syntheses included studies on chronic liver diseases from various etiologies and did not comprehensively explore all liver-related outcomes[5,6]. Types of NITs investigated in these meta-analyses were also limited. In this present review, we provided an updated systematic review and meta-analysis to assess the importance of validated NITs in risk stratification specific to CHC patients.

MATERIALS AND METHODS

Literature search

The protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews; no. CRD42019128176). The systematic review was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines[7]. Search was performed using MEDLINE and EMBASE databases from the inception of databases to February 25, 2020. The NITs for hepatic fibrosis included in our review were retrieved from the European Association for the Study of the Liver, Asociación Latinoamericana para el Estudio del Hígado Clinical Practice Guidelines [1]. The list of serum biomarkers and respective formulae are provided in Supplemental Table 1. In addition to the list of NITs, the terms prognosis, decompensation, hepatocellular cancer, chronic hepatitis C, and their related terms were selected as keywords. The details of the search strategy are provided in Supplemental Table 2. We restricted our search to cohort studies. Publications in the reference list of our included studies, publications that cited the included studies, and publications that were included in recent meta-analyses[8,9] of NITs and chronic liver diseases were also reviewed.

Study selection

Two reviewers (TY and CT) independently searched for studies on the prognosis of CHC patients based on non-invasive staging of liver fibrosis. Title and abstract of the studies were initially screened. The full-text of these studies were then independently assessed for eligibility by the two reviewers. Cohort studies that met the following criteria were included: (1) NITs documented and used to identify CHC patients who had a risk of developing LREs including hepatic decompensation, HCC, and/or mortality. Hepatic decompensation (HD) was defined as the development of variceal bleeding, hepatic encephalopathy, ascites, spontaneous bacterial peritonitis, jaundice, and/or hepatorenal syndrome; (2) Patients were free of HCC and HD at enrollment; (3) Development of HD, HCC and mortality were assessed; and (4) Outcomes of interest were reported by hazard ratio (HR), relative risk, or area under the receiver operating characteristic (AUROC). Whereas studies of any size or language were included, the following studies were excluded: (1) Case-control studies, cross-sectional studies, case series, and conference abstracts; and (2) Trials enrolling patients with no evidence of HCV infection or when more than 10% of the patients were co-infected with HBV. Publications detailing the same patient cohorts but reporting different outcomes of interest were selected for separate analysis. When publications from the same cohort described the same outcomes, the study with the most comprehensive data or with the longest follow-up was selected for each outcome[10]. Any disa-

greement over study eligibility between reviewers was resolved through discussion with a third reviewer (PL).

Data extraction

A standardized form was used to extract data from the selected papers. Data included study characteristics (primary author, country, publication year, patient enrollment period, duration of follow-up), patient characteristics (age, sex, co-infection, baseline levels of NITs, fibrosis stages, HCV treatment regimen, response), method of NITs, endpoint (HD, HCC, overall and liver-related mortality), HR and AUROCs with 95% confidence intervals (95%CI), and control variables used for the adjusted analysis. Two reviewers (TY and CT) extracted the data independently, discrepancies were identified and discussed with a third reviewer (PL). Any missing data from the publications were requested from the study authors.

Risk of bias

A quality assessment of prognostic studies was performed independently by TY and CT using the Quality In Prognosis Studies tool[10]. Any disagreements between the reviewers over the risk of bias in particular studies were resolved *via* discussion with a third reviewer (PL).

Statistical analysis

Primary analysis assessed the performance of NITs in the prediction of LRE development in CHC patients. The analysis of each outcome was computed using a random-effects model. Since relative risk was provided by only one study[11], it was not included in our meta-analysis. Inverse variance method was used to pool the results. Unadjusted and adjusted HRs were pooled separately. Additionally, the significance of each NIT's prognostic value was assessed *vs* the random value (mean AUROC of each NIT was compared with 0.50 or the "random" value representing the absence of prognostic value). We then pooled the results, and 0.50 was added back to illustrate the overall prognostic value of each NIT. The AUROCs of different NITs were then compared using *t*-tests to identify any statistical difference in terms of prognostic ability. Subgroup analyses based on timing of liver fibrosis assessment (before or after HCV treatment) were performed when possible. Heterogeneity between studies was considered when I^2 value was greater than 50%. Publication bias was first evaluated by constructing funnel plots. Egger's linear regression test was also performed due to possible bias ascertained from funnel plots. All analyses were conducted using Review Manager (RevMan) [Computer program], Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014, and ProMeta (Version 3) [Computer software] (Internovi, Cesena, Italy).

RESULTS

Study selection

After removing duplicate publications, 17248 papers were identified and screened by title and abstract. Of these, 104 full articles met our predefined selection criteria and were further examined. We further excluded 65 publications due to the following reasons: Non-relevant outcomes (n = 32), outcomes not reported as risk ratio (n = 13), patients meeting our exclusion criteria, *e.g.*, prior history of HCC (n = 10), studies of the same patient cohorts (n = 5), and NITs being used as diagnostic tests for HCC or HD (n = 5) (Figure 1).

Among the 39 cohort studies matching our selection criteria, 29 studies (69339 HCV-infected patients) were selected for quantitative analysis, with the 10 remaining studies slated only for qualitative analysis.

These 39 included studies enrolled a total of 77920 participants between 1990 and 2015. Seventeen and 22 studies were conducted in Western[12-28] and Asian countries [11,29-49], respectively (Table 1, Supplemental Table 3).

The performance of the Fibrosis-4 (FIB-4) index, aspartate aminotransferase to platelet ratio (APRI) score, and LSM tests for the prediction of LREs and mortality were characterized in 20, 11, and 19 studies, respectively. LSM was mainly performed by ultrasound-based transient elastography (TE), except in two studies that used either magnetic resonance elastography (MRE)[30], or 2D-shear wave elastography (2D-SWE)[29].

Table 1 Characteristics of the cohort studies included in the systematic review

Ref.	Country	*n*	NITs	Outcomes
Chun *et al*[49], 2020	South Korea	669	FIB-4	HCC
Chalouni *et al*[18], 2019	France	998	APRI, FIB-4, TE	LRE
Chen *et al*[45], 2019	China	691	FIB-4	OM
Hansen *et al*[20], 2019	Denmark	591	TE	OM, LRD, HD
Ioannou *et al*[13], 2019	United States	48135	FIB-4	HCC
Na *et al*[33], 2019	South Korea	295	APRI, FIB-4	HCC
Nakagomi *et al*[34], 2019	Japan	1146	TE	HCC
Ogasawara *et al*[38], 2019	Japan	398	FIB-4, TE	HCC, HD
Ogasawara *et al*[47], 2019	Japan	457	FIB-4	OM
Peleg *et al*[23], 2019	Israel	515	TE	HCC, OM, HD
Pons *et al*[14], 2019	Spain	572	TE	HCC
Rinaldi *et al*[15], 2019	Italy	258	TE	HCC
Shili-Masmoudi *et al*[28], 2019	France	1062	TE	OM, LRM
Sou *et al*[41], 2019	China	1884	APRI, FIB-4	HCC
Tamaki *et al*[30], 2019	Japan	346	FIB-4, MRE	HCC
Watanabe *et al*[44], 2019	Japan	1174	APRI, FIB-4	HCC
Bloom *et al*[17], 2018	Australia	780	TE	LRE
Hamada *et al*[29], 2018	Japan	196	FIB-4, SWE	HCC
Munteanu *et al*[22], 2018	France	3449	Fibrotest	OM, LRM
Cepeda *et al*[25], 2017	United States	964	TE	OM
Gomez-Moreno *et al*[19], 2017	Spain	343	TE	HCC, HD, LRM
Merchante *et al*[26], 2017	Spain	446	TE	HD
Thandassery *et al*[43], 2017	Qatar	1605	APRI, FIB-4	HCC, HD, LRE
Akuta *et al*[39], 2016	Japan	958	FIB-4	HCC
Lee *et al*[31], 2016	South Korea	598	APRI	HCC
Lee *et al*[46], 2016	South Korea	190	TE	LRE
Ng *et al*[36], 2016	China	105	APRI	HCC
Pérez-Latorre *et al*[24], 2016	Spain	957	TE	LRE, OM
Sato *et al*[40], 2016	Japan	355	APRI, FIB-4	HCC
Tada *et al*[48], 2016	Japan	1723	FIB-4	LRM, OM
Berenguer *et al*[12], 2015	Spain	903	FIB-4	LRE, OM
Macías *et al*[21], 2015	Spain	1046	TE	HD, OM
Narita *et al*[35], 2014	Japan	151	TE	HCC
Nojiri *et al*[37], 2014	Japan	142	APRI, FIB-4, Forns index	HCC
Tamaki *et al*[42], 2014	Japan	1046	FIB-4	HCC
Bambha *et al*[16], 2012	United States	450	APRI, FIB-4	OM
Nunes *et al*[27], 2010	United States	303	APRI, FIB-4	LRM
Masuzaki *et al*[32], 2009	Japan	984	TE	HCC
Yu *et al*[11], 2006	China	1338	APRI	HCC, OM

N/A: Not available; APRI: Aspartate aminotransferase to platelet ratio index; FIB-4: Fibrosis-4 index; HCC: Hepatocellular carcinoma; HD: Hepatic

decompensation; LSM: Liver stiffness measurement; LRM: Liver-related mortality; LRE: Liver-related event; NIT: Non-invasive test; OM: Overall mortality; TE: Transient elastography; MRE: Magnetic resonance elastography.

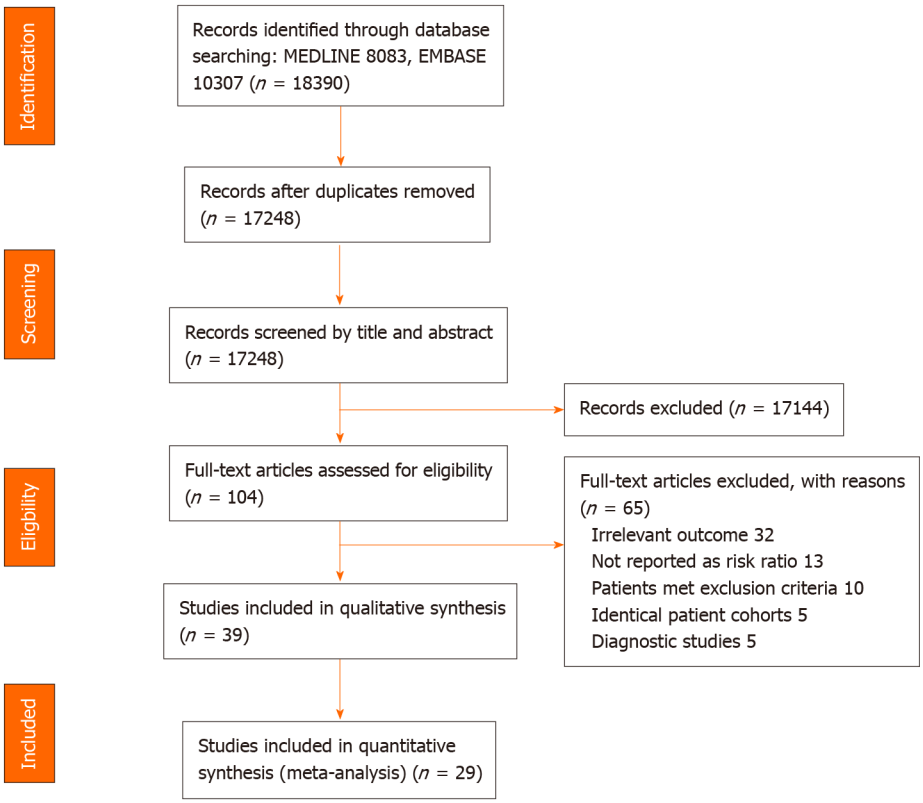


Figure 1 Flow diagram of search methodology and selection process.

The primary outcomes of interest were HCC, overall mortality, and liver-related mortality in 21[11,13-15,29-44,49], 12[3,12,17-24,38,46], and 10[16,18,20,21,25,27,28,45,47,48] studies, respectively. Twelve studies selected HD or a compound of LREs as relevant outcome(s)[12,17-21,23,24,26,38,43,46]. Characteristics of all the studies are summarized in Table 1 and Supplemental Table 3.

Eleven studies enrolled patient cohorts with HCV and human immunodeficiency virus co-infection[12,16,18,21,22,24-28,45]. Fifteen reports included only patients who were successfully treated, *i.e.* having SVR[13,14,23,29-31,33,36,38-40,44,46,47,49], while two studies enrolled only patients with cirrhosis[13,15]. All studies had a mean or median follow-up time of at least 1 year.

FIB-4, APRI, and LSM were among the most extensively explored NITs (Table 2). We did not conduct quantitative analysis using other NITs due to their very limited usage (n = 1 for Forns index[37] and Fibrotest[22], n = 0 for other NITs).

The included studies were mostly rated as low risk of bias (n = 27)[11-14,16,19-23,25,26,28-30,32,33,35-39,42-44,46,48] (Supplementary Table 4, Supplementary Figure 1). However, five studies were rated as high risk of bias because of concerns about selective reporting of multivariate analysis and other biases[34,40,41,45,49]. Only 13 studies provided the number of patients lost to follow-up[13,14,17,20-22,24,28,32,36,37,44,45]. The agreement between the two reviewers' assessment was excellent (93%).

Association between NITs and HCC risk

Among NITs included in the present analysis, FIB-4 score was the most studied NIT for its role in HCC prediction. Eleven studies including 1891 HCC cases examined the relationship between FIB-4 values and HCC development[13,29,30,33,38-42,44,49]. The FIB-4 cutoffs selected in these studies ranged from 2.5 to 4.5. All these studies reported a significant positive association between high FIB-4 values and risk of HCC development, with pooled unadjusted and adjusted HRs of 5.17 (95%CI: 4.03-6.63, I^2 = 76%) and 2.48 (95%CI: 1.91-3.23, I^2 = 96%), respectively (Figure 2A).

Table 2 Pooled unadjusted and adjusted hazard ratios of pre- and post-treatment fibrosis-4 index, aspartate aminotransferase to platelet ratio index, liver stiffness measurement for the prediction of hepatocellular carcinoma development

Analysis	HR				aHR			
	Pooled HR (95%CI)	I^2 (%)	Ref.	No. of cases	Pooled aHR (95%CI)	I^2 (%)	Ref.	No. of cases
FIB-4	5.17 (4.03-6.63)	76	[13,29,30,38,40-42]	1831	2.48 (1.91-3.23)	96	[13,33,39-42,44,49]	1842
pre-Rx	4.91 (3.71-6.49)	81	[13,38,40-42]	1781	3.20 (1.77-5.80)	97	[13,33,39-40,42,44]	1699
post-Rx with SVR	5.44 (2.25-13.15)	69	[29,30,38,41]	173	3.01 (0.32-28.61)	89	[33,49]	21
APRI	5.27 (2.34-11.83)	91	[31,40,41]	150	4.24 (2.15-8.38)	20	[33,36,41]	149
pre-Rx	4.23 (1.42-12.62)	83	[31,40,41]	142	-	-	[33]	12
post-Rx with SVR	9.33 (5.85-14.88)	0	[31,41]	130	9.88 (2.21-44.16)	24	[33,41]	134
LSM	9.45 (4.49-19.92)	70	[14,15,29,30,34,38]	301	7.90 (3.98-15.68)	52	[15,29,30,32,34,35,38]	362
pre-Rx	4.68 (2.00-10.96)	40	[15,38]	54	3.76 (1.77-8.02)	7	[15,35,38]	63
post-Rx with SVR	8.90 (4.10-19.33)	36	[14,29,30,38]	76	6.33 (2.57-15.59)	17	[29,30,38]	51

aHR: Adjusted hazard ratio; APRI: Aspartate aminotransferase to platelet ratio index; FIB-4: Fbrosis-4 index; HCC: Hepatocellular carcinoma; LSM: Liver stiffness measurement; pre-Rx: Pre-treatment; post-Rx with SVR: Post-treatment with sustained virologic response.

Five studies totaling 169 HCC cases evaluated the prognostic value of APRI and found a statistically significant positive association between high APRI values and HCC occurrence[31,33,36,40,41]. The APRI cutoffs used in these studies ranged from 0.5 to 2.0. The overall pooled unadjusted and adjusted HRs were 5.27 (95%CI: 2.34-11.83, I^2 = 91%) and 4.24 (95%CI: 2.15-8.38, I^2 = 20%), respectively (Figure 2B).

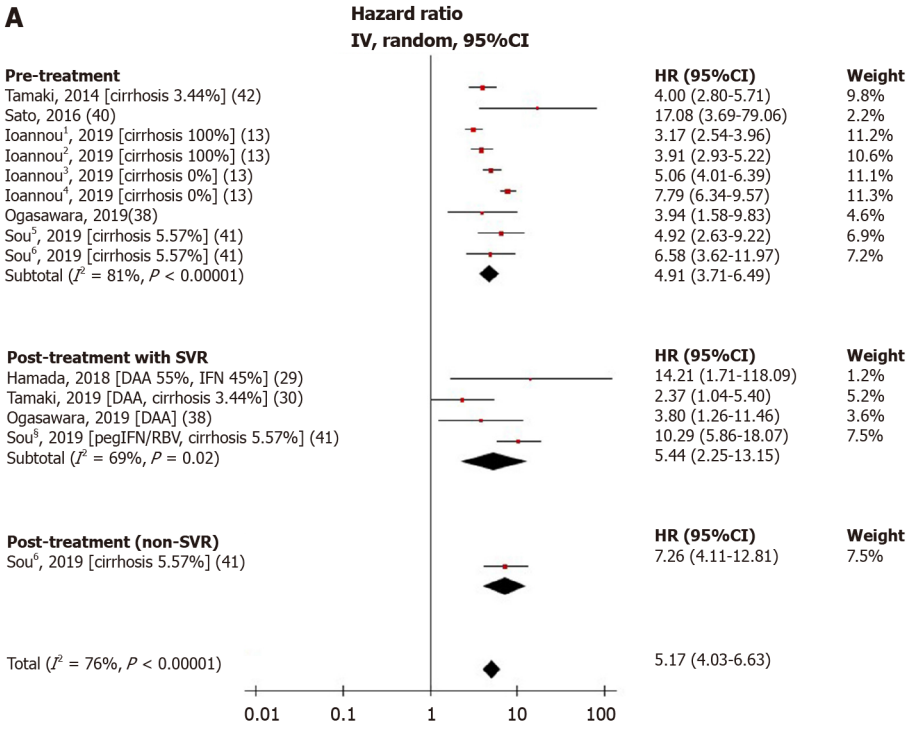
Eight studies with 387 HCC cases investigated the association between LSM and HCC risk[14,15,29,30,32,34,35,38]. The LSM cutoffs chosen for each study were all unique and ranged from 3.75 to 30. Consistent with FIB-4 score and APRI results, the overall pooled unadjusted and adjusted HRs were 9.45 (95%CI: 4.49-19.92, I^2 = 70%) and 7.90 (95%CI: 3.98-15.68, I^2 = 52%), respectively (Figure 2C).

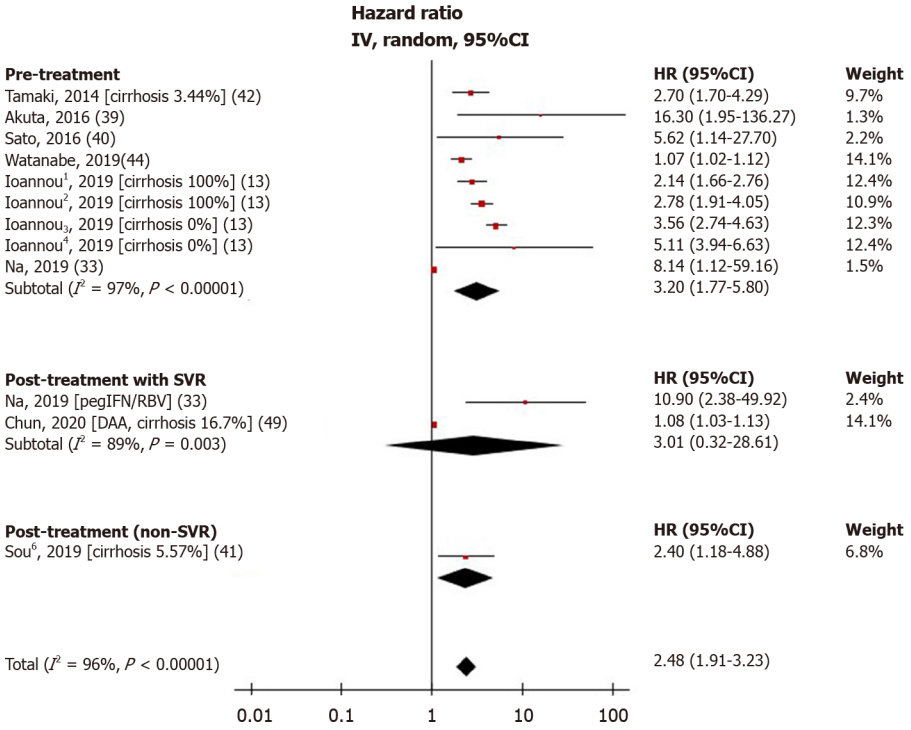
Subgroup analyses were performed for NITs assessed at pre-treatment and post-treatment with SVR. Pooled adjusted HRs for pre-treatment FIB-4 and LSM were 3.20 (95%CI: 1.77-5.80, I^2 = 97%) and 3.76 (95%CI: 1.77-8.02, I^2 = 7%), respectively. Pooled adjusted HRs for post-treatment with SVR FIB-4, APRI, and LSM were 3.01 (95%CI: 0.32-28.61, I^2 = 89%), 9.88 (95%CI: 2.21-44.16, I^2 = 24%), and 6.33 (95%CI: 2.57-15.59, I^2 = 17%), respectively (Figure 2). The prognostic ability of these NITs remains valid even after the introduction of direct-acting antiviral (DAA) therapy. Pooled unadjusted and adjusted HRs for LSM in patients with SVR following DAA therapy were 6.80 (95%CI: 3.54-13.05, I^2 = 0%) and 5.55 (95%CI: 1.47-21.02, I^2 = 36%), respectively (Supplementary Figure 2).

To determine the optimal cutoff for HCC prediction, we pooled the results using a pre-treatment FIB-4 cutoff of 3.25 as this cutoff was applied in four studies, accounting for over 51360 CHC patients (Supplementary Figure 3). We found that the pooled, unadjusted and adjusted HRs were 4.79 (95%CI: 3.58-6.42, I^2 = 85%) and 3.22 (95%CI: 2.32-4.47, I^2 = 80%), respectively, for predicting HCC development.

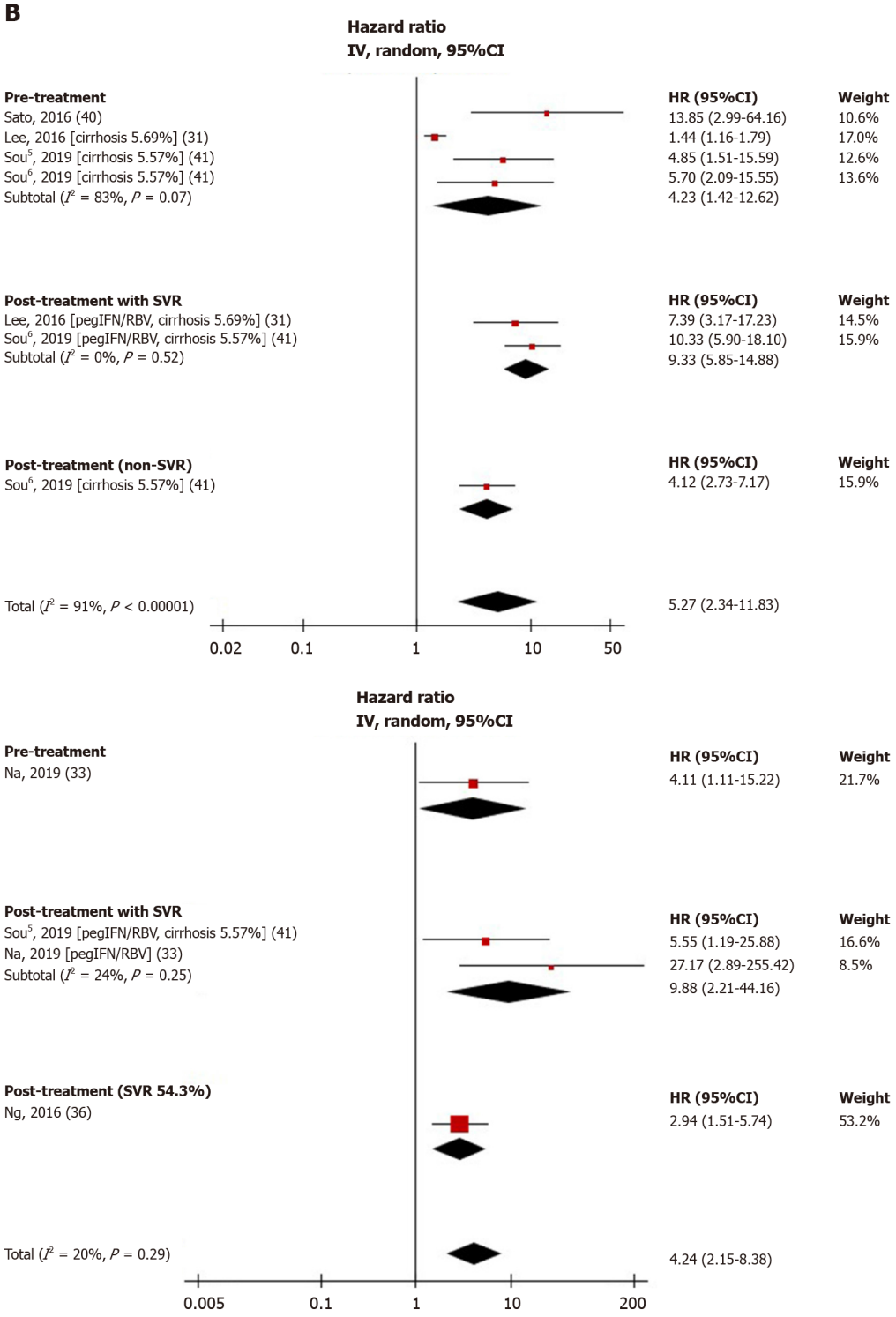
Given the high heterogeneity of the analysis of pre-treatment FIB-4, we performed subgroup analyses by location of study. We found that, in the subgroup of Asian countries, pooled unadjusted and adjusted HRs of 4.91 (95%CI: 3.60-6.70, I^2 = 18%) and 3.12 (95%CI: 1.31-7.42, I^2 = 87%) for the pre-treatment FIB-4 and HCC development (Supplementary Figure 4). The I^2 of pooled unadjusted HR decreased from 76% to 18%, while the I^2 of pooled adjusted HR slightly decreased from 97% to 87%. We hypothesized that the remaining high heterogeneity stemmed from the variety of FIB-4 cutoff used in the different studies.

Figure 3 shows the performance of NITs for HCC prediction. FIB-4 score, APRI, and LSM was significantly greater than random (AUROC = 0.5), with pooled AUROCs of 0.81 (95%CI: 0.73-0.89, I^2 = 77%), 0.81 (95%CI: 0.75-0.87, I^2 = 68%), and 0.79 (95%CI: 0.63-0.96, I^2 = 90%), respectively. The pooled AUROCs of FIB-4 and APRI were both statistically higher than that of the LSM, P < 0.0001 for both, respectively.


A
Hazard ratio
IV, random, 95%CI
Pre-treatment
HR (95%CI)
Weight
Tamaki, 2014 [cirrhosis 3.44%] (42) 4.00 (2.80-5.71) 9.8%
Sato, 2016 (40) 17.08 (3.69-79.06) 2.2%
Ioannou[1], 2019 [cirrhosis 100%] (13) 3.17 (2.54-3.96) 11.2%
Ioannou[2], 2019 [cirrhosis 100%] (13) 3.91 (2.93-5.22) 10.6%
Ioannou[3], 2019 [cirrhosis 0%] (13) 5.06 (4.01-6.39) 11.1%
Ioannou[4], 2019 [cirrhosis 0%] (13) 7.79 (6.34-9.57) 11.3%
Ogasawara, 2019(38) 3.94 (1.58-9.83) 4.6%
Sou[5], 2019 [cirrhosis 5.57%] (41) 4.92 (2.63-9.22) 6.9%
Sou[6], 2019 [cirrhosis 5.57%] (41) 6.58 (3.62-11.97) 7.2%
Subtotal (I^2 = 81%, P < 0.00001) 4.91 (3.71-6.49)
Post-treatment with SVR
HR (95%CI)
Weight
Hamada, 2018 [DAA 55%, IFN 45%] (29) 14.21 (1.71-118.09) 1.2%
Tamaki, 2019 [DAA, cirrhosis 3.44%] (30) 2.37 (1.04-5.40) 5.2%
Ogasawara, 2019 [DAA] (38) 3.80 (1.26-11.46) 3.6%
Sou[§], 2019 [pegIFN/RBV, cirrhosis 5.57%] (41) 10.29 (5.86-18.07) 7.5%
Subtotal (I^2 = 69%, P = 0.02) 5.44 (2.25-13.15)
Post-treatment (non-SVR)
HR (95%CI)
Weight
Sou[6], 2019 [cirrhosis 5.57%] (41) 7.26 (4.11-12.81) 7.5%
Total (I^2 = 76%, P < 0.00001) 5.17 (4.03-6.63)
0.01
0.1
1
10
100


Hazard ratio
IV, random, 95%CI
Pre-treatment
HR (95%CI)
Weight
Tamaki, 2014 [cirrhosis 3.44%] (42) 2.70 (1.70-4.29) 9.7%
Akuta, 2016 (39) 16.30 (1.95-136.27) 1.3%
Sato, 2016 (40) 5.62 (1.14-27.70) 2.2%
Watanabe, 2019(44) 1.07 (1.02-1.12) 14.1%
Ioannou[1], 2019 [cirrhosis 100%] (13) 2.14 (1.66-2.76) 12.4%
Ioannou[2], 2019 [cirrhosis 100%] (13) 2.78 (1.91-4.05) 10.9%
Ioannou[3], 2019 [cirrhosis 0%] (13) 3.56 (2.74-4.63) 12.3%
Ioannou[4], 2019 [cirrhosis 0%] (13) 5.11 (3.94-6.63) 12.4%
Na, 2019 (33) 8.14 (1.12-59.16) 1.5%
Subtotal (I^2 = 97%, P < 0.00001) 3.20 (1.77-5.80)
Post-treatment with SVR
HR (95%CI)
Weight
Na, 2019 [pegIFN/RBV] (33) 10.90 (2.38-49.92) 2.4%
Chun, 2020 [DAA, cirrhosis 16.7%] (49) 1.08 (1.03-1.13) 14.1%
Subtotal (I^2 = 89%, P = 0.003) 3.01 (0.32-28.61)
Post-treatment (non-SVR)
HR (95%CI)
Weight
Sou[6], 2019 [cirrhosis 5.57%] (41) 2.40 (1.18-4.88) 6.8%
Total (I^2 = 96%, P < 0.00001) 2.48 (1.91-3.23)
0.01
0.1
1
10
100

B


Hazard ratio
IV, random, 95%CI
Pre-treatment
Sato, 2016 (40)
Lee, 2016 [cirrhosis 5.69%] (31)
Sou[5], 2019 [cirrhosis 5.57%] (41)
Sou[6], 2019 [cirrhosis 5.57%] (41)
Subtotal (I² = 83%, P = 0.07)
HR (95%CI)
13.85 (2.99-64.16)
1.44 (1.16-1.79)
4.85 (1.51-15.59)
5.70 (2.09-15.55)
4.23 (1.42-12.62)
Weight
10.6%
17.0%
12.6%
13.6%
Post-treatment with SVR
Lee, 2016 [pegIFN/RBV, cirrhosis 5.69%] (31)
Sou[6], 2019 [pegIFN/RBV, cirrhosis 5.57%] (41)
Subtotal (I² = 0%, P = 0.52)
HR (95%CI)
7.39 (3.17-17.23)
10.33 (5.90-18.10)
9.33 (5.85-14.88)
Weight
14.5%
15.9%
Post-treatment (non-SVR)
Sou[6], 2019 [cirrhosis 5.57%] (41)
HR (95%CI)
4.12 (2.73-7.17)
Weight
15.9%
Total (I² = 91%, P < 0.00001)
5.27 (2.34-11.83)
0.02
0.1
1
10
50
Hazard ratio
IV, random, 95%CI
Pre-treatment
Na, 2019 (33)
HR (95%CI)
4.11 (1.11-15.22)
Weight
21.7%
Post-treatment with SVR
Sou[5], 2019 [pegIFN/RBV, cirrhosis 5.57%] (41)
Na, 2019 [pegIFN/RBV] (33)
Subtotal (I² = 24%, P = 0.25)
HR (95%CI)
5.55 (1.19-25.88)
27.17 (2.89-255.42)
9.88 (2.21-44.16)
Weight
16.6%
8.5%
Post-treatment (SVR 54.3%)
Ng, 2016 (36)
HR (95%CI)
2.94 (1.51-5.74)
Weight
53.2%
Total (I² = 20%, P = 0.29)
4.24 (2.15-8.38)
0.005
0.1
1
10
200

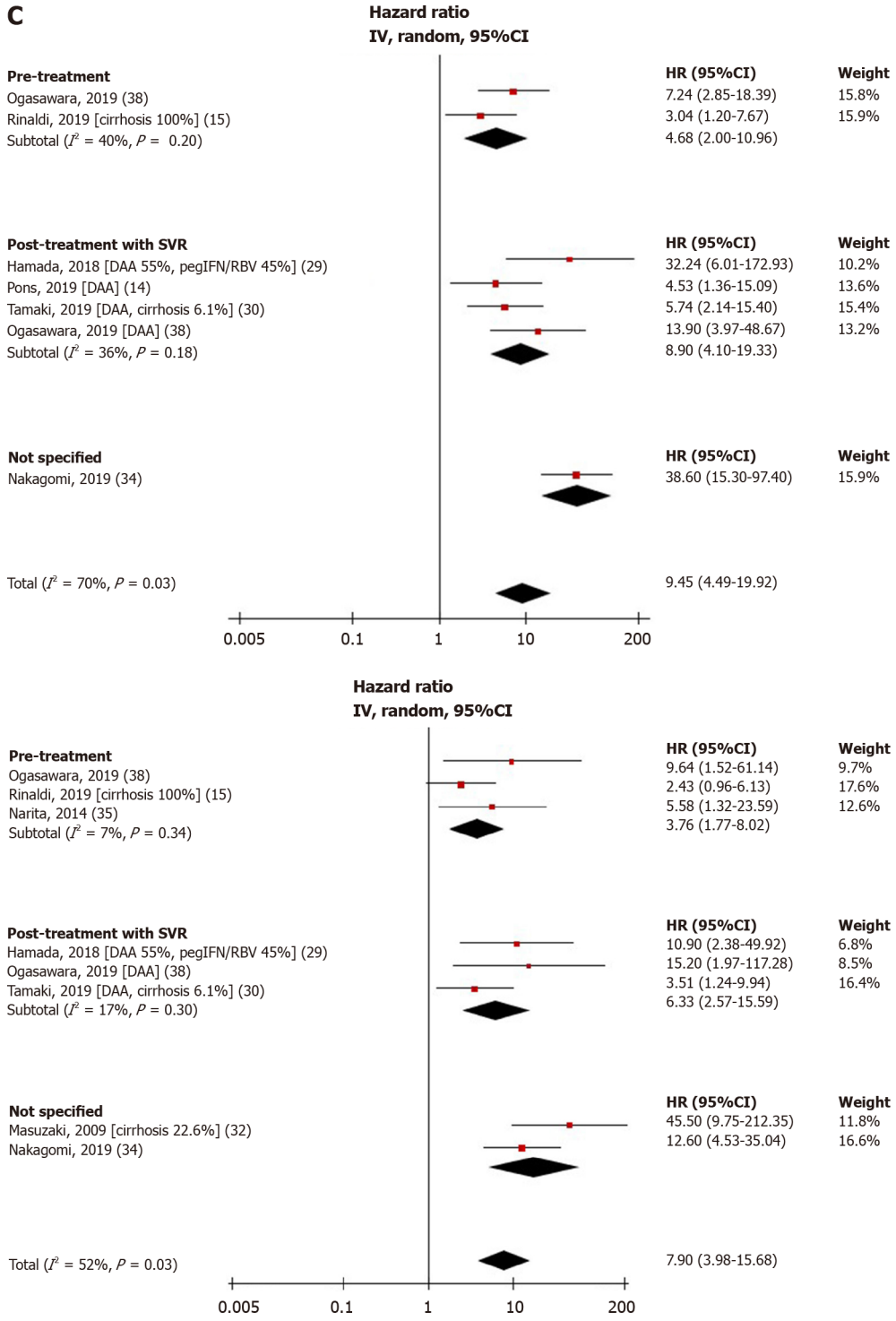


Figure 2 Unadjusted and adjusted hazard ratios of fibrosis-4 index (A), aspartate aminotransferase to platelet ratio score (B), liver stiffness measurement (C), and hepatocellular carcinoma risk. [1]Cirrhosis and direct acting antiviral-treated cohort. [2]Cirrhosis and interferon-treated cohort. [3]Non-cirrhotic and direct acting antiviral-treated cohort. [4]Non-cirrhotic and interferon-treated cohort. [5]Sustained virologic response cohort. [6]Non-sustained virologic response cohort. DAA: Direct-acting antiviral; FIB-4: Fibrosis-4 index; pegIFN/RBV: Pegylated interferon and ribavirin; SVR: Sustained virologic response.

We further analyzed the prognostic values of NITs before and after HCV treatment. For the pre-treatment period, the pooled AUROC of FIB-4 score was significantly greater compared to APRI (0.88, (95%CI: 0.83-0.92, I^2 = 0%) *vs* 0.77, (95%CI: 0.70-0.84, I^2 = 36%), P < 0.0001). For NITs assessed at post-treatment among patients with SVR, the pooled AUROC of LSM was 0.84 (95%CI: 0.66-1.03, I^2 = 88%), which was statistically higher than that of FIB-4 (pooled AUROC 0.75, 95%CI: 0.55-0.95, I^2 = 88%), P < 0.0001. The pooled AUROC of pre-treatment LSM and post-treatment APRI score was not estimated due to the limited number of studies (n = 1 each).

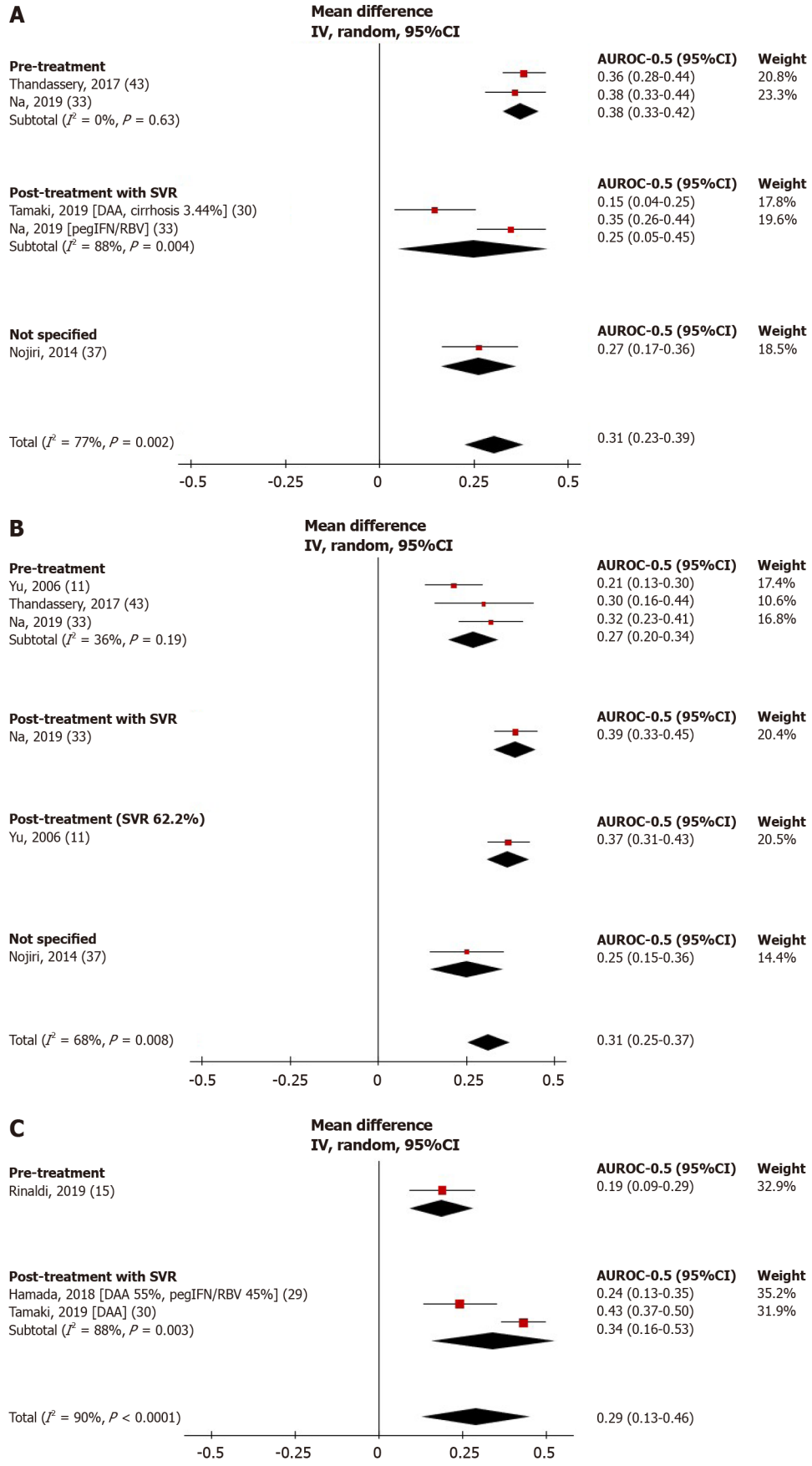


Figure 3 Forest plots showing hepatocellular carcinoma predictive performance *vs* random of fibrosis-4 (A), random of aspartate aminotransferase to platelet ratio (B), and random of liver stiffness measurement (C). DAA: Direct-acting antiviral; FIB-4: Fibrosis-4 index; pegIFN/RBV: Pegylated interferon and ribavirin; SVR: Sustained virologic response. APRI: Aspartate aminotransferase to platelet ratio index; LSM: Liver stiffness measurement.

Association between NITs and overall mortality

Four studies identifying 823 deaths among 3321 patients reported a significant positive association between FIB-4 score and overall mortality with pooled unadjusted and adjusted HRs of 3.06 (95%CI: 1.38-6.67, I^2 = 90%) and 2.07 (95%CI: 1.49-2.88, I^2 = 27%), respectively (Supplementary Figure 5)[16,45,47,48]. Likewise, a significant positive association between LSM and overall mortality was reported from four studies containing 3663 patients with 368 deaths[20,21,25,28], with pooled unadjusted and adjusted HRs of 5.52 (95%CI: 2.81-10.85, I^2 = 74%) and 4.04 (95%CI: 2.40-6.80, I^2 = 63%), respectively (Supplementary Figure 6).

The pooled HR and AUROC of APRI performance for the prediction of mortality was not estimated because only one study was included in this meta-analysis. The AUROCs for predicting overall mortality reported in individual studies are shown in Table 3.

Liver-related mortality, decompensation of cirrhosis, and composite outcomes

Due to the broad definitions of HD and LRE outcomes, we did not perform a meta-analysis on these outcomes. However, taken individually, any NIT showed statistically significant positive associations and predictive values for their respective outcomes. The HRs and AUROCs of NITs and liver-related outcomes are summarized in Tables 4 and 5[12,16-21,23-28,38,43,45-48].

Publication bias

Publication bias was assessed through Deeks funnel plots for unadjusted and adjusted HRs of NITs and LREs. The distribution of studies was symmetrical for all analyses, except for adjusted HRs of FIB-4, APRI, LSM, and HCC development, which showed asymmetry (Figure 4). Egger's regression asymmetry test detected publication bias in adjusted HRs of FIB-4 (P < 0.001) but not in HRs of APRI or LSM (P = 0.081 and 0.097, respectively). We found that five out of eight studies that reported an adjusted HR for FIB-4 score each had more than 1000 participants[33,39-41,49]. When only studies with > 1000 participants were selected for the subgroup analysis of adjusted HRs of FIB-4 and HCC development, publication bias was no longer detected (P = 0.12), suggesting that bias resulted from the inclusion of small studies.

DISCUSSION

NITs for liver fibrosis assessment play an important role in the management of HCV infection. Liver fibrosis staging is determinant for treatment prioritization and regimen in low- and middle-income countries as well as HCC surveillance. In addition to fibrosis staging, NITs are increasingly evaluated for their prognostic value. Our systematic review highlighted the potential use of FIB-4, APRI, and LSM to guide risk-stratified strategies in HCV-infected patients.

We found that LSM had a higher pooled HR for HCC development than APRI and FIB-4. TE is the most validated method for LSM as judged by its clinical implementation since 2003[3]. Other techniques such as MRE and 2D-SWE were also shown to have a better performance than TE in differentiating stages of fibrosis[50,51], but they are not as widely available. All of the studies included in our review performed LSM by TE, with the exception of those from Tamaki *et al*[30] and Hamada *et al*[29], which used MRE and real-time SWE, respectively. Although both studies[29,30] evidenced higher HRs for HCC development, the difference in prognostic ability compared to TE was not explored in our meta-analysis due to the limited number of studies using MRE and 2D-SWE.

Although LSM is the most commonly used and validated NIT for liver fibrosis staging, several drawbacks can limit its use in practice such as costly equipment and maintenance, need for frequent calibration and skilled operators, and limited performance in obese patients. Therefore, the use of serologic markers such as APRI or FIB-4 score were recommended by the World Health Organization (WHO)[52] to assess hepatic fibrosis in resource-limited settings. Indeed, these scores can be easily calculated using only patient age and common laboratory data (aspartate aminotransferase, alanine aminotransferase, platelets). Considering the current recommendation to measure the degree of liver fibrosis prior to HCV treatment[2], we found that in a pre-treatment setting APRI and FIB-4 score performed well in terms of HCC prediction, with AUROCs of 0.77 and 0.88, respectively. They could provide similar, if not higher, prognostic value in comparison to LSM.

WHO has committed to eradicate viral hepatitis by 2030. Since the introduction of direct acting antiviral (DAA) therapy, the number of treated CHC patients achieving

Table 3 Area under the receiver operating characteristic curves of non-invasive tests for overall mortality, liver-related mortality, and composite outcomes

Ref.	NIT[1]	Outcome	AUROC (95%CI)
Chalouni *et al*[18], 2019	APRI	OM	0.58 (N/A)
		LRM	0.80 (N/A)
		LRE	0.75 (N/A)
	FIB-4	OM	0.66 (N/A)
		LRM	0.88 (N/A)
		LRE	0.78 (N/A)
	TE	OM	0.69 (N/A)
		LRM	0.88 (N/A)
		LRE	0.88 (N/A)
Hansen *et al*[20], 2019	TE	OM	0.70 (0.62–0.78)
		LRM	0.93 (0.89–0.98)
		HD (HCC included)	0.89 (0.82–0.97)
Munteanu *et al*[22], 2018	Fibrotest	OM	0.74 (0.71-0.77)
		LRM	0.88 (0.85-0.90)
Thandassery *et al*[43], 2017	APRI (Pre-Rx)	HD	0.54 (0.06–0.78)
	FIB-4 (Pre-Rx)	HD	0.85 (0.74–0.96)
Pérez-Latorre *et al*[24], 2016	TE	OM	Estimation cohort 0.87 (0.84-0.90)
			Validation cohort 0.88 (0.84-0.91)
Lee *et al*[46], 2016	TE (Post-Rx)	A composite outcome of HD, HCC, and/or LRM	0.92 (0.84-1.00)
Berenguer *et al*[12], 2015	FIB-4 (Pre-Rx)	LRE (HD or HCC)	0.75 (0.72-0.78)
Yu *et al*[11], 2006	APRI (Pre-Rx)	OM	0.53 (0.35-0.72)
	APRI (Post-Rx)	OM	0.87 (0.81-0.93)

[1]NITs are not classified as either pre-treatment or post-treatment once the study did not specify when the NIT measurement regarding the initiation of hepatitis C virus therapy was done. N/A: Not available; APRI: Aspartate aminotransferase to platelet ratio index; FIB-4: Fibrosis-4 index; HCC: Hepatocellular carcinoma; HD: Hepatic decompensation; LSM: Liver stiffness measurement; LRM: Liver-related mortality; LRE: Liver-related event; NIT: Non-invasive test; OM: Overall mortality; pre-Rx: Pre-treatment; post-Rx: Post-treatment; AUROC: Area under the receiver operating characteristic curves; TE: Transient elastography.

SVR has greatly increased. SVR is independently associated with improved hepatic function and prognosis[35,36]. Despite achieving SVR, some patients can develop HCC or LREs suggesting that regular follow-up remains necessary[13,30,31,33,39,41,49]. Non-invasive assessment of residual fibrotic burden in post-therapy patients who achieved SVR is currently unreliable[2]. This issue could explain at least partly the decision of international guidelines not to recommend NITs for monitoring of post-treatment residual fibrosis[1,2]. Despite its questionable diagnostic potential, we found that among patients with SVR, APRI and LSM can predict HCC development with AUROC values of 0.75 and 0.84, respectively. This was shown to be helpful even in the DAA era, as shown in our study that the adjusted HR of LSM and HCC risk in patients achieving SVR after DAA era was 5.55.

Large variations in NIT cutoffs were observed in the studies included in our meta-analysis. For example, the cutoff of FIB-4 score recommended by WHO for predicting significant fibrosis (METAVIR ≥F2) is 1.45 for high sensitivity and 3.25 for high specificity[52]. We found that five out of 11 studies included in this meta-analysis chose the cutoff of 3.25[13,33,41,42,49], while no studies used the cutoff of 1.45. Accordingly, we pooled the results for unadjusted and adjusted HRs of pretreatment FIB-4 using the 3.25 cutoff and found that this cutoff had a statistically significant potential to be used clinically for HCC risk stratification, with a pooled adjusted HR of 3.22 (no subgroup analysis of post-treatment SVR population was done due to the lack

Table 4 Unadjusted and adjusted hazard ratios of non-invasive test for the prediction of liver-related mortality

Unadjusted hazard ratio (HR)				
Ref.	**NIT[1]**	**HR (95%CI)**	***P* value**	
Hansen *et al*[20], 2019	TE	97.00 (13.20–713.00)	< 0.005	
Shili-Masmoudi *et al*[28], 2019	TE	29.65 (8.88–99.01)	< 0.001	
Nunes *et al*[27], 2010	APRI	10.18 (4.86–21.32)	N/A	
	FIB-4	9.45 (4.51–19.79)	N/A	
Adjusted hazard ratio (aHR)				
Ref.	**NIT[1]**	**aHR (95%CI)**	***P* value**	**Adjustment variables**
Hansen *et al*[20], 2019	TE	11.00 (1.22–98.60)	0.018	SVR
Shili-Masmoudi *et al*[28], 2019	TE	20.60 (5.99–70.78)	< 0.001	Gender, alcohol consumption, drug consumption, CD4 count, HCV genotype, metabolic disorders, previous HCV treatment
Macías *et al*[21], 2015	TE	29.90 (4.30–217.00)	0.001	Age, gender, platelet counts, AIDS at baseline, alcohol use, treatment against HCV, time-varying CD4 cell counts, undetectable HIV RNA
Tada *et al*[48], 2016	FIB-4 (Pre-Rx)	13.02 (4.16–40.77)	< 0.001	Age, gender, AST concentration, ALT concentration, albumin, total bilirubin concentration, prothrombin time, platelet count, AFP concentration, FIB-4 index
Nunes *et al*[27], 2010	FIB-4	1.19 (1.12–1.27)	< 0.001	Gender, MELD
	FIB-4	1.13 (1.05–1.21)	0.001	Gender, CPT
	APRI	1.11 (1.01–1.22)	0.035	Gender, CPT
	APRI	1.25 (1.15–1.35)	< 0.001	Gender, MELD

[1]Non-invasive tests are not classified as either pre-treatment or post-treatment if the study did not specify when the non-invasive test measurement was done with regards to the initiation of hepatitis C virus therapy. NIT: Non-invasive test; HR: Hazard ratio; AFP: alpha-fetoprotein; APRI: Aspartate aminotransferase to platelet ratio index; CTP: Child-Turcotte-Pugh score; FIB-4: Fibrosis-4 index; N/A: Not available; LSM: Liver stiffness measurement; MELD: Model for end-stage liver disease score; pre-Rx: Pre-treatment; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; SVR: Sustained virologic response; TE: Transient elastography.

of studies). Notably, this does not justify excluding patients with FIB-4 below this cutoff from HCC screening, as it is still debatable whether this cutoff adequately identifies the at-risk population. Decisions regarding HCC screening in patients with low FIB-4 should be individualized based on patient risk profile.

The strength of this meta-analysis resides in the inclusion of all recently validated noninvasive fibrosis tests, including both radiological and serological tests, as we aimed to make this review as comprehensive as possible. There are some limitations. Although the present meta-analysis extensively assessed several clinically relevant outcomes including HCC, HD, and overall and liver-related mortality, our analysis was nevertheless narrowed by several unavailable data such as the timing in which NITs were assessed after receiving treatment or achieving SVR. Statistical heterogeneity was found in some of our analyses. However, this could be explained by subgroup-analyses of the following factors: NITs assessed at pre-treatment or post-treatment with SVR, treatment with either pegylated interferon and ribavirin or DAA, Eastern or Western countries, and different cutoff points. For instance, statistical heterogeneity found in the analyses of pre-treatment FIB-4 and HCC development is partially explained by country of study. In the subgroup analysis on Eastern countries, there was a reduction of I^2 from 76% to 18% for the unadjusted HR. Since the majority of studies are from Eastern countries with Asian participants, further studies conducted in other ethnicities are needed. Residual statistical heterogeneity seen in some of the analyses could also be explained by factors such as the presence of cirrhotic patients in the study and the type of HCV treatment regimen. Due to the limited number of studies and lack of information provided in some studies, we were unable to perform subgroup analysis on these factors. Instead, we provided this information in the figures, wherever subgroup analysis was not possible. More studies are needed to make it possible for us to explore the remaining statistical heterogeneity,

Table 5 Unadjusted and adjusted hazard ratios of non-invasive tests for the prediction of hepatic decompensation and other composite outcomes

Unadjusted hazard ratio (HR)

Ref.	Outcomes	NIT[1]	HR (95%CI)	*P* value
Hansen *et al*[20], 2019	HD (HCC included)	TE	59.00 (17.40–200.00)	< 0.005
Ogasawara *et al*[38], 2019	HD	TE (Pre-Rx)	7.77 (1.29–46.20)	0.025
		TE (Post-Rx)	17.80 (1.85–171.30)	0.013
Bloom *et al*[17], 2018	LRE (HD, HCC and OM)	TE	56.00 (7.00–415.00)	< 0.001
Gomez-Moreno *et al* [19], 2017	LRE (HD, HCC or LRM)	TE	33.27 (7.25–152.63)	< 0.001
Pérez-Latorre *et al* [24], 2016	HD or HCC, whichever occurred first	TE (Post-Rx)	37.76 (17.87–79.80)	< 0.001
Macías *et al*[21], 2015	HD (HCC included)	TE	39.90 (5.50–291.00)	< 0.0001

Adjusted hazard ratio (aHR)

Ref.	Outcomes	NIT[1]	aHR (95%CI)	*P* value	Adjustment variables
Hansen *et al*[20], 2019	HD (HCC included)	TE	9.00 (2.49-32.20)	0.001	Age, SVR, hyaluronic acid
Ogasawara *et al*[38], 2019	HD	TE (Pre-Rx)	4.85 (0.80–29.40)	0.086	Platelet count, albumin
		TE (Post-Rx)	14.90 (1.45-152.10)	0.023	Platelet count, albumin
Peleg *et al*[23], 2019	OM or HCC	TE (Post-Rx)	2.32 (0.97-6.59)	0.062	liver steatosis, baseline serum platelets
Gomez-Moreno *et al* [19], 2017	LRE (HD, HCC and OM)	TE	30.97 (6.73-142.51)	< 0.001	Age, gender, time since HCV diagnosis, HCV genotype, injection drug use, high alcohol intake, HCV antiviral therapy
Merchante *et al*[26], 2017	HD	TE	1.90 (1.04–3.64)	< 0.001	Age, gender, SVR during follow-up
Lee *et al*[46], 2016	HD, HCC, and/or LRM	TE (Post-Rx)	9.47 (1.02-88.13)	0.048	Age, AFP
Macías *et al*[21], 2015	HD (HCC included)	TE	59.50 (8.30-427.00)	< 0.001	Age, gender, platelet counts, AIDS at baseline, alcohol use, treatment against HCV, time-varying CD4 cell counts and undetectable HIV RNA.
Berenguer *et al*[12], 2015	OM/LRE (HD or HCC), whichever occurred first.	FIB-4 (Pre-Rx)	3.90 (2.46-6.16)	< 0.001	Age, gender, HIV transmission category, Centers for Disease Control and Prevention HIV clinical category, CD4 cell nadir, HCV genotype, HCV RNA, alcohol intake, methadone use, SVR

[1]Non-invasive tests are not classified as either pre-treatment or post-treatment if the study did not specify when the NIT measurement was done with regards to the initiation of hepatitis C virus therapy. APRI: Aspartate aminotransferase to platelet ratio index; FIB-4: Fibrosis-4 index; HCC: Hepatocellular carcinoma; HD: Hepatic decompensation; LSM: Liver stiffness measurement; LRM: Liver-related mortality; LRE: Liver-related event; NIT: Non-invasive test; OM: Overall mortality; pre-Rx: Pre-treatment; post-Rx: Post-treatment; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; SVR: Sustained virologic response; TE: Transient elastography.

by either subgroup analysis or meta-regression.

The publication bias in adjusted HR for FIB-4 index could be explained by biased selection of outcomes in four studies. Notably, only adjusted HRs for significant variables were reported, while non-significant variables were either omitted or considered as non-significant without providing a numerical adjusted HR[39-41,49]. However, through subgroup analysis, we have concluded that the publication bias detected was due to the inclusion of small studies.

CONCLUSION

FIB-4, APRI, and LSM showed predictive value in stratifying risk for CHC patients, particularly for pre-cirrhotic patients with significant fibrosis. Patients with a higher degree of fibrosis based on NITs were found to be at increased risk of complications, regardless of treatment regimen and response. Therefore, liver fibrosis measurement

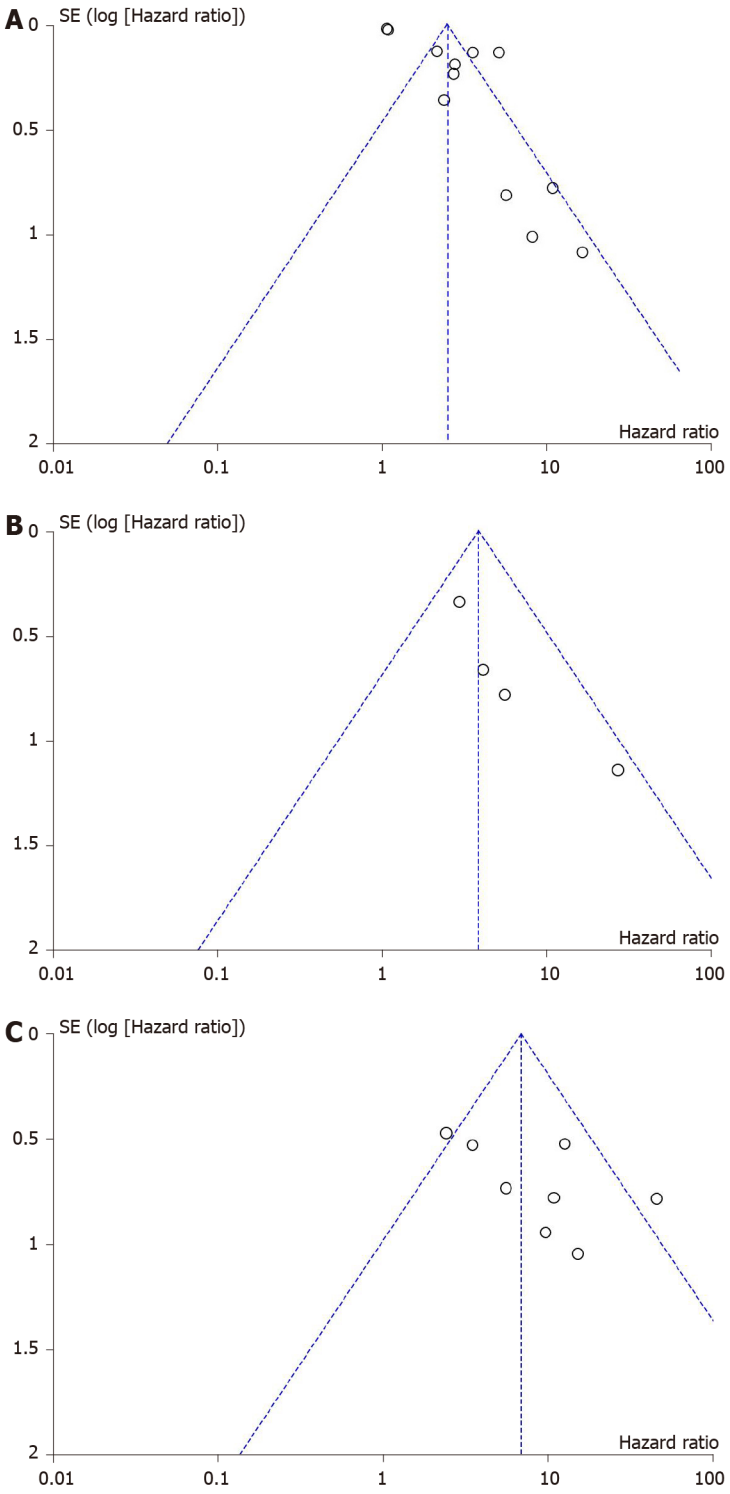


Figure 4 Funnel plots for adjusted hazard ratios of Fibrosis-4 (A), aspartate aminotransferase to platelet ratio (B), and liver stiffness measurement (C) for the evaluation of hepatocellular carcinoma development.

by NITs could benefit any HCV patient as it can determine the priority to monitor for the development of HCC and other LREs. The clinical implementation of these NITs does require future studies that can validate their respective cutoff levels.

ARTICLE HIGHLIGHTS

Research background

Non-invasive tests (NITs) have reduced the need for liver biopsy in chronic hepatitis C

(CHC) patients. Despite its limited diagnostic performance in patients with an intermediate degree of fibrosis or in post-treatment setting, previous meta-analyses have evidenced the potential of NITs in determining prognosis. However, these studies focused on chronic liver diseases from various etiologies and did not comprehensively explore all liver outcomes.

Research motivation

The authors aimed to explore all validated NITs for liver fibrosis, specifically their ability to predict liver-related outcomes in CHC patients.

Research objectives

The main goal was to determine the prognostic value of NITs for risk stratification in CHC patients.

Research methods

A literature search was performed to identify CHC cohort studies that reported an association between liver fibrosis assessment by NITs and outcomes such as hepatocellular carcinoma. Hazard ratios (HR) and area under the receiver operating characteristic from those studies were then pooled using the random effects model. Subgroup analyses were performed based on treatment status, treatment regimen, countries, and different cutoff points.

Research results

Fibrosis-4 (FIB-4) index, aspartate aminotransferase to platelet ratio (APRI) score, and liver stiffness measurement (LSM) were found to have hepatocellular carcinoma predictive potential with pooled adjusted HR of 2.48 (95%CI: 1.91-3.23, I^2 = 96%), 4.24 (95%CI: 2.15-8.38, I^2 = 20%) and 7.90 (95%CI: 3.98-15.68, I^2 = 52%) and area under the receiver operating characteristic of 0.81 (95%CI: 0.73-0.89, I^2 = 77%), 0.81 (95%CI: 0.75-0.87, I^2 = 68%) and 0.79 (95%CI: 0.63-0.96, I^2 = 90%), respectively.

Research conclusions

FIB-4, APRI, and LSM were found to have prognostic value, and can potentially be used to stratify risk for CHC patients, regardless of their treatment status or regimen.

Research perspectives

To facilitate clinical implementation, validation of FIB-4, APRI and LSM cutoff levels are needed.

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OPINION REVIEW

Coronavirus disease 2019 and non-alcoholic fatty liver disease

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic may present with a broad range of clinical manifestations, from no or mild symptoms to severe disease. Patients with specific pre-existing comorbidities, such as obesity and type 2 diabetes, are at high risk of coming out with a critical form of COVID-19. Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, and, because of its frequent association with metabolic alterations including obesity and type 2 diabetes, it has recently been re-named as metabolic-associated fatty liver disease (MAFLD). Several studies and systematic reviews pointed out the increased risk of severe COVID-19 in NAFLD/MAFLD patients. Even though dedicated mechanistic studies are missing, this higher probability may be justified by systemic low-grade chronic inflammation associated with immune dysregulation in NAFLD/MAFLD, which could trigger cytokine storm and hypercoagulable state after severe acute respiratory syndrome coronavirus 2 infection. This review focuses on the predisposing role of NAFLD/MAFLD in favoring severe COVID-19, discussing the available information on specific risk factors, clinical features, outcomes, and pathogenetic mechanisms.

Key Words: Non-alcoholic fatty liver disease; Metabolic-associated fatty liver disease; COVID-19; SARS-CoV-2; Liver injury; Immune dysregulation

Core Tip: Non-alcoholic fatty liver disease is the most widespread hepatic disorder. Recently re-named as metabolic-associated fatty liver disease, it has been lately pointed out as a predisposing factor for severe coronavirus disease 2019 (COVID-19). We herein discuss the epidemiology and possible underlying pathways predisposing severe COVID-19 in non-alcoholic fatty liver disease/metabolic-associated fatty liver disease patients.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) was declared as a global pandemic by the World Health Organization (WHO) on March 11, 2020[1]. Indeed, after the first diagnosis of COVID-19 case in Wuhan (China) in December 2019, the virus spread quickly, affecting 220 countries and territories[2]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative virus of COVID-19, whose most likely origin is natural selection in an animal host followed by zoonotic transfer[3]. Features of SARS-CoV-2 infectivity and transmissibility, as well as multiple clinical presentations of COVID-19, represent burning research topics, especially with the alarming rise of new variants. Severe COVID-19 most frequently presents with acute respiratory failure, even though several non-respiratory manifestations may characterize both the acute phase of the disease and the post-COVID syndrome (or long COVID)[4].

COVID-19 patients may show hepatic injury – largely characterized by a mild increase in serum aminotransferase levels – or may experience worsening of a pre-existing liver disease[5]. Most patients presenting with moderate-severe COVID-19 are old and/or affected by metabolic comorbidities, such as diabetes mellitus and obesity [6]. These conditions are also strongly associated with unrecognized underlying liver disease, mostly non-alcoholic fatty liver disease (NAFLD)[7,8]. Affecting almost 1 billion people, NAFLD is considered as the most common chronic liver disease all over the world, and its prevalence is estimated to become higher together with the epidemics of type 2 diabetes and obesity[9]. Recent international consensus panel proposed to rename NAFLD to metabolic-associated fatty liver disease (MAFLD), giving importance to the underlying systemic metabolic dysfunction rather than alcohol abstinence[10]. Of interest, NAFLD/MAFLD patients are more likely to develop liver damage when infected by SARS-CoV-2[11].

To date, the available reviews on this topic focused on the impact of COVID-19 infection on NAFLD/MAFLD worsening and progression. The present review aims to consider the ongoing relationship between COVID-19 and NAFLD/MAFLD, targeting the predisposing role of NAFLD/MAFLD in favoring severe COVID-19. The available information since the beginning of pandemic, specific risk factors, clinical features, outcomes, and pathogenetic mechanisms will be analyzed and discussed.

EPIDEMIOLOGY

Epidemiology of NAFLD/MAFLD

NAFLD/MAFLD is characterized by steatosis in > 5% of liver parenchyma, in association with metabolic alterations (mostly type 2 diabetes and obesity), without any chronic liver disease, and with ethanol intake not exceeding 30 g/d for men and 20 g/d for women[12]. In the histological spectrum of NAFLD/MAFLD, steatosis may be accompanied by mild inflammation (non-alcoholic fatty liver) or necro-inflammation with hepatocyte ballooning (non-alcoholic steatohepatitis, NASH)[13].

Being the most widespread chronic liver disease worldwide, NAFLD/MAFLD prevalence ranges from 13.5% in Africa to 31.8% in the Middle East, consistent with differences in genetic predisposition, caloric intake, physical activity, body fat distribution, and socio-economic status[14]. In the general population, NAFLD/MAFLD prevalence increases with age, and it is higher in men than women (particularly in the pre-menopausal period)[15,16]. NAFLD/MAFLD is diagnosed in 47.3%-63.7% of type 2 diabetes patients and up to 80% of obese people[17,18]. Type 2 diabetes is rising worldwide, affecting more than 400 million people and representing the ninth main cause of death[19]. Even though type 2 diabetes is closely related to obesity, its significance in NAFLD is two-fold. Indeed, other than a high prevalence of NAFLD in these patients, type 2 diabetes accelerates NAFLD progression and is a predictor of advanced fibrosis and mortality[20]. Similar to type 2 diabetes, obesity prevalence has doubled in the last 40 years, so that approximately a third of the population can be classified as overweight or obese[21]. Even though its prevalence is higher in older

people, obesity rates increased in all ages and both sexes, regardless of country, ethnicity, or socioeconomic status[21].

Epidemiology of COVID-19

COVID-19 has been declared as a global pandemic by the WHO in March 2020, since cases are reported in all continents[1]. To date, there have been 168509636 confirmed cases of COVID-19, including 3505534 deaths, reported to WHO[22]. Nevertheless, the reported case counts undervalue the global burden of COVID-19, since only a small percentage of acute infections is diagnosed[23]. COVID-19 severity is related with increasing age, male sex, and pre-existing medical diseases[24,25]. Severe COVID-19, defined as intensive care unit or hospital admission, mechanical ventilation, or death, is associated with underlying conditions as diabetes mellitus and obesity[26,27]. Indeed, prevalence studies are not conclusive on increased risk of SARS-CoV-2 infection in patients affected by diabetes mellitus, but this condition may worsen the outcome of COVID-19[28]. Similarly, investigations do not show that obesity increases the risk of contracting COVID-19, but that it may exacerbate the disease severity[27].

NAFLD/MAFLD in COVID-19 patients

The diagnosis of NAFLD/MAFLD requires: (1) the presence of hepatic steatosis detected by liver imaging or histology; and (2) exclusion of significant alcohol intake, other causes of steatosis, or chronic liver disease[29]. Even though liver histology is the gold standard for the diagnosis of NAFLD/MAFLD, to differentiate NASH from simple steatosis and to assess fibrosis, liver biopsy is limited to selected patients due to its invasiveness and costs[29]. Thus, available data on NAFLD/MAFLD prevalence in COVID-19 patients are limited to non-invasive diagnosis.

The frequency of hepatic steatosis fortuitously detected by chest computed tomography in COVID-19 patients was 4.7 times higher than that in age- and sex-matched non-infected patients (31.9% *vs* 7.1%)[30]. This result is confirmed by further studies in which NAFLD/MAFLD was diagnosed by the hepatic steatosis index in 30.7%-37.6% COVID-19 patients from China, even though (differently from the previous investigation) associated with higher risk of disease progression[11,31]. Other studies from China demonstrated that the presence of NAFLD/MAFLD is independently associated with severe COVID-19[32,33]. These latter observations suggest that a huge percentage of patients is at risk of developing the severe form of COVID-19 due to the increasing worldwide occurrence of NAFLD/MAFLD. Nevertheless, results from a study performed in Qatar could not demonstrate that NAFLD/MAFLD was an independent predictor of mortality or COVID-19 severity [34]. A further study conducted at the Imperial College Healthcare NHS Trust in London assessed that NAFLD/MAFLD *per se* was not associated with adverse outcomes in COVID-19 patients[35]. Two systematic reviews with meta-analysis considered several studies to conclude that NAFLD/MAFLD was associated with increased risk of severe COVID-19[36,37].

To answer the question whether NAFLD/MAFLD could increase the risk of contracting COVID-19, the impact of genetic risk score was analyzed in hospitalized participants of the UK Biobank cohort, resulting in no evident association between genetic predisposition of NAFLD/MAFLD and severe COVID-19[38]. A review on data from a huge commercial database including electronic records from 26 national healthcare systems demonstrated that the diagnosis of NASH increases 4.93 times the risk of COVID-19[6].

Several studies tried to point out if there are any risk factors predictive of severe COVID-19 in NAFLD/MAFLD patients (summarized in Table 1). According to the results of a pooled analysis, the risk of severe disease in COVID-19 patients affected by NAFLD/MAFLD seems independent of obesity[39]. Nevertheless, a systematic review showed that obesity, together with hepatic fibrosis and younger age, are associated with increased risk of severe COVID-19[40]. A subsequent study performed in a tertiary care center from Mexico showed that the presence of liver fibrosis in NAFLD/MAFLD patients is associated with severe COVID-19[41]. A further study from three Chinese hospitals suggested that high serum interleukin-6 (IL-6) levels at admission represents an independent risk factor for severe COVID-19 in NAFLD/MAFLD patients[42]. In NAFLD/MAFLD patients, male sex and a noticeable inflammatory response were associated with high COVID-19-related mortality[35]. A retrospective study showed that NAFLD/MAFLD rose the risk of hospitalization in all racial subgroups, even though the highest increase was observed among black people [43].

Table 1 Risk factors associated with severe coronavirus disease 2019 in patients with non-alcoholic fatty liver disease/metabolic-associated fatty liver disease

Risk factors	Ref.
Obesity	[40]
Younger age	[40]
Black race	[43]
Liver fibrosis	[40,41]
High serum IL-6 at admission	[42]
Male sex	[35]
High ferritin at admission	[35]
High EWS at admission	[35]

EWS: Early warning score; IL-6: Interleukin-6.

COVID-19 AND NAFLD/MAFLD: PATHOGENETIC LINKS

As the risk of severe COVID-19 increases in patients affected by NAFLD/MAFLD, it is conceivable that specific joint pathogenic mechanisms could be involved (Figure 1).

SARS-CoV-2 virus entry and cleavage

During the initial phase of COVID-19 infection, pathogenesis of the disease relies on binding of spike SARS-CoV-2 protein to angiotensin I converting enzyme 2 (ACE2) receptors, through which the virus enters target cells[44-46]. Even though ACE2 receptors are mainly expressed in epithelial cells of the upper respiratory tract, in type 2 alveolar epithelial cells, and in ciliated cells, they can also be found on the brush border of enterocytes and in cholangiocytes[45]. Following the binding with ACE2 receptor, the SARS-CoV-2 spike protein undergoes a cleavage by the host's *FURIN* serine protease, a critical process in promoting spike-mediated entry of the virus[47]. Likewise, cleavage of SARS-CoV-2 spike protein by the serine protease two key host factors of SARS-CoV-2 (transmembrane serine protease 2, TMPRSS2) is determinant for its fusogenic activity[46]. Of great interest, it has been evidenced that patients with NAFLD/MAFLD present with an increased expression of *ACE2*, *FURIN*, and *TMPRSS2* genes[48]. The enhanced expression of receptors that mediate SARS-CoV-2 cellular entry can explain the increased susceptibility of NAFLD/MAFLD to COVID-19. Moreover, increased levels of *FURIN* and *TMPRSS2* may boost the processing of SARS-CoV-2 spike, further improving its cellular entry. It is worth to note that analysis of data from rodent models and NAFLD/MAFLD patients could not show any increased hepatic expression of *ACE2*, *FURIN*, and *TMPRSS2* genes[49]. On the contrary, the upregulation of these genes in multiple tissues probably represents an additional mechanism of increased susceptibility to severe COVID-19 in NAFLD/MAFLD patients[50].

Immune cell response

Several authors suggested that individuals with NAFLD/MAFLD may present with a dysregulation of both innate and adaptive immune response, which could predispose to worse outcomes in COVID-19. Innate immune response is particularly mediated by Kupffer cells in the liver, which represent the major number of resident macrophages in a single organ[51,52]. Kupffer cells are located within the hepatic sinusoids as part of the reticuloendothelial system, constituting the first line of defense against microorganisms, and regulating immune homeostasis in the liver with the involvement of other immune cells such as neutrophils[53]. In NAFLD/MAFLD, macrophages are polarized towards a pro-inflammatory (M1, or classically activated) rather than anti-inflammatory (M2, or alternatively activated) phenotype[54]. Activation and hyperplasia of Kupffer cells was documented in patients with COVID-19 by several histopathological findings[55,56]. Nevertheless, the impact of COVID-19 on Kupffer cell polarization has not been fully characterized. Of note, ACE2 receptor is detected on the surface of Kupffer cells, leading to hypothesize that hepatic macrophages could be infected by SARS-CoV-2, triggering the primary defense response to the host[57]. This response is mostly mediated by type-I and type-III interferons, leading to the

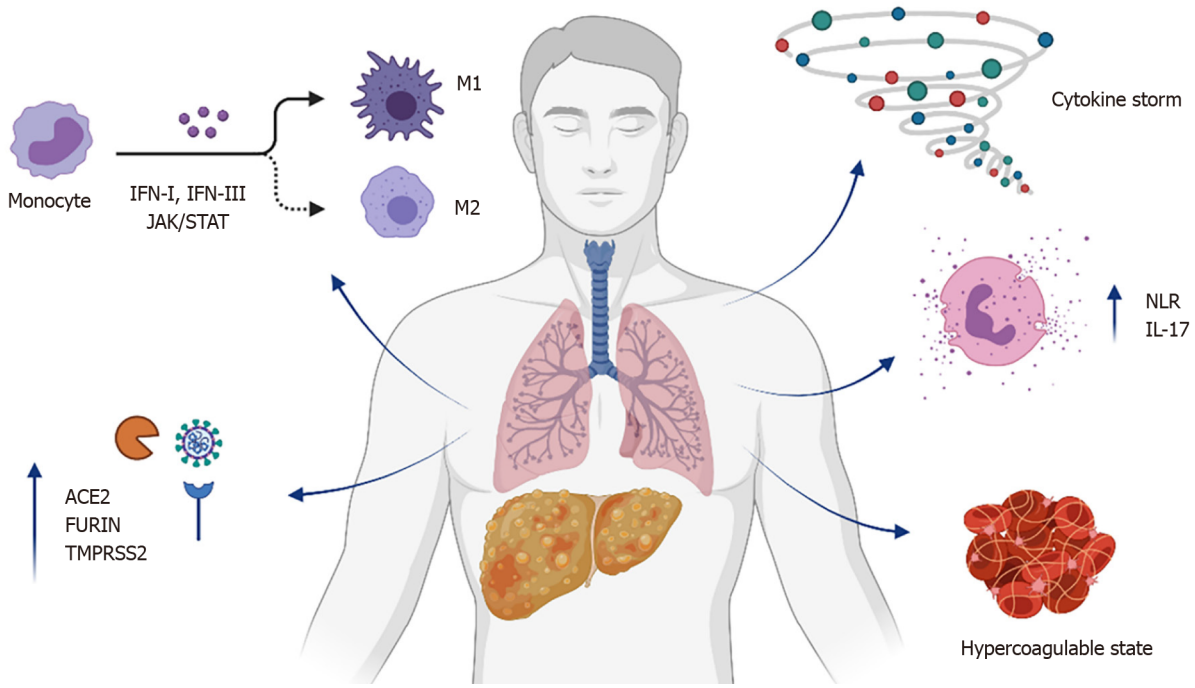

Figure 1 Mechanisms supporting severe coronavirus disease 2019 in non-alcoholic (or metabolic-associated) fatty liver disease. Non-alcoholic fatty liver disease/metabolic-associated fatty liver disease may present with systemic overexpression of genes involved in severe acute respiratory syndrome coronavirus 2 entry and cleavage (such as angiotensin I converting enzyme 2, FURIN, and transmembrane serine protease 2), interferon-mediated polarization of macrophages toward a pro-inflammatory M1 phenotype, elevated circulating levels of pro-inflammatory cytokines, increased neutrophil-to-lymphocyte ratio with activation of the pro- interleukin-17 axis, and enhanced production of pro-coagulant molecules. Taken together, these pathways increase susceptibility of severe coronavirus disease 2019 in non-alcoholic fatty liver disease/metabolic-associated fatty liver disease patients. ACE2: Angiotensin I converting enzyme 2; IFN: Interferon; IL-17: Interleukin-17; JAK/STAT: Janus kinase/signal transducer and activator of transcription; NLR: Neutrophil-to-lymphocyte ratio; TMPRSS2: Transmembrane serine protease 2.

activation of janus kinase (JAK)-signal transducer and activator of transcription (STAT)-driven transcription of cytokines[58,59]. The expression of both *JAK1* and *STAT1*, as well as interferon-encoding genes, are increased in NAFLD/MAFLD patients[48]. Of interest, a significant relationship between ACE2 and JAK-STAT signaling was described, suggesting that this pathway may be involved in the downstream action of ACE2 overexpression[60].

Cytokine storm

The progression from a mild to a severe form of COVID-19 is associated with a cytokine storm, characterized by elevated IL-6, IL-8, and tumor necrosis factor (TNF) levels[61]. Several cytokines are involved in NAFLD/MAFLD, determining a low-grade systemic inflammation that favors disease progression and comorbidities[62]. Circulating IL-6 is high in several chronic conditions, including metabolic syndrome, cardiovascular diseases, and chronic inflammatory airways diseases[63]. Furthermore, fatty liver is independently associated with elevated IL-6 levels[64]. Serum IL-6 is strongly and independently associated with COVID-19 severity, and treatment with a monoclonal antibody directed against IL-6 receptor (tocilizumab) improves clinical outcomes in patients affected by serious disease[65]. Indeed, while in physiological conditions the hepatic production of cytokines is nonexistent or mild, lipid accumulation leads to the release of pro-inflammatory molecules as TNF and IL-6 by hepatocytes, Kupffer cells, and adipose tissue, with reduced levels of the anti-inflammatory cytokine IL-10[66]. It is worth to note that adipose tissue is mainly characterized by dysfunctional and inflammatory immune response in patients affected by morbid obesity. In particular, both adipose and mesenchymal stem cells from obese patients are characterized by increased secretion of pro-inflammatory cytokines, including IL-6, IL-8, and TNF[67]. This may contribute to explain the increased probability of severe SARS-CoV-2 infections in NAFLD/MAFLD patients, but further studies are required to improve knowledge about the pathogenetic link between the altered innate liver immunity and COVID-19.

Neutrophils and IL-17

The neutrophil-to-lymphocyte ratio (NLR) is a biomarker of cellular immune imbalance in NAFLD/MAFLD[68]. A high NLR is associated with severity of disease, worse outcomes, and mortality in NAFLD/MAFLD patients[69,70]. Of interest, the presence of NAFLD/MAFLD and a NLR > 2.8 is associated with higher risk of severe COVID-19 with respect to patients not affected by NAFLD/MAFLD and normal NLR [33]. It is worth to note that NLR is also an easy-to-use prognostic biomarker in the early stage of SARS-CoV-2 infection[71]. Neutrophils are a crucial source of IL-17, especially in the liver but also in the airway[72,73]. The pro-inflammatory IL-17 axis may drive the progression of NAFLD/MAFLD, and also COVID-19 severity[74,75]. Activation of the IL-17 axis in NAFLD/MAFLD, other than complemented with the increase of additional pro-inflammatory cytokines as IL-6 and TNF, occurs with the imbalance of T helper lymphocyte subsets[76]. Hospitalized COVID-19 patients show a dysregulation in the balance of T lymphocytes, characterized by a reduced proportion of Treg cells as compared to non-hospitalized individuals[77]. Taken together, these observations suggest that the cellular immune imbalances described in NAFLD/MAFLD could predispose to severe COVID-19, even though further research is needed to clarify this aspect.

Hypercoagulable state

Cytokine release by pro-inflammatory cells may lead to enhanced production of pro-coagulant molecules such as the tissue factor and the von Willebrand factor, with consequent hypercoagulable state and resulting widespread micro-/macrovascular thrombosis[78,79]. NAFLD/MAFLD patients exhibit coagulation disorders, including elevated circulating levels of both tissue factor and von Willebrand factor, as well as increased platelet activation and plasmatic concentration of plasminogen activator inhibitor type 1[80-82]. COVID-19 patients affected by NAFLD/MAFLD present with higher level of circulating D-dimer with respect to those without NAFLD/MAFLD, suggesting that the NAFLD/MAFLD-associated pro-coagulant state may contribute to COVID-19 severity[83]. Results from a retrospective study on a cohort of COVID-19 patients revealed that the prevalence of NAFLD/MAFLD was higher in individuals presenting with Doppler ultrasound documented deep vein thrombosis[84]. Furthermore, mean admission and peak serum D-dimer concentration was more elevated in COVID-19 patients with NAFLD/MAFLD with respect to those without NAFLD/MAFLD[84]. It is conceivable that COVID-19 may further increase production of pro-inflammatory cytokines in NAFLD/MAFLD subjects, with consequent activation of the coagulation cascade and thrombosis. Indeed, histologic study of pulmonary vessels described widespread thrombosis with microangiopathy in COVID-19 patients, who also presented with hepatic steatosis involving 50%-60% of liver parenchyma[85]. To confirm this report, an Italian post-mortem analysis found hepatic steatosis and pulmonary thrombi in 55% and 73% COVID-19 patients, respectively[86]. These observations strongly suggest that these diseases are interlinked; the proinflammatory hypercoagulable state representing a mutual pathogenetic pathway to severe COVID-19, contributing to thrombosis and disease progression.

CONCLUSION

Since COVID-19 may present with severe disease and high mortality rate, several studies addressed predisposing factors and underlying pathways to identify patients at high risk. The severe form of SARS-CoV-2 infection occurs in individuals preliminary affected by metabolic diseases, including NAFLD/MAFLD. Chronic low-grade inflammation is suggested as the main leading process to trigger immune dysregulation, cytokine storm, and hypercoagulability in NAFLD/MAFLD patients with COVID-19. Other than being considered for specific therapeutic approaches against COVID-19, subjects affected by NAFLD/MAFLD should be acknowledged among groups with high-risk medical conditions in SARS-CoV-2 vaccination programs. Even though several concerns were raised about SARS-CoV-2 vaccine responses, vaccination with the alum-adjuvanted inactivated COVID-19 vaccine (Beijing Institute) resulted as effective and safe in NAFLD/MAFLD patients[87]. Nevertheless, further investigations are necessary to clarify whether NAFLD/MAFLD patients should be prioritized for SARS-CoV-2 vaccination.

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REVIEW

Epigenetic mechanisms of liver tumor resistance to immunotherapy

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver tumor, which stands fourth in rank of cancer-related deaths worldwide. The incidence of HCC is constantly increasing in correlation with the epidemic in diabetes and obesity, arguing for an urgent need for new treatments for this lethal cancer refractory to conventional treatments. HCC is the paradigm of inflammation-associated cancer, since more than 80% of HCC emerge consecutively to cirrhosis associated with a vast remodeling of liver microenvironment. In the recent decade, immunomodulatory drugs have been developed and have given impressive results in melanoma and later in several other cancers. In the present review, we will discuss the recent advancements concerning the use of immunotherapies in HCC, in particular those targeting immune checkpoints, used alone or in combination with other anti-cancers agents. We will address why these drugs demonstrate unsatisfactory results in a high proportion of liver cancers and the mechanisms of resistance developed by HCC to evade immune response with a focus on the epigenetic-related mechanisms.

Key Words: Liver cancer; Immunotherapies; Epigenetics; Resistance; Hepatocellular carcinoma

Core Tip: Although our understanding of hepatocellular carcinoma (HCC) pathogenesis has improved, this aggressive tumor is still devoid of effective treatments and remains a major health problem. Despite the justified hopes on immunotherapies, only a limited number of HCC patients respond to treatments. The characterization of the molecular mechanisms displayed by tumor cells to evade immune response will help to consider new combinations of therapies. In recent years, a growing body of evidence argues for a modulation of tumor immune privilege by several epigenetic events and renders drugs targeting these regulators as a partner of choice for immunotherapy combination

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver tumor with 800000 newly diagnosed people per year in the world[1]. HCC also stands fourth in rank of deaths related to cancer worldwide, accounting for more than 700000 deaths per year. Liver cancer incidence has tripled since the 80s and reaches a high incidence in western countries consequently to obesity and diabetes epidemic, supporting the need of novel effective strategies for this cancer refractory to the majority of conventional anticancer treatments. HCC is a complex disease but its mutational landscape has been extensively uncovered these two last decades with advances in deep-sequencing technologies. The most recurrent mutations identified in HCC are mutations in *TERT*, *CTNNB1* and *TP53*[2], but other frequent mutations in epigenetic modifiers and chromatin remodelers are also encountered (*e.g.*, *ARID1A, ARID2, MLL2*)[3,4]. Other crucial epigenetic modulators, the non-coding RNAs (ncRNAs), are also largely deregulated during hepatocarcinogenesis, reprogramming tumor cells but also modifying the surrounding cells and secondary sites of metastasis *via* their secretion [5].

Integrating outside and inside signals in time and space, the epigenetic regulations of gene expression is a crucial determinant of tumor cell fate regarding differentiation, proliferation, metabolism, migration and immunosurveillance. Epigenetic modifications are categorized into three main mechanisms: DNA methylation, histone modifications mainly on H3 and H4 histones (acetylation, methylation, *etc.*) and control by ncRNAs. There is a growing body of evidence that epigenetic modifiers play key roles during cancer, including in HCC. Therefore, they constitute attractive therapeutic options, alone or in combination with other anti-cancer agents, such as drugs targeting DNA methylation and histone acetylation, which have already been approved for hematological cancers[6]. These recent years, it has been extensively documented that the immune response is epigenetically controlled and plays critical roles in tumor immunosurveillance. Among others, epigenetic changes impact macrophage polarization, myeloid-derived suppressor cell (MDSC) function, genesis of cancer-associated fibroblasts and function of T cell populations, either CD4+, CD8+ and T regulators (Tregs). Of note, subsets of inflammatory gene promoters have been found epigenetically deregulated in cancer. In particular, aberrant DNA methylation of interferon-γ (IFNγ) is associated with exhausted phenotype of T cells[7]. The cytokines involved in T_H response have been found epigenetically inhibited by EZH2 (Enhancer of zeste homolog 2) and DNMT1 (DNA methyltransferase 1)[8]-infiltration of CD8+ cells being inversely associated with the high expression of EZH2. In addition to cytokines, the expression of immune checkpoints such as the program cell death 1 (PD-1)/program cell death ligand 1 (PD-L1) axis is also regulated by epigenetic modifications. DNA methylation in the promoter region of *CD274* encoding PD-L1 predicts patient survival in multiple cancers. EZH2 modifies its H3K27 trimethylation status in hepatoma cells[9], while the BET protein BRD4 (bromodomain-containing protein 4), found overexpressed in HCC and enriched on super-enhancers driving oncogene expression[10], suppressed PD-L1 expression[11].

HCC is the paradigm of inflammation-associated cancer, since more than 80% of HCC emerge consecutively to cirrhosis associated with a vast remodeling of liver microenvironment. Immune cell remodeling is a consequence of chronic hepatitis or liver disease associated with alcohol consumption, genotoxic exposure or metabolic disorders[12]. Even if liver parenchyma harbors a specialized and protective immune system to manage its constant exposure to toxins and bacteria susceptible to trigger deleterious inflammation, the chronicity of hepatic injuries sensitizes to HCC. In liver cancers, as in a number of other cancers, tumor microenvironment differs accordingly to the driven oncogenic mutations and thus impacts response to treatments, notably to immunomodulatory drugs[13]. Cancers with *CTNNB1* mutations have been defined as

cold tumors with lower immune cell infiltration and refractoriness to immune checkpoint inhibitors (ICIs)[14,15]. Indeed, the Wnt/β-catenin pathway plays a major role in the specification of a multitude of immune cells including macrophages, dendritic cells (DC) and lymphocytes[16].

In the present review, we will discuss the recent advances on immunotherapies in clinical practice, successfully used alone or in combination with other anti-cancers agents in several cancers. We will also address why these drugs demonstrate unsatisfactory results in a high proportion of liver cancers, which shown innate or acquired resistance to immunomodulatory agents. We will thus detail the mechanisms of resistance developed by HCC and particularly the epigenetic-related mechanisms.

MECHANISMS OF T CELL ACTIVATION AND ATTENUATION

T cell activation needs two signals from antigen presenting cells (APC). The initial signal is based on antigen recognition through interaction between T cell receptor (TCR) complexed to CD3 subunits on T lymphocytes and its cognate antigen/MHC (major histocompatibility complex) on APC (Figure 1). This interaction promotes CD3 phosphorylation on ITAM motifs (immunoreceptor tyrosine-based activation motifs) which serve as docking sites for the recruitment of ZAP-70 (TCR-ζ chain-associated 70-kDa tyrosine phosphoprotein) and subsequent phosphorylation by Lck (lymphocyte-specific protein tyrosine kinase) and autophosphorylation. Once fully activated ZAP-70 phosphorylates LAT (linker of activated T cells) and SLP-76 (SH2 domain-containing leukocyte protein of 76 kDa), two adaptors for the assembly of the complete TCR signalosome. Secondary signals are required to fully activate LAT. The costimulatory signals are mostly provided by members of the immunoglobulin superfamily such as CD80(B7-1)-CD86(B7-2) bound to CD28, ICOSL to ICOS (inducible T-cell costimulator) (respectively on APC and T cell), or those of the tumor necrosis factor (TNF) receptor superfamily (*e.g.*, OX40L-OX40, CD40/CD40L).

To avoid excessive immune response, co-inhibitory molecules, including CTLA-4 (cytotoxic T lymphocyte antigen 4), PD-1 and LAG-3 (lymphocyte-activation gene 3), act as negative immune counterweights (Figure 1). Inhibitory receptors mediate their negative regulation through inhibitory motifs located in their cytoplasmic tails such as immunoreceptor-based inhibitory motif (ITIM) to recruit phosphatases containing Src homology-2 domains, such as SHP-1 and SHP-2 (small heterodimer partner). The recruited phosphatases dephosphorylate several molecules involved in the TCR signaling such as the TCR itself or ZAP-70. This interrupts downstream cascades such as the PI3K (phosphoinositide-3-kinase)/AKT and the rat sarcoma virus (Ras)/rapidly accelerated fibrosarcoma (Raf)/mitogen activated protein kinase kinase (MEK)/extracellular signal regulated kinase (ERK) and leads to reduction in T cell activation, proliferation, metabolism, differentiation, survival, and cytokine production. In addition, PD-1 as well as CTLA-4 are also able to directly regulate signaling pathways in lymphocytes such as the PI3K and MAP kinase pathways[17-19]. While CTLA-4 is the leading player of the ICIs limiting priming of naive T cells notably in lymph nodes, PD-1/PD-L1 interaction results in exhaustion of activated T cells in peripheral tissues and within the tumor microenvironment.

PD-1/PD-L1 axis

PD-1, also known as CD279, is low or undetectable in naive T cells and rapidly induced following TCR activation, in a process partially regulated by transforming growth factors β (TGF-β)[20]. PD-1 is also expressed on other several cells such as B lymphocytes, natural killer (NK), macrophages, DC and monocytes and tumor-specific T cells. At the transcriptional level, PD-1 expression is regulated by nuclear factor of activated T-cells (NFAT)[21], forkhead box O (FOXO)[22] and interferon regulatory factor 9 (IRF9)[23], STAT3/4 (signal transducer and activator of transcription 3 and 4) and CTCF (CCCTC- binding factor)[24] (Figure 2). PD-1 content is also dependent on microRNAs (miRNAs) such as miR-28[25], miR-138 and miR-4717 in glioma[26] and HCC respectively[27]. Differential level of the repressive H3K9me3 mark has been observed in the promoter region of PD-1 in colorectal cancer[28].

PD-1 triggers immunosuppressive signals upon binding to its ligands, PD-L1 (CD274 or B7-H1) and PD-L2 (CD273). A soluble form of PD-L1 (sPD-L1) is secreted in the blood and could compete for PD-1 binding with membranous PD-L1. PD-L2 is restricted to APCs and B lymphocytes, while PD-L1 is usually expressed by macrophages, DC, epithelial cells, activated T cells and B cells. To escape anti-tumor response, PD-L1 expression is highly induced in tumor cells. This could result from

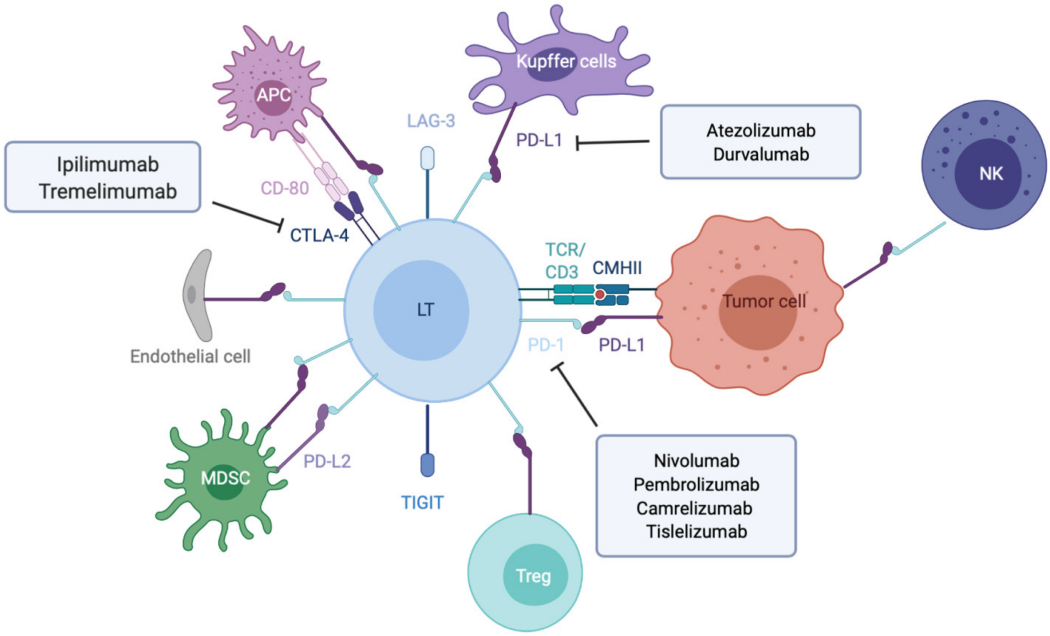

Figure 1 Overview of the main immune checkpoint and their respective targeted therapies. Made with biorender.com. APC: Antigen presenting cell; LT: T lymphocyte; MDSC: Myeloid derived suppressive cell; NK: Natural killer; Treg: Lymphocyte T regulator; LAG-3: Lymphocyte-activation gene 3; PD-L1: Program cell death ligand 1; TCR: T cell receptor.

genomic alterations such as amplification of translocation including in HCC[29]. Gain in PD-L1 copy number is also a frequent alteration across many cancers, which influences PD-L1 expression levels and correlates with higher number of mutated genes[30]. Nevertheless, such a correlation is not observed in HCC. *CD274* expression is controlled by DNA methylation and could constitute a prognosis factor in colon[31] or prostate cancers[32]. Several signaling pathways are also well documented to induce PD-L1 expression in tumor microenvironment such as interferon signaling, PI3K-AKT, MEK-ERK, JAK-STAT, c-MYC and NF-kB (nuclear factor-kappa B)[33]. This transcriptional regulation is regulated by a plethora of cytokines and growth factors such as IFN-γ, interleukin (IL)-6, IL-17, IL-25, TNF-α or epidermal growth factor (EGF)[34]. PD-L1 expression is also regulated by several miRNAs found implicated in cancers: miR-15/miR-16/miR-193a[35], miR-17[36], miR-34[37], the miR-25/miR-93/miR-106b cluster[38], miR-138-5p[39], miR-140[40], miR-142-5p[41], miR-152[42], miR-197[43], miR-200[44], miR-217[45], miR-324-5p/miR-338-5p[46], miR-424 [47], miR-513[48], and miR-570 in HCC[49].

CTLA4/CD80-CD86 axis

CTLA-4 is a CD28 homolog which interacts with CD80 and CD86 with higher affinity and avidity than CD28. Therefore, CTLA-4 enters in competition and prevents the stimulatory signals induced by CD28:CD80/CD86 complexes. Membranous CTLA-4 expression is very low in resting T cells, consequently to clathrin-dependent recycling, and increases following T-cell activation[50]. CTLA-4 is thus mostly localized in intracellular compartments such as lysosomal and endosomal vesicles and the trans Golgi network. CTLA-4 expression is also regulated at the transcriptional level by NFAT[51]. Importantly, CTLA-4 expression has also been detected on tumor cells, including melanoma, colon and renal cancers[52]. In cancer cells, notably in melanoma, CTLA-4 expression is regulated by IFN-γ signaling pathway and DNA methylation[53] but also induced by β-catenin binding on a lymphoid enhancer factor-1 (LEF-1) binding site in its promoter region[54]. In line with these regulations, the *CTLA4* gene displays several SNPs (single-nucleotide polymorphism) associated with disease and cancer in its promoter as well as in its first exon. In particular, the *CTLA-4 318C > T* SNP creates a LEF-1 binding site in its promoter and increase CTLA-4 expression and antitumor activity[55]. CTLA-4 expression is also epigenetically regulated with lower level of repressive H3K27me3 mark detected in CTLA-4 promoter in colorectal cancers[28]. CTLA-4 expression is also post-transcriptionally regulated by miR-9/miR-155[56], miR-138[26] and miR-487a-3p[57].

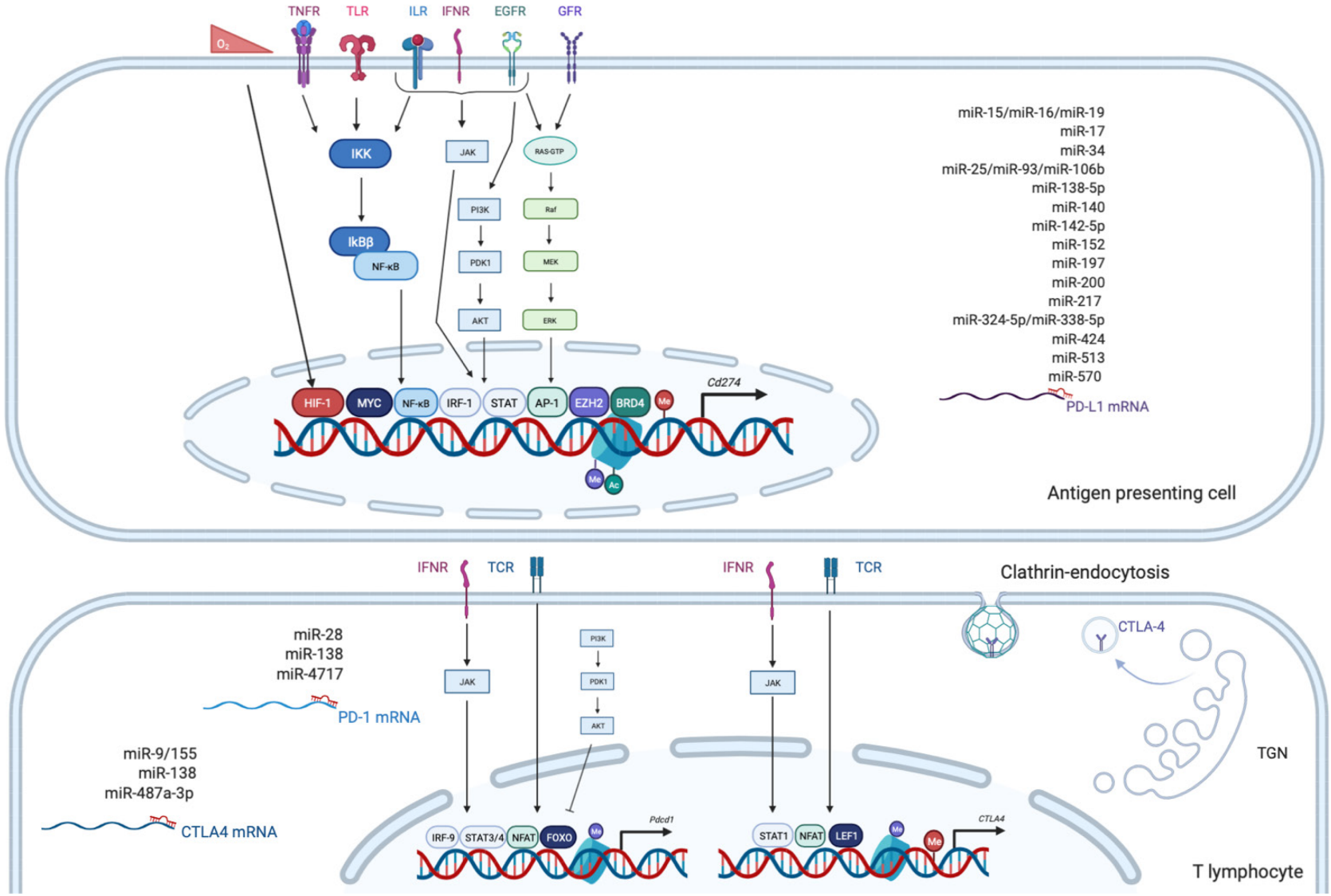

Figure 2 Overview of the main epigenetic and transcriptional regulations of program cell death 1, program cell death ligand 1 and cytotoxic T lymphocyte antigen 4. Made with biorender.com. Ac: Acetylation; Me: Methylation of DNA or histone; EGFR: Epidermal growth factor receptor; GFR: Growth factor receptor; ILR: Interleukin receptor; IFNR: Interferon receptor; TCR: T cell receptor; TGN: Trans-Golgi Network; TLR: Toll like receptor; TNFR: Tumor necrosis factor receptor.

Regarding CTLA-4 ligands, contrary to PD-L1, CD80 and CD86 are restricted to lymphoid cells. While CD80 is generally poorly detected on resting cells and upregulated after activating signals, CD86 is ubiquitously expressed on DCs, monocytes and activated B cells and induced at high levels upon activation. The regulation of these molecules is less detailed. In DCs, CD80 expression is reduced in response to miR-424[47]. Low levels of CD80 and CD86 have been detected on melanoma and colon cancer cells, where low level of CD80 expression favors tumor growth[58] but also on HCC cells, as shown by a pioneer study supporting the potential of CTLA-4 axis targeting as anticancer therapy[59].

MECHANISMS OF IMMUNE ESCAPE AND IMMUNOTHERAPY

The goal of immunotherapies is to boost ability of the immune system to detect tumors and limit their progression. They might counteract the evasion mechanisms mediated by the suppressive molecules rolled out by tumor cells. Different therapeutic strategies have been developed but ICIs, designed to block the co-inhibitory signals of T-cell activation (*e.g.*, CTLA-4, PD-1 and PD-L1), are the preferred methods in clinical practice. These drugs have given very impressive results with cancers of bad prognosis and with few therapeutic options, such as melanoma, and have been rapidly tested in several other tumors with high clinical efficacy in most cases.

Mechanisms of tumor immune evasion

Tumor development and progression is a complex process resulting from the interplay between cancer cells and its surrounding environment including endothelial cells, fibroblasts, and a plethora of immune cells with suppressive, regulatory, killing and either anti or pro-inflammatory functions. All types of immune cells are present in the tumor or in the invasive margin, including macrophages, DCs, mast cells, NK cells, naive and memory lymphocytes, B cells, and effector T cells (*e.g.*, Th1, Th2, Th17, Treg and cytotoxic T cells). Therefore, the strength of anti-tumor immune response is governed by the level and the composition of immune cell infiltrated in the tumors and the degree of T cell activation.

As previously mentioned, tumor cells are able to express co-inhibitory ligands such as PD-L1 or PD-L2, and sometimes inhibitory receptors such as PD-1 including in HCC[60,61]. This prevents T cell activation and modulates the activity of recruited immune cells, which express the cognate molecules and play suppressive activities such as tumor-associated macrophages (TAM), myeloid-derived suppressive cells or Tregs[62] (Figure 3). Accumulation of suppressive cells and T dysfunction are also sustained by several molecules secreted by tumor cells such as PGE2 (prostaglandin E2), COX2 (cyclooxygenase 2), nitric oxide, TGF-β and IL-10[63]. Additionally, multiple cancers are associated with chronic inflammation, particularly HCC related to hepatitis infection. Chronic disease results in an ineffective T response and T cell exhaustion mostly due to persistent inflammatory signals, antigen exposure and suppressive cytokines such as IL-10 and TGF-β. It has also been described that chronic disease modifies PD-1 promoter status in exhausted T cells that remains demethylated and poised to facilitate its rapid expression[64,65]. Progressively, exhausted T cells lose their proliferative capacity and effector function related to decrease in IL-2, TNF-α and IFN-γ.

Tumor cells are also able to modify T cell expansion through metabolic alterations. In particular, an overexpression of IDO (indoleamine-2,3-dioxygenase), an enzyme involved in tryptophan conversion, is frequently observed in tumors[66] as well as overexpression in arginase, particularly in MDSC[67]. The depletion of tryptophan and arginine in tumor microenvironment reduces T cell proliferation[68,69].

Tumor immune privilege is also the consequence of decrease in the expression of recognition molecules including MHC, tumor-associated antigens (TAA) and tumor-specific antigens. It is well described that changes in antigens expressed by tumor cells are detected by the immune system, which further develop autoantibodies against TAAs as reporters to control the transformation process. The typical antigen with autoantibodies identified in cancer is p53[70]. Antigens in HCC could be categorized from cancer testis origin such as SSX-2 (synovial sarcoma, X breakpoint 2) and MAGE (melanoma antigen gene), or oncofetal antigens such as α-fetoprotein and glypican 3 or overexpressed tumor antigens such as annexin A2 and epithelial cell adhesion molecule. They constitute promising targets for adoptive cell therapies such as chimeric antigen receptor T cells or tumor-infiltrating lymphocytes (TILs)[71]. A higher expression of TAAs in HCC patients is correlated with higher immune infilt-

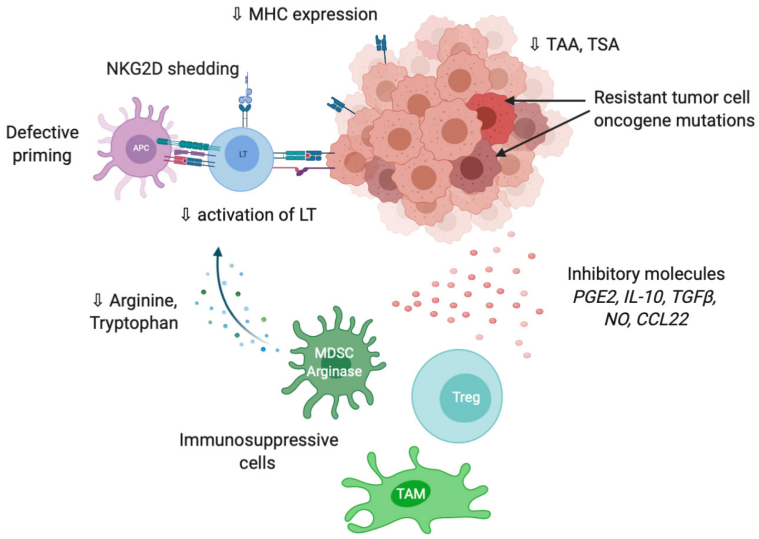

Figure 3 Overview of the main mechanisms involved in tumor evasion to immune response. Made with biorender.com. APC: Antigen presenting cell; ICI: Immune checkpoint inhibitors; LT: T lymphocyte; MHC: Major histocompatibility complex; MDSC: Myeloid derived suppressive cell; NK: Natural killer; NKG2D: Natural killer group 2D; NO: Nitric oxide; TAA: Tumor-associated antigens; TAM: Tumor-associated macrophage; TSA: Tumor-specific antigen; Treg: Lymphocyte T regulator.

ration and better prognosis[72]. The loss or modification of antigens promote immune evasion *via* a defect of tumor recognition. Shedding of natural killer group 2D (NKG2D) ligands into the tumor microenvironment is another way to evade immune recognition. Following proteolysis by matrix metalloproteinases, tumor cell death or exosome secretion, the soluble form of NKG2D ligand induces internalization and degradation of NKG2D and decrease the subsequent cytotoxic effects of T cells[73].

Independently from tumor microenvironment, tumor cells resist to destruction through additional mutations in oncogenes (*BRAF, EGFR, HER2*, *etc.*) that give proliferative advantage. Inversely, mutations in tumor suppressive molecules in particular in damage sensors and pro-apoptotic actors (*TP53, BCL2*, *etc.*) also limits the cytotoxic activity of the immune system[74].

Tumor-infiltrating immune cells

Tumor immune response and subsequent efficacy of ICI treatment is also highly dependent on the immune cell spectrum and its localization within or around the tumors. Indeed, pathological characterization of various solid tumors has shown a great diversity in immune cell types and density between tumors, which could be dependent on driver oncogenes. Three groups have been characterized either as immune desert, immune excluded or inflamed tumors – each group being associated with differential response to ICIs[75].

The inflamed tumors are characterized by the presence of CD8+ and CD4+ T cells with suppressive cells including macrophages, MDSC and Treg that promote T cell dysfunction and exhaustion[76]. In immune-excluded tumors, aggregates of immune cells are at the tumor boundaries. Immune cells are not recruited in the vicinity of tumors consequently to physical hindrance associated with dense and stiff extracellular matrix fibers, defect in neo-vasculature, hypoxia, low level of chemoattractive molecules for T cells such as C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10, insufficient level of antigens or exposure to microbes or virus. In immune desert or cold tumors, there is a low density of immune cells inside and outside the tumors. Tregs, MDSCs and macrophages interplay to inhibit DC maturation and impair T cell expansion and activation. Growing body of data have shown that EMT (epithelial-to-mesenchymal transition) and mesenchymal traits of tumor cells favor immune exclusion and resistance to ICIs[77].

In 2017, a new molecular HCC classification has been proposed on the basis of immune traits, with approximately 30% of HCCs enriched in TILs and defined as HCC immune class[15]. Thirty percent of patients inversely showed exclusion of TILs and frequent mutations in *CTNNB1* gene. This subgroup of tumors are resistant in first-intention to ICIs[13], as it was previously observed in melanoma[78]. This was confirmed with a hydrodynamic mouse model of HCC in which β-catenin activation promotes immune evasion and resistance to anti-PD-1 therapy[79].

In addition to CD8 T cells, the distribution pattern of myeloid cells has also been associated with HCC prognosis. A recent work of Wu and collaborators proposed a myeloid response score (MRS) associated with T cell activity and which could serve as a prognosis signature[80]. HCC were classified as HCCs with low, intermediate, and high MRS, which displayed patterns of immunocompetent, immunodeficient, and immunosuppressive microenvironment. MRS^{low} tumors present an intratumor contexture equivalent to the peritumor tissue containing CD169+CD163+CD14+$CD11b^{low/-}$ macrophages with antitumor activity and CD8+ T cells. Inversely, as compared to non-tumor tissue MRS^{high} tumors are enriched in CD11b+CD15+ polymorphonuclear leukocytes and CD169-CD11b+CD163+ myeloid cells associated with pro-tumoral activation of TAM. These tumors are also characterized by gene signatures related to immunosuppression.

The expression of co-inhibitory molecules within the tumor is an important prognosis factor. HCC with high expression of PD-L1 on tumor/immune cells in immunohistochemistry together with high expression of PD-1 on lymphocytes also exhibit markers of aggressiveness such as poor differentiation and vascular invasion [81]. In addition, if PD-L1 is overexpressed by HCC cells, this predicts early recurrence. Importantly, in this study, no correlation between glutamine synthetase, a direct positive target of the β-catenin, and PD-L1 labeling was observed meaning that the immunosuppressive activity of the Wnt/β-catenin could thus be linked to an immune checkpoint other than PD-L1/PD-1 axis. Another study performing cytometry analysis on HCC tumors confirmed that PD-L1 was both expressed by tumor cells and immune cells and mostly on CD68+ myeloid cells[82]. The presence of PD-L1 on tumor cells correlates with tumor progression, while PD-L1+ macrophages play a protective role in HCC associated with immune response and T activation signature. Recently, a TCGA analysis showed that a high correlation between all negative checkpoints such as PD-L1, PD-1, CTLA-4, LAG-3 and T infiltration in tumors is associated with an immunosuppressive and exhausted tumor microenvironment [83]. Nevertheless, the application of ICIs would be of survival benefit for these patients.

IMMUNOTHERAPY SUCCESSES AND LIMITATIONS IN HCC

Development of immune checkpoints inhibitors constitutes a major breakthrough in oncology that leads to revisit therapeutic strategies and clinical practice for various cancers particularly those of poor prognosis with few therapeutic options, following impressive results obtained in melanoma. ICIs have resulted in increased patient survival in melanoma, kidney and non-small cell lung cancer as well as Hodgkin's lymphoma in comparison with conventional chemotherapies. Other cancers present a more heterogenous response to ICIs such as ovarian, breast, pancreatic and liver cancers. More promising data have been obtained with combination of treatments including ICIs. Microsatellite instability has been evidenced as a biomarker for ICI response[84]-tumors with a low mutation rate having less neoantigens and thus being less immunogenic. Another biomarker is TMB (Tumor mutational burden) has been recently found correlated with ICI sensitivity[85].

Anti-CTLA-4 therapy is the first generation of ICI since antitumor regression after blocking co-inhibitory molecules was firstly evidenced with the anti-CTLA-4 antibody ipilimumab in melanoma[86]. It was the first ICI approved by the Food and Drug Administration (FDA) for the treatment of advanced melanoma. Therapeutic strategies against PD-1 are the second generation of ICI with nivolumab and pembrolizumab lately approved by FDA for advanced melanoma[87]. Since then, the impacts of both therapies have been explored in various cancers and several others surface molecules have been targeted: Inhibitory co-receptors such as VISTA (V-domain Ig suppressor of T cell activation)[88], TIGIT (T Cell Immunoreceptor With Ig And ITIM Domains)[89], TIM-3 (T cell immunoglobulin and mucin domain-containing protein 3)[90] and LAG-3[91] or costimulatory receptors like CD28, OX40[92] or GITR (glucocorticoid-induced TNFR-related protein)[93].

Ipilimumab was the first blocking antibody to significantly promote a regression of lesions in metastatic melanoma with a complete remission in some patients[94]. A 3-year overall survival (OS) rate of around 20% was observed[95]. In HCC, the first anti-CTLA-4 tested was tremelimumab, a fully human IgG2 monoclonal antibody. Response rates were more modest in advanced hepatitis C virus-related HCC, with a median OS of 8.2 mo and survival rate of 43% at 1 year[96]. Another study conducted on hepatitis B virus and hepatitis C virus-associated HCC combined tremelimumab

with tumor ablation at day 36[97]. Twenty-six percent of patients achieved a partial response with an OS of 12.3 mo. Inversely to melanoma, extensive studies were not conducted in HCC with anti-CTLA-4 antibodies as monotherapies. Ipilimumab is now approved, in combination with the anti-PD-1 nivolumab for previously treated advanced HCC, as detailed below.

The significant results obtained with anti-CTLA-4 therapies are also accompanied with severe adverse events. Dogmas that patients with immune-related adverse events have higher response rates have not been confirmed. Adverse events are mainly immune-related such as rash, thyroiditis and frequent complications of the gastrointestinal tract, including aphthous ulcers, esophagitis, gastritis, diarrhea and colitis in around 20% of patients[98]. These adverse effects could be linked to high expression of CTLA-4 on mucosal Tregs[99]. Liver toxicity with ICI-related hepatitis is also a severe adverse effect of anti-CTLA-4 treatment that could be life-threatening in case of delayed management[100]. Oral glucocorticoids or additional immunosuppressants are usually administered to those patients. After adverse effects, an important question is to restart treatment or not. The decision depends on the severity of the complications and the cancer status[101]. Importantly, retreated patients could develop the same adverse event and others new complications. However, an alternative ICI could be administered to patients with adverse effects, *i.e.* anti-PD-1 is safety after deleterious ipilimumab treatment in melanoma patients[102].

To limit those toxicities, targeting TILs rather than peripheral populations will be preferred with antibodies against the PD-1/PD-L1 axis, which exhibit less severe adverse events[103]. In addition to fewer immune related adverse events, PD-1/PD-L1 inhibitors also produced greater anticancer activity. Since PD-1 is more broadly expressed than CTLA-4, on tumor cells in particular, and its expression is also induced by chronic antigen exposure, anti-PD-1 antibodies may exert additional anti-tumor effects and exhibits superior clinical activity and safety when compared to anti-CTLA4 [104]. The rationale of combining anti-CTLA-4 with anti-PD-1 therapies is also supported by the differential immune patterns observed in individual monotherapies [105].

Another important decision is the selection of anti-PD-1 or anti-PD-L1 therapies. Indeed, PD-L1 inhibition preserves the interaction between PD-1 and its other ligand PD-L2, while it blocks its interactions with CD80, an alternative interaction that has been recently reported to promote T-cell responses[106]. Conversely, PD-1 inhibition blocks the interaction of PD-1 with its two ligands but preserves anti-tumor PD-L1/CD80 complexes. Therefore, these antibodies may drive differential anti-tumor immune response. For instance, in non-small-cell lung carcinoma, anti-PD-1 therapies exert better anti-tumor response, while anti-PD-L1 antibodies demonstrate less severe adverse effects[107]. In HCC, three drugs are currently authorized in the United States: The two anti-PD1 nivolumab and pembrolizumab for advanced HCC and one anti-PD-L1, atezolizumab approved in combination with the anti-vascular endothelial growth factor (anti-VEGF) bevacizumab. Nivolumab and pembrolizumab approval has been accelerated by FDA after promising results obtained in preclinical studies on sorafenib refractory HCCs, respectively in Checkmate 040[108] and KEYNOTE-224 [109] (20% of overall response rate and 60% of disease control rate). However, in phase 3 trials both agents did not achieve statistical significancy according to the registered statistical plan (CheckMate-459[110] and KEYNOTE-240[111]). New phase 3 trials are conducted for these two drugs as an adjuvant in CheckMate-9DX for nivolumab (NCT03383458), and for pembrolizumab KEYNOTE-937 (NCT03867084) or in second-line with pembrolizumab KEYNOTE-394 (NCT03062358). New anti-PD-1 antibodies are also currently under investigation. The anti-PD-1 tislelizumab, an antibody designed to limit FcγR-mediated phagocytosis, demonstrated a good antitumor activity in a phase 1 trial — a phase 3 trial is ongoing in various solid cancers including non-small cell lung cancer, esophageal squamous cell carcinoma and HCC (RATIONALE 301)[112]. Camrelizumab is also an alternative, which has been tested in China on 220 patients from multiple centers. At a median follow-up at 12.5 mo, the objective response rate (ORR) was 14.7% and 6-mo OS rate was 74.4%. No complete response was observed, 17.6% of patients present partial response and 23.1% a stable disease. The median progression free survival (PFS) was only of 2.6 mo, shorter than other ICIs. Grade 3 and 4 adverse events occurred in 22% of patients[113].

Strategies combining anti-PD-1/PD-L1 with anti-CTLA-4 antibodies have been evaluated in various cancers and in March 2020 FDA have granted approval for nivolumab/ipilimumab (1 and 3 mg/kg) in advanced HCC patients who have priorly received sorafenib. In Checkmate-040, at a median follow-up of 30.7 mo, the combination arm demonstrated 29% ORR. The median duration of response was 21.7 mo. No adverse effects were observed for 79% of patients. An ORR of 31% with 7

complete responses was provided by Blinded independent central review per RECIST [114]. Nonetheless, it has been shown that a combination of ipilimumab and nivolumab leads to higher incidence of ICI-related hepatitis in different cancers including melanoma with 6% to 9% as compared to 1% in single therapies[115]. Rapid diagnosis and management are thus crucial for better outcomes. Another PD-1/CTLA-4 blocking strategy combining durvalumab with tremelimumab is currently under investigation in a randomized, multi-center phase 3 study called HIMALAYA (NCT03298451) to compare combination against durvalumab or sorafenib alone as a first-line therapy for advanced HCC.

Another combination of ICI successfully tested in HCC is atezolizumab plus bevacizumab (anti-VEGF) in first-line in patients with unresectable HCC. A phase III trial (IMbrave150) showed improved progression-free survival of 6.8 mo *vs* 4.3 mo for sorafenib with an OS at 12 mo of 67.2% *vs* 54.6%[116]. Hypertension, a typical adverse effect of bevacizumab, occurred in 15.2% of patients receiving the combination therapy.

Another intensively tested strategy is to combine ICIs with locoregional treatment, which have demonstrated synergistic activities. Tumor destruction by locoregional treatments releases TAAs promoting immune cell priming, which could be even more enhanced by ICIs. Phase 1, 2 and 3 clinical trials are now conducted with anti-PD-1 or anti-PD-L1, alone or combined with anti-CTLA-4 or anti-angiogenic agents, together with transarterial chemoembolization, hepatic artery infusion chemotherapy or external beam radiation therapy[117] (Table 1). Until now, the combination of ICIs with tyrosine kinase inhibitors such as sorafenib was not concluding. Three phase 3 clinical trials are now conducted to evaluate the benefit of such combinations (NCT04194775, NCT04344158, NCT03755791). However, these recent years, combination of epigenetic drugs with ICIs have emerged as potent therapeutic avenues in hematologic and solid tumors, a point that we will develop in the next paragraph.

EPIGENETICS AND HCC

These recent decades, epigenetic mechanisms have emerged as crucial decision-makers of cell fate determination and deregulations of epigenetic mechanisms could lead to modifications of gene transcription in the cell, which could favor the initiation and progression of cancers. Conventionally, the epigenetic code is divided into three major mechanisms: ncRNA driven-regulations, DNA methylation and histone modifications mainly occurring on H3 and H4 histones. Many studies have been focusing on miRNA implications in HCC but few data are currently available concerning the clinical used of ncRNA-based therapies in combination with ICIs. We will thus develop the promising results obtained regarding approaches targeting DNA methylation and histone modifiers in HCC, alone or in combination with ICIs (Figure 4).

DNA methylation and DNMT inhibitors

DNA methylation in somatic cells is regulated by DNA methyltransferases that add, in CpG dinucleotide, a CH_3 group on the 5′ position of the pyrimidine ring in cytosine residue. This modification in methylation will monitor the binding of transcription factors and DNA accessibility in the DNA regulatory region, inevitably leading to modulate gene transcription[118]. The DNMT family is composed of DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L. DNMT1 is known to act mainly as a "maintenance" methyltransferase during DNA synthesis and DNMT3A and DNMT3B act as "*de novo*" methyltransferase during development. But DNMT1 can also act as a "*de novo*" methyltransferase for genomic DNA and DNMT3A and DNMT3B can also act as "maintenance" methyltransferase during replication[119,120]. The catalytically inactive DNMT3L stimulates the activity of the DNMT3A and DNMT3B enzymes by a direct binding to their respective catalytic domains. Overexpression of DNMTs and their mutations in a variety of tumors, including HCC, modify DNA methylation profiles[121]. Inversely, modification of enzymes involved in DNA demethylation such as TETs (Ten-eleven translocation) is also frequently observed[122]. DNA hypomethylation associated with genome instability and locus-specific hypermethylation of CpG islands are an epigenetic hallmark of cancer, associated with uncontrolled cell proliferation and survival leading to tumor growth. In HCC, DNA methylation is increasingly altered from cirrhosis to preneoplastic lesions and to HCC, without etiology differences, and could be associated with tumor recurrence and

Table 1 Main clinical trials on immunotherapies and epigenetic agents in monotherapies or in combination

Clinical trial	Phase	Drugs	Line/setting	Cancer type
NCT03383458[1]	3	Nivolumab *vs* placebo	ADJ	HCC
NCT03867084[1]	3	Pembrolizumab *vs* placebo	ADJ	HCC
NCT03062358[1]	3	Pembrolizumab + BSC *vs* placebo + BSC	ADJ	HCC
NCT03412773[1]	3	Tislelizumab *vs* sorafenib	1	HCC
NCT03755791[2]	3	Cabozantinib + atezolizumab *vs* sorafenib	1	HCC
NCT04487067[2]	3	Atezolizumab + bevacizumab	1	HCC
NCT04310709[2]	2	Regorafenib + nivolumab	1	HCC
NCT04443309[2]	1-2	Lenvatinib + camrelizumab	1	HCC
NCT04393220[2]	2	Nivolumab + bevacizumab	1	HCC
NCT03778957[3]	3	TACE + durvalumab + bevacizumab	1	HCC
NCT04246177[3]	3	Lenvatinib + pembrolizumab + TACE	1	HCC
NCT04340193[3]	3	Nivolumab + ipilimumab + TACE	1	HCC
NCT04268888[3]	2-3	Nivolumab + TACE/TAE	1	HCC
NCT03482102[3]	2	Durvalumab + tremelimumab + radiation	1	HCC
NCT03298451[4]	3	Durvalumab + tremelimumab and durvalumab *vs* sorafenib	1	HCC
NCT04039607[4]	3	Nivolumab + ipilimumab *vs* SOC	1	HCC
NCT03605706[5]	3	Camrelizumab + FOLFOX4	1	HCC
NCT03439891[5]	2	Sorafenib + nivolumab	1	HCC
NCT03257761[6]	1	Guadecitabine + durvalumab	2	Liver, pancreatic, bile duct or gallbladder cancer
NCT02816021[6]	2	Azacitidine + pembrolizumab	1	Melanoma
NCT04541277[6]	2	Tislelizumab + DNMTi +/- chemotherapy	1	AML
NCT02530463[6]	2	Nivolumab and/or ipilimumab +/- azacitidine	1/2	Myelodysplastic Syndrome
NCT03552380[6]	2	Entinostat + nivolumab + ipilimumab	2	Kidney
NCT03179930[6]	2	Entinostat + pembrolizumab	2	Lymphoma
NCT02697630[6]	2	Pembrolizumab + entinostat	1	Metastatic uveal melanoma
NCT03250273[6]	2	Entinostat + nivolumab	2	Cholangiocarcinoma and pancreatic adenocarcinoma
NCT02915523[6]	1/2	Avelumab +/- entinostat	1/2	Ovarian cancer
NCT03838042[6]	1/2	Nivolumab + entinostat	1/2	CNS, solid tumors
NCT03024437[6]	1/2	Atezolizumab with entinostat and bevacizumab	1/2	Kidney
NCT01928576[6]	2	Nivolumab +/- entinostat + azacitidine	2	NSCLC
NCT02901899[6]	2	Guadecitabine and pembrolizumab	2	Ovarian, primary peritoneal, or fallopian tube cancer
NCT03179943[6]	2	Atezolizumab + guadecitabine	2	Urothelial carcinoma
NCT03576963[6]	1/2	Guadecitabine + nivolumab	2	Metastatic colorectal cancer
NCT03308396[6]	1/2	Durvalumab + guadecitabine	1/2	Kidney
NCT02935361[6]	1/2	Guadecitabine + atezolizumab	2	Myelodysplastic syndrome or chronic myelomonocytic leukemia

[1]Immune checkpoint inhibitor (ICI) monotherapy.

[2]Combination ICI with anti-angiogenic agents.
[3]Combination ICI with locoregional treatment.
[4]ICI combination.
[5]Other ICI combinations.
[6]ICI + epigenetic drugs.
AML: Acute myeloid leukemia; BSC: Best supportive care; CNS: Central nervous system; HCC: Hepatocellular carcinoma; NSCLC: Non-small-cell lung carcinoma; SOC: Standard of care; TACE: Transarterial chemoembolization; TAE: Transarterial embolization.

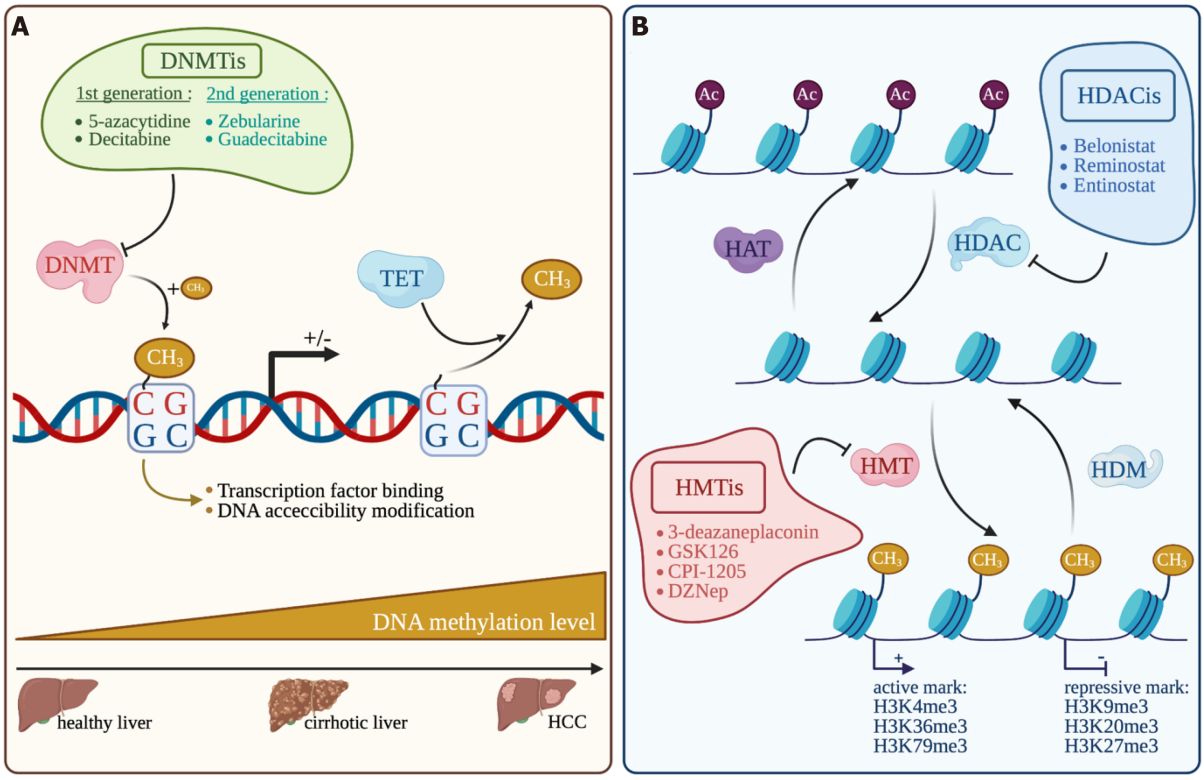

Figure 4 Overview of the main epigenetic mechanisms in hepatocellular carcinoma and their inhibitors. Made with biorender.com. A: DNA methylation; B: Histone modification. DNMT: DNA methyltransferase; TET: Ten-eleven translocation; DNMTis: DNA methyltransferase inhibitors; HAT: Histone acetyl transferase; HDAC: Histone deacetylase; HDACis: Histone deacetylase inhibitors; HMT: Histone methyl transferase; HDM: Histone demethylase; HMTis: Histone methyl transferase inhibitors; HCC: Hepatocellular carcinoma.

survival[123-125]. Promoter hypermethylation related to gene silencing is also often observed on tumor-suppressor genes and regulators of cell proliferation and survival such as *APC, CDH1, CDKN1A* and *CDKN2A*[126].

To counteract the tumoral effect of DNA methylation, several DNMT inhibitors (DNMTi) have been extensively studied and under clinical trials for hematologic cancers and increasingly tested in solid tumors. First generation DNMTis like 5-azacytidine (5-aza) and decitabine, can be incorporated into DNA and favor DNMT1 degradation by irreversible binding leading to DNA demethylations. Patients with advanced HCC treated with decitabine show significant clinical benefit from this treatment and a favorable toxicity profile[127]. Second generation DNMTis that are more stable *in vivo*, have shown interesting results. Zebularine treatment is potentially less toxic, since it does not incorporate into DNA, and gives promising results on an HCC mouse model with high degree of CpG methylation[128]. Guadecitabine was also successfully tested under the clinical trial NCT01752933 on patients which were not responsive to sorafenib with an average PFS of 2.7 mo and an OS of 8 mo[129]. Interestingly, guadecitabine promotes an innate immune response through reactivation of epigenetically silenced endogenous retroviruses and thus could improve ICI sensitivity[130].

HISTONE MODIFICATIONS AND TARGETING DRUGS

Another central epigenetic mechanism is the posttranslational modifications of histones, which control gene expression by modulating chromatin accessibility. Histone-modifying enzymes target specific residues on histone tails by acetylation, phosphorylation or methylation. Other modifications of histone residue exist but are less common, such as ubiquitination, citrullination, ADP-ribosylation, butylation[131]. First, histone acetylation is based on a reversible addition of an acetyl group on histone lysine residues that are added by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs) (Figure 4). Histone acetylation is often associated with a positive gene transcription. Secondly, like DNA methylation, histone methylation is based on the addition of a methyl group on a lysine or an arginine residue in the histone tails by histone methyl transferases (HMTs). Histone demethylases (HDMs) are responsible for methyl removing. Some histone methylation marks are associated with an active gene transcription, like H3K4me3[132], H3K36me3[133] and H3K79me3 [134] and others are rather repressive marks, like H3K27me3[135], H3K20me3[136] and H3K9me3[137]. The expression of several histone modifiers is deregulated in HCC and associated with tumor progression and prognosis, such as HAT with hMOF[138], a plethora of HDAC (HDAC1, 2, 4 and 5, and SIRT1, 2 and 7)[139]. HMT are also concerned with the best characterized EZH2 promoting gene repression through H3K27 trimethylation, G9a[140] and SUV39H1[141] mainly associated with gene repression through H3K9 modifications. Regarding histone modifications, another key actor is BRD4, which reads H3K27ac marks highly enriched in large clusters of enhancers. BRD4 was found overexpressed in HCC and required for super-enhancer-mediated expression of oncogenes[10].

As DNMTi, HDAC inhibitors (HDACi) have also been evaluated in clinical trials for hematological malignancies but also in solid cancers such as HCC. HDACis bind the zinc-containing catalytic domain of HDACs and thus modify histone acetylation status and gene transcription through HDAC inhibition. An interesting phase 2 clinical study of Yeo *et al*[142] (NCT00321594) shows the beneficial effect of belinostat in unresectable HCCs. Belinostat, a pan-HAC inhibitor against zinc-dependent HDACs, could increase PFS to 2.6 mo and OS to 6.6 mo with tumor stabilization. The SHELTER study (NCT00943449) combining sorafenib with resminostat, another pan-HDACi targeting HDAC 1, 2 and 3, doubles the OS of advanced HCC patients (8 mo instead of 4.1 mo) [143]. Interestingly, some epigenetics drugs have shown interesting results in HCC experimental studies regarding their impact on tumor microenvironment and tumor response to ICIs. The BET bromodomain inhibitor i-BET762 significantly reduces the level of Monocytic-MDSCs and enhances TILs, alone or in combination with anti-PD-L1, and consequently decreases tumor growth in two fibrotic HCC mouse models [144]. In the same way, the co-inhibitor of G9a and DNMT1 called CM-272 favors differentiated HCC and impairs the pro-tumorigenic effects of the surrounding fibrotic stroma[145]. Together, these data support the potent therapeutic benefit of targeting microenvironment remodeling together with epigenetic reprogramming during HCC, in a context of fibrogenesis in particular.

THERAPEUTIC STRATEGIES COMBINING ICI WITH EPIGENETIC DRUGS

Most immunotherapies are based on the targeting of immune checkpoints and the enhancement of immune system reaction to eradicate cancer cells but not all the patients are good responders to those cures. As mentioned previously, several treatments targeting epigenetic mechanisms allow to modify tumor progression and response to treatment. Epigenetic drugs that target DNMTs and HDACs, can in particular upregulate the expression of several immune signaling components in cancer cells such as TAAs[146], stress- and death-induced ligands and receptors, expression of co-stimulatory molecules at the cell surface but also expression of checkpoint ligands[147,148]. Therefore, epigenetic drugs have been used as neoadjuvant agent or in combination with immunotherapies to prime the immune system and create a better response to ICIs.

As previously detailed, cancer cells can evade immune surveillance by a lack of expression of TAAs. Cancer testis antigens (CTAs) are the best characterized TAAs that are regulated by epigenetic events. They are expressed in embryonic and germ cells but silenced by methylation of their promoter in mature somatic and cancer cells. The use of DNA methylation inhibitors such as DNMTis have proved CTAs re-expression in several solid tumors[146,147,149]. HDACis can also induce the re-

expression of CTAs but in a less extent than DNMTis, in human cancer cell lines[150]. Several clinical trials are already ongoing (Table 1). Other TAAs are sensitive to several DNMTis or HDACis depending on cancer type and once again DNMTis are more efficient than HDACis[151]. Those drugs can also be used to compensate the methylation deregulation of the promoter region of the APM (antigen processing machinery) component, like TAP-1, TAP-2, LMP-2, LMP-7 and MHC molecule in various tumors[152-154]. Epigenetics drugs can also facilitate tumor cells death by inducing the expression of death receptors, stress induced ligands and co-stimulatory molecules that will sensitize tumor cells to immune-mediated cells lysis[155-161]. Those drugs can also sensitize cancer cells to immune checkpoint therapies targeting PDL-1 and PDL-2, PD-1 and CTLA-4 by increasing their expression on both cancer cells and TILs favorizing their response to ICI[153,154]. Woods and collaborators show on a mouse model of melanoma that a pretreatment with HDACis upregulates PD-L1 and PD-L2 expression and favor the effect of the anti-PD1 treatment, slowing tumor progression and increasing mouse survival[162]. The co-inhibition of H3K27me3 and CTLA-4 reduces the number of Tregs in a mouse model of melanoma and limits tumor size[163]. An interesting work of Goswami and collaborators also shows that the pharmacologic inhibition of EZH2 with CPI-1205 on human T cells altered their Treg phenotype and function and enhanced T cytotoxic activity[164]. They also observe in patients with melanoma or prostate cancer that the anti-CTLA-4 ipilimumab increases EZH2 expression in peripheral T cells. Finally, they could demonstrate in their murine models that EZH2 targeting in T cells could improve the antitumor response mediated by an anti-CTLA-4 therapy. EZH2 appears to be a target of choice since several others works have unveiled its implication in ICI response. Zhou *et al*[165] also show in an anti-PD1 resistant model of head and neck cancers that EZH2 targeting can restore response to anti-PD1 treatment by increasing antigen specific CD8+ T cell proliferation. Additionally, EZH2 and DNMT1 co-inhibition increases the expression of the Th1 chemokines CXCL9 and CXCL10 in the ID8 ovarian cancer mouse model. This leads to an increase in CD8+ T cell infiltration and improves response to anti-PD-L1 treatment[8]. As previously mentioned, DNMTis also constitute promising partners for ICI, and particularly 5-azacytidine. In a transplantable mammary carcinoma and mesothelioma murine models, the use of 5-azacytidine increases the anti-CTLA-4 anti-tumor efficiency[166]. A combination of anti-CTLA-4 and anti-PD-1 together with the two epigenetic modulatory drugs 5-azacytidine and the HDACi entinostat could eradicate tumors in mice with colorectal or metastatic breast cancers. These combined strategies mainly inhibit the suppressive activity of Granulocytic-MDSCs against intratumor T cell killing[167]. Many phase 2 trials are currently testing the impact of entinostat with ICI in several cancers (Table 1).

HCC tumors arise in fibrotic livers enriched in MDSCs with less infiltrating lymphocytes inside the tumor[168]. MDSC enrichment is also correlated with an aggressive tumor phenotype and a poor survival rate. Liu *et al*[144] show on a fibrotic-HCC mouse model that inhibiting monocytic MDSCs with a combination of molibresib, a BET bromodomain inhibitor, with an anti-PD-L1 therapy could enhance TILs and extend mouse survival even with a complete tumor regression[144]. Inhibition of EZH2 and DNMT1 by DZNep and 5-azacytidine respectively, led to tumor regression after anti-PD-L1 treatment of a subcutaneous HCC cell mouse model (HepG2, G-Hep3B and Hepa1-6). This increases cytotoxic T lymphocyte trafficking and promotes cancer cell apoptosis[169]. A second generation of DNMTi molecule, guadecitabine, shows interesting optimization of immunotherapy treatment. Guadecitabine is actually under a clinical trial as a monotherapy in HCC patients and shows a better stability and performance than the first generation DNMTis[130]. Other clinical trials with this DNMTi are actually ongoing in combination with ICI including in HCC (Table 1). HDACi have also been tested in HCC. In a subcutaneous Hepa129 murine model, Llopiz *et al*[170] demonstrate that the HDACi belinostat increases the anti-tumor activity of anti-CTLA-4 therapy. This combination enhances IFN-γ production by T-cells and decreases the number of Tregs. It also induces an early upregulation of PD-L1 on tumor-specific APCs and delay PD-1 expression on TILs. Furthermore, belinostat combined to CTLA-4 and PD-1 blockade leads to a complete tumor rejection[170].

CONCLUSION

The liver is a highly complex organ which orchestrates fundamental metabolisms finely regulated at the transcriptional and epigenetic level. Liver parenchyma also

harbors a specialized immune system playing a central role in liver homeostasis with the constant management of toxins, diet or bacteria susceptible to trigger deleterious inflammation. However, when toxin and pathogenic insults get into chronicity, liver inflammation could sensitize to cancer development in part by immune suppression mechanisms. Thus, this peculiar tumor microenvironment constitutes an interesting opportunity to therapeutic avenues based on ICIs. Due to its high complexity, HCC response to conventional therapies is quite heterogeneous and frequently associated with poor outcome, rendering this cancer one of the deadliest cancers in the world. While several solid tumors are good responders to immunotherapy, ICIs in HCC show disappointing results, especially on β-catenin mutated HCCs, even if ICIs have given better results than tyrosine kinase inhibitors particularly in terms of prolonged response. Contrary to other solid tumors, personalized therapies for HCC are more complex to define, in particular because of tumor appearance in a context of cirrhotic livers with high level of inflammation and damages. Even if genomic analyses of the tumor mutational background have already classified HCCs, a translational approach taking into account the immune cell pattern, inside and outside the tumor, but also their respective epigenetic state, regarding DNA methylation level or histone marks, will be of therapeutic benefit to select the more efficient therapy for each patient. The bi-therapy combining immunotherapies either with anti-angiogenic agents or epigenetic drugs currently appears as the most promising to treat HCC patients. It is now well known that multiple epigenetic modulations can lead to the modification of tumor microenvironment by expressing TAAs, immune checkpoint ligands, costimulatory molecules and death-induced ligands or receptors at the cell surface. Therefore, using epigenetic agents to prime the microenvironment before immunotherapy may favor a better outcome for patients with a re-polarization of immune cells towards an efficient anti-cancer response. Several clinical studies have already shown that these bi-therapies are efficient in different solid tumors like pulmonary cancer, melanoma and colon cancers. Recently, results from clinical trials with epigenetic drugs and immunotherapy on advanced HCC patients showed interesting results with an extension of patient OS. These new combined therapies could be the new hope for HCC treatment. However, these clinical trials were only performed on advanced HCCs and it would be necessary to test these on HCC of lower grade because these treatments may be more efficient on these subgroups. The important point in close future is to identify predictive biomarkers, based on patient responses during clinical trials, to predict patient that will respond to treatment or not. Correlative studies are thus a prerequisite to create guidelines for personalized treatments and sequencing therapies to counteract immune dysfunction and overcome the current barriers to immunotherapies in HCC.

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REVIEW

Advances in the management of cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a primary malignancy of the bile ducts with three anatomically and molecularly distinct entities: Intrahepatic CCA (iCCA), perihilar CCA (pCCA), and distal CCA. As a result of phenotypic and anatomic differences they differ significantly with respect to management. For each type of CCA there have been significant changes in management over the last several years which will be discussed in this review. Although resection remains the standard of care for all types of CCA, liver transplantation has been established as curative treatment for selected patients with pCCA and is being evaluated for iCCA with early success. With respect to systemic therapy capecitabine is now first line adjuvant therapy for all biliary tract malignancies after curative intent resection. Progress in exploiting the pathologic mutations and molecular abnormalities has also yielded regulatory approval of targeted therapy for CCA in patients with acquired alterations in the fibroblast growth factor receptor. There is also increased consensus in managing malignant biliary obstruction associated with CCA where pre-operative biliary stenting is not beneficial while self-expanding metal stents have been shown to be superior to plastic stents in patients who are not surgical candidates.

Key Words: Cholangiocarcinoma; Intrahepatic cholangiocarcinoma; Perihilar cholangiocarcinoma; Liver transplantation; Chemotherapy

Core Tip: This review presents recent advances in the management of cholangiocarcinoma with particular focus on the expanding role for liver transplantation, updated guidelines in the use of chemotherapy, novel applications of individualized therapy targeting the specific mutation profile of tumors, and management of malignant biliary obstruction.

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INTRODUCTION

Cholangiocarcinoma (CCA) is an epithelial cell malignancy of the biliary tree and is the second most common primary hepatic malignancy[1,2]. The management of CCA depends largely on anatomic location and stage of disease. Anatomic location is significant not only because it dictates if a tumor can be resected, but also because different anatomic locations are associated with distinct molecular and biological characteristics which are increasingly important in determining optimal systemic therapy[3]. Intrahepatic CCA (iCCA) arises from the second order bile ducts within the liver and account for 10%-20% of CCAs, perihilar CCA (pCCA) originates between first order bile ducts and the cystic duct accounting for 50%-60% of CCA, and distal CCA (dCCA) arises distal to the cystic duct and account for 20%-30% of CCA[4]. Resection remains the best curative option for all types of CCA but is only possible in about 35% because symptoms occur late, the tumor progresses rapidly, and CCA is difficult to definitively diagnose[1,5]. Despite a historically low 5 year survival of 7%-20% and median survival of unresectable CCA of less than a year there has been significant progress in the management of CCA primarily in the use of liver transplantation and systemic therapy including targeted molecular therapy show promise to improve outcomes in the future[4,6].

iCCA

iCCA generally presents at later stages than other types of CCA because tumor growth is often intrahepatic and causes obstructive jaundice less frequently. When iCCA is diagnosed at early stages, it is often as an incidental finding or in patients with cirrhosis found during routine screening for hepatocellular carcinoma (HCC)[4]. Staging of iCCA should be done in accordance with the American Joint Committee on Cancer/International Union Against Cancer 7th edition staging manual as it has been validated and correlates with prognosis[7].

Surgical resection

Liver resection is the only widely accepted curative treatment for iCCA. Staging laparoscopy is recommended prior to resection in patients with high risk features such as multicentric disease, high CA19-9, questionable vascular invasion, or suspicion for peritoneal disease, because peritoneal or extrahepatic metastases are identified in 27-38% of patients[8]. However, because iCCA presents in advanced stages, only approximately 15% of patients with iCCA are candidates for liver resection[9]. The aim of surgical resection is complete removal of the tumor both grossly and microscopically, termed R0 resection. Resections which have microscopically positive margins are denoted R1 and if all gross tumor cannot be removed R2[10].

In planning liver resection, the location of the tumor in relation to biliary and vascular structure as well as the quality and size of the remaining liver parenchyma after resection are critically important[11]. In patients with inadequate future liver remnant, portal vein embolization can be attempted to allow for hypertrophy of the liver remnant[12]. However, this is associated with significant dropout of 20%-30% due to tumor progression and lack of adequate liver regeneration[13]. In smaller lesions and peripheral lesions anatomic resection is associated with lower recurrence and improved survival compared to non-anatomic resections[11]. Open and minimally invasive resection are associated with similar outcomes and both are endorsed by international consensus[14]. Hilar lymphadenectomy of at least 6 lymph nodes is recommended for accurate staging because imaging has low sensitivity for detecting nodal disease and because a recent multicenter retrospective review demonstrated removal of > 3 Lymph nodes is associated with improved survival compared to those where 1-2 lymph nodes were removed[1,15,16]. In patients with multifocal iCCA, the risk of recurrence is high and resection does not improve overall or recurrence free survival comparted to locoregional therapy (LRT)[17].

Although most patients are not candidates for surgical resection, the frequency of liver resection for iCCA is increasing[18]. The 5 year survival after curative intent liver resection is 25%-40% with a median survival of 40 mo[19-21]. However, recurrence remains high at 50%-70%[22]. Tumor recurs most frequently in the remnant liver and can be often be treated with repeat resection which is associated with improved survival of 26.1 mo compared to 9.6 in patients treated with chemotherapy and 18.6 in patients treated with LRT[23].

Liver transplantation

Liver transplantation for iCCA was initially associated with survival as low as 53% at 1 year[24]. As a result liver transplantation was not recommended for the treatment of iCCA and remains a contraindication for liver transplant except as part of research protocols[1]. Subsequently a multicenter series of patients who underwent liver transplantation for presumed HCC but explant pathology showed iCCA demonstrated 1-year, 3-year, and 5-year actuarial survival rates of 93%, 84%, and 65% respectively in patients with tumor < 2 cm[25]. More recently a retrospective series from France demonstrated lower recurrence (18% *vs* 46%, $P = 0.01$) and improved recurrence free survival (75% *vs* 36%, $P = 0.004$) in cirrhotic patients with iCCA who underwent liver transplantation compared to resection[26]. A trend toward reduced recurrence was maintained in patients with tumors 2-5 cm (21% *vs* 48%, $P = 0.06$). Data such as this as well as improved survival after liver transplantation for pCCA prompted a re-examination of the role of liver transplantation for iCCA.

There is currently very limited prospective data for liver transplantation in patients with iCCA. A prospective series of 6 patients with iCCA treated with gemcitabine based neoadjuvant chemotherapy demonstrated excellent post-transplant survival: 100% at 1 year, 83.3% at 3 years, and 83.3% at 5 years[27]. It should be noted that median time from diagnosis to transplantation was 26 mo, which speaks to the value of assessing response to chemotherapy and tumor biology during an initial waiting period before liver transplantation. There are currently ongoing clinical trials to more thoroughly define the role for liver transplantation for iCCA. However, because iCCA is not accepted as an indication for liver transplantation and patients do not receive MELD exception points, organ allocation remains an obstacle and relies largely on marginal donor grafts.

Systemic therapy

The performance status of the patient and disease distribution are the primary determinants of candidacy for systemic therapy. In patients where iCCA is resected with curative intent, neoadjuvant therapy is not recommended but 6 mo of capecitabine should be offered to patients with R0 or R1 resections as adjuvant chemotherapy[28]. This recommendation is based largely on the BILCAP study which included 447 patients with biliary tract cancer including iCCA (19%), pCCA (28%), dCCA (35%), and muscle invasive gallbladder cancer (18%) and compared capecitabine to observation[29]. This demonstrated improved overall survival of 51 mo in the capecitabine group compared to 36 in the observation group. Because this data was not available when the National Comprehensive Cancer Network guidelines were published in 2019, the American Society of Clinical Oncology convened an expert panel who recommended capcitabine for all biliary tract cancers after R0 or R1 resection[28].

In patients who have acceptable performance status but are not candidates for resection, gemcitabine-cisplatin based palliative chemotherapy is recommended as first line[1]. This recommendation is supported by trials such as ABC-02 which included 410 patients where gemcitabine-cisplatin demonstrated improved overall compared to gemcitabine alone (11.7 mo *vs* 8.1 mo)[30]. Recent data from the phase III ABC-06 trial has established FOLFOX (leucovorin, Fluorouracil, and Oxaliplatin) as the preferred second line chemotherapeutic regimen[31]. This trial included 162 patients with advanced biliary tract cancer who progressed on a gemcitabine-cisplatin regimen. The one-year survival of patients randomized to FOLFOX was 25% compared to 11% in patients treated with supportive care. The similar benefit was maintained in the iCCA subgroup but did not achieve statistical significance.

Improved understanding of the molecular pathogenesis of iCCA has allowed for development of targeted therapies. Targeted and immunotherapy is a rapidly developing field with multiple agents under investigation therefore agents which are furthest along in the development/approval process will be reviewed here. Early attempts to use targeted therapy aimed at epidermal growth factor receptor (EGFR) and vascular epidermal growth factor (VEGF) pathways were unsuccessful. Cediranib, bevacizumab, sunitinib and vandetanib which target VEGF and VEGF receptor and

the EGFR inhibitor erlotinib have not shown survival benefit[32,33].

Point Mutations in the isocitrate dehydrogenase (IDH) 1 and 2 genes, present in 28% of iCCA and 7% of pCCA, result in increased production of the oncometabolite hydroxyglutarate[3,34]. Ivosidenib, a small molecule inhibitor of mutant IDH-1, was compared to placebo in patients with advanced IDH-1 positive CCA who progressed on first line therapy. Patients treated with ivosidenib had improved progression free survival compared to placebo (2.7 mo *vs* 1.4 mo $P \leq 0.0001$) and progression free survival at 6 mo was 32% in the ivosidenib group compared to 0 in the placebo group [35]. This provides strong evidence for targeted therapy and benefit of molecular profiling in CCA and led to approval of ivosidenib in the United States by the Food and Drug Administration (FDA) for treatment of IDH-1 positive CCA.

Acquired alterations in the fibroblast growth factor receptor (FGFR) gene are associated with tumorigenesis through a variety of mechanisms including angiogenesis and enhancing cellular proliferation, migration, survival and invasion[36]. FGFR2 fusions and rearrangements are present in up to 45% of patients with iCCA but are rarely seen in pCCA and dCCA[37,38]. Of the several agents under investigation targeting this pathway pemigatinib, a FGFR 1-3 inhibitor, is the first to receive FDA approval for the treatment of CCA with FGF/FGFR alterations based on results showing 35% objective response in patients with locally advanced or metastatic CCA [39,40]. There is some concern that tumors could acquire resistance to FGFR inhibitors due to mutations in the FGFR kinase domain to early FGFR inhibitors such as infigratinib, but more recently developed irreversible FGFR inhibitor TAS-120 with high specificity for FGFR 1-4 has shown efficacy in patients with treatment failure due to FGFR kinase domain mutations[41,42]. This also suggests that these agents could be intentionally sequenced in order to prolong duration of response.

Immunotherapy has shown efficacy in an increasing number of malignancies and in some has become standard of care. Although the immune micro environment of iCCA is quite variable, it often displays features associated with responsiveness to immune checkpoint inhibitors (ICI)[43]. Although there are several ongoing phase 2 and 3 trials of ICIs in CCA, the review of which is beyond the scope of this review, published data remains limited to multi-tumor basket trials and single arm studies[32]. There is promise in patients with microsatellite instability (MSI) where 40% objective response was seen in tumors, including CCA, with MSI treated with pembrolizumab[44]. Targeting these mutations may have limited application as only 5-10% of biliary tract tumors have these mutations[45]. However, more recently combined anti- PD-1/CTLA-4 blockade with Nivolumab and Ipilimumab showed efficacy in a phase II trial of patients of patients with advanced biliary tract cancer without MSI demonstrated an objective response rate of 23% and disease control in 44%[46]. Interestingly, all of the responders had either gallbladder or intrahepatic tumors again emphasizing that intra and extrahepatic malignancies are phenotypically distinct tumors.

To allow for improved individualization next generation sequencing should be performed early in order to identify targetable aberrations since mutational profiles can already yield actionable mutations in > 40% of biliary tract tumors (Table 1)[47]. Because of the rapidly changing landscape of treatment and increasing number of mutational targets for therapy the importance of early testing, dedicated centers and a multidisciplinary approach is increasing.

Tumor directed therapies

In patients with unresectable tumors liver directed therapies are a possible adjunct to systemic therapy and have demonstrated efficacy in multicenter retrospective and phase II prospective experiences. Although there is increasing interest in these modalities for treatment of iCCA they have not yet become standard of care. Liver directed therapies include trans-arterial radioembolization (TARE), trans-arterial chemoembolization (TACE), thermal ablation, external beam radiation, and intra-arterial pump chemotherapy. TARE delivers a high dose of localized radiation to the target tumor *via* yttrium-90 coated microspheres. A multicenter retrospective review including 115 patients with unresectable iCCA treated with TARE in addition to standard of care treatment demonstrated median overall after treatment was 11 mo and 1-year overall survival was 44%, which compares favorably to historical data[48]. Treatment with TACE involves intraarterial injection of embolic beads impregnated with a chemotherapeutic agent resulting in embolic tumor kill augmented by high dose localized chemotherapy. TACE use in CCA has been limited but have generally shown that TACE is well tolerated and is associated with median overall survival of up to 15 mo in patients without extra-hepatic disease[49]. Thermal therapy involves either radiofrequency or microwave induced thermal ablation with an image guided

Table 1 Targetable genomic alterations in cholagiocarcinoma under investigation

Alterations	iCCA	pCCA/dCCA	Products under investigation
FGFR fusion	15%-20%	< 5%	Pemigatinib[1], Derantinib (ARQ-087), Infigrantinib[1] (BGJ398), Erdafitinib, TAS-120, ADZ4547
IDH1/2 mutation	20%	< 5%	Ivosidenib1, Enasidenib (AG-221), BAY 1436032, IDH305
ErbB2 (HER-2) amplification	< 5%	10%-15%	Trastuzumab, iapatinib, TAS0728, A166, PRS-343, ZW25
BRAF mutation	5%	< 5%	Dabrafenib + trametinib
DNA damage repair gene mutation (*ARID1A*, *BRCA1/2*)	25%	10%-15%	PARP inhibitors (olaparib, rucaparib)

[1]FDA approved.

iCCA: Intrahepatic cholangiocarcinoma; pCCA: Perihilar cholangiocarcinoma; dCCA: Distal cholangiocarcinoma; FGRR: Fibrobast growth factor receptor; IDH: Isocitrate dehydrogenase; ERBB (HER-2): A subtype of epidermal growth factor receptor tyrosine kinase; *BRAF*: Gene for serine/threonine-protein kinase B-Raf; *ARID1A*: Gene encoding a swItch/sucrose non-fermentable ATP-dependent chromatin remodeling complex; *BRCA*: Breast cancer gene.

probe percutaneously. Although data is limited, a systematic review of observational studies evaluating 84 patients with unresectable CCA treated with radiofrequency ablation showed pooled 1 year, 3 year, and 5 year survival of 82%, 47%, and 24% respectively[50]. Thermal ablation is therefore an option in patients with smaller (less than 4 cm) more peripheral tumors who are ineligible for surgery[51]. Both intraarterial and ablative treatment have also been reported as effective in patients with recurrence after resection[52,53]. Hepatic arterial infusion of high dose chemotherapy has demon-strated promising results in phase II studies of patients with unresectable iCCA. Of the 38 patients who were treated with intra-arterial infusion of floxuridine in addition to gemcitabine and oxaliplatin 58% achieved partial radiographic response with progression free survival of 11.8 mo, overall survival 25 mo, and 1 year survival of 89.5%[54].

Radiation therapy is also increasingly being evaluated for patients with unresectable iCCA as technologic advances has improved to the ability to specifically target malignant tissue while sparing non-malignant tissue. In a phase II trial high dose hypofractonated proton beam therapy was used to treat 37 patients with localized unresectable iCCA and demonstrated progression free survival of 8.4 mo, median overall survival of 22.5 mo and 1 year overall survival of 69.7%[55]. Evaluation of stereotactic body radiotherapy has similarly demonstrated safety and improved survival when compared to historical controls and is currently an area of investigation in phase III clinical trials (NCT02200042)[56,57].

PCCA

pCCA is the most common subset of CCA accounting for approximately 50% of CCA. The most common risk factor for pCCA is primary sclerosing cholangitis (PSC)[58]. Due to the risk of peritoneal seeding, percutaneous or fine-needle aspiration during endoscopic ultrasound is not recommended. Tissue diagnosis is most commonly obtained *via* cytology from endoscopic retrograde cholangiopancreatography (ERCP). Despite good specificity (97%), sensitivity of this is relatively low (43%)[59]. However, the addition of fluorescence in situ hybridization to conventional cytology can increase the sensitivity significantly to 65% while maintaining 100% specificity[60]. There is also interest in combining cytology with other methods to detect molecular or genetic signatures of CCA to aid in diagnosis, but these methods require further study before they are widely adopted[61-63].

Surgical resection

Although both liver transplantation and surgical resection for pCCA can offer cure, resection has historically been the preferred option[64]. Contraindications to resection include underlying PSC (because of high rates of multifocal disease) and presence of metastatic disease. Staging laparoscopy or laparotomy is recommended because occult metastatic disease or vascular involvement prior to surgical resection[65]. Despite this, recurrence is common with estimates based on long term follow up of 306 patients

who underwent curative intent surgery is 76%[66]. Patients with tumors involving both right and left intrahepatic ducts (Bismuth type IV) were previously not considered for resection however successful resection of these tumors has been described, primarily from centers in Asia. In one series from Japan 216 patients with Bismuth IV tumors treated with resection had 5 years survival of 32.8% and 53% in those who were negative for nodal and metastatic disease compared to 1.5% in those with unresected tumors[67]. Survival in Bismuth IV stage disease in this series was similar to earlier stage disease from other centers and suggests that presence of ductal invasion should not necessarily determine respectability if there is a high degree of local expertise[68]. Similarly advances in vascular reconstruction has allowed for resection of tumors with some degree of vascular involvement. While unilateral portal vein involvement does not impact overall survival in patients undergoing resection, there is decreased survival in patients with bilateral/main portal vein involvement or any hepatic artery involvement[69].

Liver transplantation

Although resection has been considered the standard of care for pCCA, only 20% of patients are candidates for surgical resection and of those who undergo surgical resection only 60%-80% achieve free margins (R0). Because survival after R0 resection is 20%-40% at 5 years and approaches 0% in those without R0 resection, there is significant interest in the use of liver transplantation for pCCA[70]. However, early experience with liver transplantation for pCCA resulted in recurrence rates of approximately 50% and poor long term survival[71]. Subsequently incorporating neoadjuvant chemoradiation prior to liver transplantation demonstrated favorable survival with multi-center experience from the United states showing 5-year disease free survival of 65% at 5 years following liver transplantation[72]. Based on this and other similar data, pCCA has been accepted by the United Network for Organ Sharing in the United States as an indication for liver transplantation and receives standard MELD exception points. In order to qualify, patients must have unresectable disease based on technical considerations or underlying liver disease, meet diagnostic criteria for pCCA less than 3 cm in size, be treated with neoadjuvant therapy, undergo operative staging to rule out intraperitoneal/lymph node metastases after neoadjuvant therapy, and be otherwise a candidate for liver transplantation. This approach has been criticized because a pathologic diagnosis is not required to qualify and residual tumor is found in only 52% of explants, therefore patients may undergo transplant without truly having CCA[72]. It has been argued that lack of pathologic evidence of CCA on explant may also be due to effective pre-transplant neoadjuvant therapy. There are no prospective comparisons of liver transplantation and surgical resection, however a multicenter retrospective comparison of curative intent resection (R0, R1) and transplantation for unresectable disease showed an improved overall survival of 77.4 mo compared to 17.1 mo ($P \leq 0.001$) and five year overall survival was 53% compared to 17%[73]. Survival advantage was maintained when limiting resections to only tumors < 3 cm with negative lymph nodes ($P = 0.002$) and non-PSC patients ($P = 0.049$). It should be noted that in this comparison, all patients had pathologically confirmed CCA. This data raises the possibility that liver transplantation will have an increasing role in the management of pCCA, but further study of this topic is required.

Systemic therapy

There is currently very little data regarding the use of neoadjuvant chemotherapy for pCCA prior to resection and reported experiences are from single centers and with small sample sizes[74]. However, these experiences suggest that there may be a role for neoadjuvant therapy in patients with initially unresectable disease. Neoadjuvant therapy with 5-FU and radiation therapy prior to liver transplantation for pCCA has become standard of care since initial positive experiences were reported[75]. Based on the BILCAP study which was previously described, adjuvant therapy with capecitabine is recommended for 6 mo following curative intent resection regardless of R0 or R1 status[28]. Adjuvant therapy after liver transplantation is not recommended. Reports of adjuvant therapy is primarily from prior to wide application of neoadjuvant therapy or small series where patients had significantly more or more advanced disease than suspected pre-transplant[76]. First and second line systemic therapy for patients with advanced pCCA who are not candidates for liver transplantation or resection are the same as for iCCA, gemcitabine/cisplatin and FOLFOX respectively [31,77] (Table 2).

Table 2 Role of treatment modalities in the management of cholangiocarcinoma

Tumor location	Surgery	Liver transplantation	Systemic therapy			Radiation therapy
			NeoAdjuvant	Adjuvant	Palliative	
Intrahepatic	Liver resection is first line management, anatomic resection is preferred	Clinical trials and select centers only	Not indicated	Capecitabine	Gemcitabine/Cisplatin; FOLFOX or evaluate for targetable mutations	External beam radiation reduces recurrence in R1 resection
Perihilar	Liver resection is first line management	Consider if not resection candidate, PSC	Only prior to liver transplant	Capecitabine	Gemcitabine/Cisplatin; FOLFOX	External beam radiation required pre liver transplant
Distal	Pancreaticoduodenectomy is first line management	Not indicated	Not indicated	Capecitabine	Gemcitabine/Cisplatin; FOLFOX	No defined role

PSC: Primary sclerosing cholangitis; FOLFOX: Leucovorin, fluorouracil, and oxaliplatin.

Tumor directed therapy

In patients who are candidates for surgical resection, neo adjuvant radiation therapy is not recommended while the role for radiation therapy is well established in prior to liver transplantation for pCCA. Although there are no randomized trials evaluating adjuvant radiation therapy in patients with complete resection of extrahepatic CCA, it has not been shown to improve survival in review of the SEER database[78]. In patients with incomplete surgical resection adjuvant radiation therapy is recommended and was found to reduce post resection local recurrence in retrospective series [64]. Data specific to patients with locally advanced unresectable pCCA is limited however based on small series of patients including pCCA and evidence of benefit of radiation and chemotherapy (capecitabine plus cisplatin) compared to chemotherapy alone (overall survival 9.3 mo *vs* 6.3 mo) in iCCA, radiation therapy is often used in patients with unresectable pCCA[79,80]. There is even less data for TARE and other intra-arterial therapies for pCCA, but based on experience in iCCA, this can also be used in selected patients.

Management of biliary obstruction

Biliary obstruction is a common complication of CCA given the presence of advance disease at the time of diagnosis. Proximal malignant biliary obstruction (MBO) secondary to pCCA accounts for roughly 60% of all MBO, whereas distal MBO is caused by dCCA and account for 20%-30% of cases[3]. Although endoscopic stenting is the mainstream endoscopic approach for MBO, numerous clinical studies have failed to show any benefits of routine pre-operative endoscopic stenting[81-83]. However, since most patients are not candidates for curative surgical resection, endoscopy provides a minimally invasive, cost-effective, and safe intervention for palliative biliary drainage (BD) with the aim of improving the patient's quality of life (QOL)[81].

The optimal approach for proximal MBO remains controversial with conflicting results on whether percutaneous transhepatic biliary drainage (PTHD) or ERCP with biliary stenting is superior[84,85]. The choice between these two strategies depends on multiple factors, including local expertise availability. When available, the potential advantage of an endoscopic approach may include minimally invasiveness, lower risk for leakage and higher patient satisfaction when compared to PTHD[85].

Several randomized clinical trials on patients with hilar MBO support the use of self-expanding metal stents (SEMS) over plastic stents (PS). SEMS are associated with higher stent patency, lower rate of adverse events, and improved survival[86-88]. SEMS can be broadly divided into two types: uncovered (USEMS) or fully-covered (FCSEMS). USEMS are routinely used, as FCSEMS pose the risk of iatrogenic biliary obstruction of the contralateral and/or branch ducts.

The choice between unilateral *vs* bilateral drainage remains a point of debate given the conflicting data. When compared to bilateral stenting, De Palma *et al*[89] demonstrated that unilateral stenting was associated with a higher technical success rate (88.6% *vs* 76.9%; P = 0.04) and less adverse events (18.9% *vs* 26.9%; P = 0.03). However, recent randomized studies from Asia suggest that bilateral stenting, particularly in patients with Bismuth type III-V strictures, result in fewer interventions,

improved stent patency and BD[90,91]. There are currently two main strategies for bilateral endoscopic drainage: The stent-in-stent (SIS) or stent-by-stent (SBS) techniques. With SIS, a USEMS is placed through the mesh of the first indwelling USEMS into the contralateral hepatic duct. This method requires the use of large cell-sized SEMS to facilitate the introduction of the second stent in the SIS fashion. This type of stents is commonly available in Asia but not in the United States. As opposed to the SIS technique, with SBS, both stents are inserted and deployed simultaneously into two opposite lobes of the liver. Both techniques appear to be associated with similar rates of technical success, adverse events and stent occlusion[92-94]. In clinical practice, the choice between these two techniques is often based on endoscopist's preference and device availability.

In all, the optimal treatment strategy will vary and should be individualized. From a broad perspective, the goal is to drain at least 50% of the total liver volume, as this is associated with improved clinical outcomes and survival[95]. Considering the high degree of technical difficulty of ERCP in this patient population, referral to high-volume centers is recommended. High quality cross-sectional imaging are crucial for pre-procedural planning to determine the extent of the liver volume involved by the strictures and whether BD of those segments is indicated.

Several studies have reported a possible role for endobiliary ablation with different modalities (*i.e.*, radiofrequency ablation, cryoablation, photodynamic therapy, intraluminal brachytherapy) as a primary palliative treatment for CCA or as and adjunct therapy for SEMS occlusion[96]. Several studies suggest that endobiliary ablation combined with palliative stenting may improve stent patency and prolong patient survival without an increase in adverse events[97,98]. Ablative therapies may be of particular benefit for patients with comorbidities who are not surgical candidates. Nonetheless, few prospective comparative trials are available and high-quality studies evaluating endobiliary ablation with standard palliative treatments with QOL and survival endpoints are necessary to better define their role in the management of these patients.

Endoscopic ultrasound guided BD (EUS-BD) has recently emerged as an alternate endoscopic option for the primary palliation of MBO or as rescue therapy in those who have failed conventional ERCP with transpapillary BD[99-101]. The various EUS-BD approaches (*i.e.*, choledochoduodenostomy, hepaticogastrostomy, antegrade biliary stenting and rendezvous procedure) are beyond the scope of this review. Overall, the route of approach and site of BD are largely dependent on local expertise and the level of the obstruction (*i.e.*, distal *vs* proximal MBO). A recent systematic review and meta-analysis of nine studies and 483 patients demonstrated similar technical success between EUS-BD and PTHD, albeit the former was associated with lower rate of adverse events and fewer interventions[102]. Furthermore, EUS-BD obviates the need for an external drain as in PTHD thereby enhancing patient's QOL[102]. EUS-BD may also confer some additional benefits when compared to ERCP. Unlike ERCP, EUS-BD does not require transpapillary access, which increases the likelihood of procedural success when concomitant duodenal obstruction is present and reduces the risk of iatrogenic pancreatitis. Furthermore, EUS-BD can be achieved without strictly placing a SEMS across the MBO, which potentially reduces stent issues associated with tumor overgrowth/ingrowth. Noteworthy, EUS-BD is a technically demanding procedure and should be limited to centers with adequate advanced endoscopy expertise.

DISTAL CCA

Although dCCA and pCCA are similar with respect to the pathologic mutations and cells of origin, they differ significantly in their surgical management largely because of their distinct anatomic location[4]. Lesions suspicious for dCCA are evaluated similarly to pCCA with EUS, ERCP, computed tomography, and magnetic resonance imaging for definitive diagnosis, staging, and determining resectability. In evaluations of radiation therapy for CCA, dCCA and pCCA are generally referred to as extrahepatic CCA. This data was reviewed above, therefore will not be repeated in this section.

Surgical management

As with other types of CCA, the treatment of choice for dCCA is surgical resection. However, patients with dCCA are typically treated with pancreaticoduodenectomy rather than liver resection. Complete R0 resection is more common in patients with dCCA and is achieved in approximately 78% of patients[10]. The five-year survival of

patients who have curative intent surgery remains relatively poor at 37% with median survival of 33 mo[103]. Because the tumor does not involve the liver or require biliary reconstruction, liver transplant is not necessary or beneficial in the management of distal CCA.

Systemic therapy

Patients who undergo curative intent resection should be treated with capcitabine which has been shown to improve survival compared to observation[29]. In patients who are not candidates for resection and have good performance status, first line systemic therapy gemcitabine and cisplatin. Data regarding survival in patients with advanced unresectable dCCA treated with this regimen is difficult interpret due to pCCA and dCCA often being classified together and one trial in which the 95% confidence interval of the hazard radio for death crossed 1 in patients with extrahepatic CCA[30]. However, survival for patients with advanced unresectable biliary tract cancers treated with gemcitabine/cisplatin is approximately 11 mo[77]. Because of the limited data for survival benefit specific to patients with dCCA treated with gemcitabine/cisplatin consideration should be given to enroll patients in clinical trials and evaluate for targetable mutations, when available.

Management of biliary obstruction

ERCP with biliary stenting is the preferred approach for the management of patients with distal MBO. When compared to PTHD, ERCP is associated with less adverse events (8.6% *vs* 12.3%), lower cost and shorter hospitalization, and improved QOL[82,83,104-106].

Recent data support the use of SEMS over PS for the management of distal MBO, although it largely includes patients with .biliary obstruction secondary to pancreatic malignancy. Overall, there is no significant difference in terms of technical success between the two approaches; however, SEMS are associated with longer stent patency, fewer adverse events, and less reinterventions[107,108].

Several studies have evaluated outcomes between uncovered *vs* covered metal stents for distal MBO[109-112]. In a randomized trial of 129 patients with distal MBO, there was no difference in stent patency or survival rates between uncovered *vs* partially covered SEMS; albeit the latter were associated with a higher rate of stent migration (0% *vs* 12%)[111]. Similarly, in another randomized trial of 400 patients, USEMS and FCSEMS had similar stent failure rates and time to re-occlusion, with no differences in survival time. Notably, stent migration was also more frequent with FCSEMS *vs* USEMS (3% *vs* 0%)[112]. Since MBO secondary to CCA is primarily a consequence of tumor growth within the bile duct lumen, placement of a FCSEMS may be preferable as to reduce the risk of tumor ingrowth.

CONCLUSION

Over the past several years there has been significant progress in the management of CCA. The role of liver transplantation has been clearly established for the management of pCCA and in some series rivaling the success of surgical resection. Transplantation is also being evaluated for iCCA with encouraging early results. Capecitabine has become first line adjuvant chemotherapy for all patients with curative intent resections of biliary tumors. With increasing understanding of mutational pathogenesis of the CCA, targeted therapies are showing significant promise and has led to the first FDA approved therapy for CCA targeting a specific mutation, pemigatinib. The use of SEMS has also improved management of obstructive symptoms over PS and advanced biliary stent design, endobiliary ablation, and EUS guided BD are avenues of investigation that may further improve management.

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REVIEW

Herbal and dietary supplement induced liver injury: Highlights from the recent literature

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Abstract

Herbal-induced liver injury (HILI) is an important and increasingly concerning cause of liver toxicity, and this study presents recent updates to the literature. An extensive literature review was conducted encompassing September 2019 through March 2021. Studies with clinically significant findings were analyzed and included in this review. We emphasized those studies that provided a causality assessment methodology, such as Roussel Uclaf Causality Assessment Method scores. Our review includes reports of individual herbals, including *Garcinia cambogia*, green tea extract, kratom as well as classes such as performance enhancing supplements, Traditional Chinese medicine, Ayurvedic medicine and herbal contamination. Newly described herbals include ashwagandha, boldo, skyfruit, and 'Thermo gun'. Several studies discussing data from national registries, including the United States Drug-Induced Liver Injury (DILI) Network, Spanish DILI Registry, and Latin American DILI Network were incorporated. There has also been a continued interest in hepatoprotection, with promising use of herbals to counter hepatotoxicity from anti-tubercular medications. We also elucidated the current legal conversation surrounding use of herbals by presenting updates from the Federal Drug Administration. The highlights of the literature over the past year indicate interest in HILI that will continue as the supplement industry in the United States grows.

Key Words: Herbal-induced liver injury; Dietary supplement-induced liver injury; Drug-induced liver injury; Roussel Uclaf Causality Assessment Method; Hepatotoxicity; Liver toxicity

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Core Tip: Herbal-induced liver injury is a growing concern worldwide with increasing rates of reported cases. Here we provide an encompassing review of reported new cases of well-established herbals along with newly described herbals causing liver injury over the past year. Causality assessment was emphasized. New studies addressing the hepatocytoprotective effects in human studies are also emphasized.

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INTRODUCTION

Reports of herbal-induced liver injury (HILI) and dietary and weight loss supplement liver injury (DSLI) continue to be published at an increasing rate, highlighting the growing interest in the field, as well as enhanced recognition of HILI by clinicians. For example, a routine PubMed search revealed eight systematic reviews and meta-analyses on HILI published in 2020 and four in 2019, compared to none published earlier than 2002[1-8]. In this review, we discuss the highlights chosen from the recent literature regarding HILI and DSLI liver injury since our last review period[9]. New information on the incidence of HILI and DSLI, reports of new herbal hepatotoxins and updates on previously described HILI are included, along with the current regulatory status of kratom and other agents.

METHODOLOGY

A literature review for this paper was performed utilizing PubMed and Google Scholar search engines spanning September 1, 2019 through March 31, 2021. Keywords utilized included "hepatotoxicity," "hepatic toxicity," "liver toxicity," "herbal induced liver injury," "HILI" and "dietary supplements." Using both search engines, we came across approximately 1800 publications. In order to narrow down this extensive search we focused on case reports, case series, review articles and original research that were published in journals with an impact factor ≥ 1 based on listings contained in Scholar One[10]. Of note, seven of the 85 discrete journals that we reviewed had an impact factor < 1 or none at all. However, we felt the information within those particular articles was important enough to include in our review. The range of journal impact factors (IF) was 0.28-60 (Zhongguo Zhong Yao Za Zhi and Lancet respectively), mean IF was 5.46, and median was 3.37[10]. Additionally, we focused on recent literature reporting new cases of HILI/DSLI along with particular herbal agents of interest such as green tea extract (GTE) and kratom along with performance enhancing supplements (PES), traditional Chinese medicines (TCM) and Ayurvedic medicines. Many reports described the cytoprotective effects of herbal compounds, and we focused on those utilized in human studies. Legal and regulatory ramifications were also addressed in particular with regard to kratom. As in past years, we emphasized those studies that provided a causality assessment methodology, such as RUCAM scores, believing that this enhanced their validity[11]. Through this selection process we narrowed our review to approximately 150 publications (Figure 1). Given the number of publications reviewed, the omission of any specific article should not be viewed as lacking importance or significance.

INCIDENCE RATES OF HILI/DSLI

Data on the true frequency of HILI/DSLI are generally lacking, in part due to under-diagnosis and under-reporting[12]. The incidence of HILI in mainland China, which we would expect to be among the highest worldwide, is estimated to be 6.38 per 100000 based on the large retrospective study by Shen *et al*[13], who described DILI

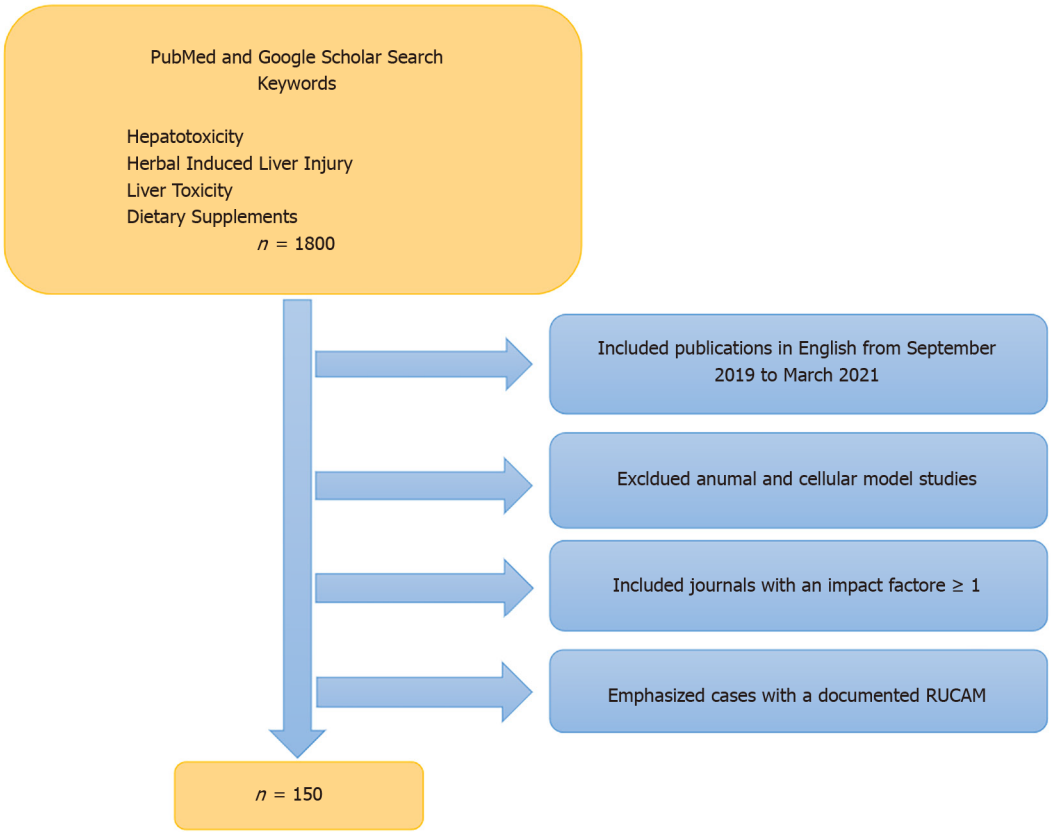

Figure 1 Study selection flowchart.

incidence to be 23.8 per 100000 of which 26.8% of single agents were TCM. In the United States, the estimated incidence of HILI was 1.16 per 100000 based on a small prospective study conducted in Delaware[14]. Perhaps a better estimation for a Western country comes from a prospective population-based study from Iceland that found an incidence of 3 per 100000[15]. While these estimates are lower than China's, HILI cases reported to the United States DILI Network have increased from 7% of all drug-induced liver injury cases in 2005 to 20% in 2014, with herbal and dietary supplements (HDS) representing the second leading class of compounds causing liver injury after antibiotics[16]. The most recent update of the United States DILI Network contained 404 cases of HILI enrolled between 2003 to 2019[17].

Registry-based frequency data demonstrates HDS responsible for 8% and 4% of DILI cases reported by the Latin DILI and Spanish DILI Networks, respectively[18]. Data from a single German hospital dedicated to TCM indicates a HILI frequency of 0.12% over a 20-year period[19]. The increasing number of reports of HILI are likely explained by the combination of more widespread HDS use as well as clinician awareness[13].

An ongoing difficulty with assessing a true incidence of HILI relates to the fact that herbal supplements commonly contain multiple ingredients, and several products are often used concurrently. As a result, it is challenging, if not impossible, to determine which specific HDS component might be responsible for the hepatotoxicity[16]. Frequent mislabeling of supplements, patient non-disclosure, and physician lack of awareness further complicate the diagnosis of HILI[16,20]. Nevertheless, it is crucial that clinicians maintain awareness of HILI, as it may have a greater potential for acute liver failure than DILI[16].

REGULATORY STATUS OF HERBAL AND HDS PRODUCTS

In contrast to the United States, herbal supplements undergo much more regulatory scrutiny in member states of the European Union (EU), where according to Directive 2004/24/EC, herbal medicinal products are required to not only register with the EU, but also comply with specific manufacturing and quality standards[21]. Herbal supplements are widely accessible to Americans both online and in nutrition stores

and pharmacies, and their appeal is heightened by marketers portraying them as natural and healthy[22]. In 2019, Americans spent $9.6 billion on herbal supplements alone, (exclusive of vitamins and other complementary and alternative therapies, which represented an 8.6% increase from the previous year[23]. As the use of HDS continues to climb, clinicians and patients alike will be faced with the challenge of recognizing and managing potential hepatic injury. The relative lack of regulatory control over HDS in the US compared to conventional medications, means there are fewer protections available to the consumer, such as quality control.

Despite the general lack of regulation of the herbal and supplement industry, the Food and Drug Administration (FDA) does maintain a role in providing for their safety. Herbal medications and vitamin supplements have long been categorized as food supplements and thus have a lower threshold required to maintain evidence for safety[24]. This was changed when the Dietary Supplement Health and Education Act of 1994 (DSHEA) was passed that named the FDA as responsible for safety concerns and for taking action against dietary supplements if needed[25]. Unfortunately, the provision only takes effect after supplements reach the market and supplement companies are not required to register themselves or their products with the FDA before offering them for sale. Until recently, the FDA mainly monitored product information through a voluntary dietary supplement adverse reporting system and took action retroactively against companies when necessary[25,26].

In recent years however, there has been an outcry regarding the sheer number of herbal supplements that have come to market with little to no consumer protection regarding their claims[27,28]. In 2019, then FDA Commissioner, Dr. Scott Gottlieb, issued a statement announcing plans for major policy changes toward the oversight of the dietary supplement industry. This included improvements in the adverse reporting system and a proposal to require the listing of the ingredients of dietary supplements with the FDA[29]. Since then, the proposal to register ingredients of various supplements has been a primary objective of the FDA with both the 2020 and 2021 budget proposals to Congress including a provision for this. In addition, they have asked for a mandate to allow them to act against products and manufacturers providing misleading information to the FDA[30]. However, both proposals have been met with significant resistance from the industry and have yet to become enacted into law.

CAUSALITY ASSESSMENT OF HILI AND DSLI

Diagnosing HILI remains a challenge and while there are several assessment tools used to determine causality, ultimately it is a diagnosis of exclusion[31]. Currently, Roussel Uclaf Causality Assessment Method (RUCAM), designed in 1993, is the most widely used assessment tool for determining causality[32]. Indeed, Teschke *et al*[33] identified 12068 HILI cases reported in the recent literature in which RUCAM was used as the basis of causality.

In another retrospective review, Teschke *et al*[34] analyzed 11,160 HILI cases from Asian countries - mainland China, Hong Kong and Taiwan, Korea, Singapore, and Japan - collected from 1964 to 2019. They identified China and Korea as being exemplary in their use of RUCAM to evaluate HILI cases. They suggest that RUCAM will be a particularly valuable tool when assessing causality of liver injury occurring during the COVID-19 pandemic, which may confound findings given the high incidence of liver test abnormalities associated with the infection[34,35]. Anirvan *et al*[36] described the effects of COVID-19 on the liver, concluding it has both direct viral cytopathic mechanisms and also acts indirectly, through immune-mediated, drug-induced, and other pathways. These investigators suggest that acute non-icteric hepatitis may precede pulmonary symptoms in COVID-19 infection[36].

RUCAM, however, is an imperfect tool, and some authors argue that it should be developed further as some of its criteria are not evidence-based[31]. For example, RUCAM does not accommodate evaluation of the several individual hepatotoxins that may comprise a single HDS[4]. Other assessment tools include the Clinical Diagnostic Scale (CDS) and Digestive Disease Week Japan 2004 Scale (DDW-J). Liu *et al*[37] compared RUCAM, CDS, and DDW-J in a cohort of 458 DILI patients at a hospital in Tianjin, China and found the CDS to be the most accurate in diagnosing DILI. The six variables that CDS employs are comparable to RUCAM's seven, though the former allocates different point values for timing of drug administration to onset of symptoms in addition to assigning points for extrahepatic manifestations including rash, fevers, eosinophilia, arthralgia, and cytopenia[38]. Of note, the most common causative agents

for liver injury in this cohort were TCM, used in 52.41% of patients.

BIOMARKERS AND GENETICS

This past year showed continued interest in innovative tools for diagnosing HILI. Liu *et al*[37] investigated the potential role of an *in vitro* monocyte-derived hepatocyte-like (MH) cell test in diagnosing HILI. Investigators identified 47 patients in Munich and Hong Kong who were determined by RUCAM to have had HILI. Among these patients, the MH cell test exhibited sensitivity and specificity of 90.6% and 86.7%, respectively. In a prior study, the MH cell test was shown to have higher specificity than RUCAM[39]. Thus, the MH cell test may be a valuable test in diagnosing HILI in the future.

Studies have investigated potential biomarkers for specific agents. Pyrrolizidine alkaloids (PA) are hepatotoxins commonly found in food items and herbs used in TCM, including *Gynura japonica (G. japonica)*. Pyrrole-hemoglobin adducts and three miRNAs - has-miR-148a-3p, has-miR-362-5p, and hs-miR-194-5p - have been shown to increase diagnostic accuracy of PA-induced liver injury. Similarly, *Polygonum multiflorum* (*P. multiflorum*) is an herbal popular in TCM. Metabolomics profiling has shown to successfully differentiate between DILI caused by *P. multiflorum* and autoimmune hepatitis (AIH) as well as hepatitis B virus[40]. Given the widespread ingestion of PA and *P. multiflorum*, these pioneering diagnostic tests may help guide clinicians in managing liver injury caused by these herbals.

UPDATES IN HILI REGISTRIES

United States

The United States DILI Network (DILIN) examined the association between GTE and the proinflammatory allele HLA-B*35:01 (see "Green Tea Extract")[41]. The other update focused on ashwagandha, a popular Ayurvedic medicine using data from the United States DILIN and Iceland (see "Ayurveda")[42].

Spain

Two updates from the Spanish DILI registry were published in 2020. While mainly focused on DILI, there is also comment on HILI. In a study of liver injury in the elderly, Weersink *et al*[43] found herbal products accounted for 4% of cases in younger patients, with a decreasing overall incidence with increased age. Similarly, in their comprehensive review of DILI over the span of 20 years up until 2018, Stephens *et al* [44] identified 843 cases of liver injury, 29 (3.4%) of which were attributable to HDS and an additional 22 (2.6%) were caused by selective androgen receptor modulators (SARMs).

Latin America

The Latin DILI Network (LATINDILI) comprises a group of seven countries that collect DILI cases prospectively, using RUCAM to determine causality. Bessone *et al* [18] published an analysis of HDS in Latin America from 2011 to 2019, and found that, similar to the findings from the prospective Spanish DILI and United States DILIN, HILI was more common among young women attempting to lose weight[18,45,46]. Rates of acute liver failure were 17%, 16%, and 6%, respectively for the LATINDILI, DILIN, and Spanish DILI networks. In another study using LATINDILI data, Santos *et al*[5] reviewed 17 records of HILI and found *Centiella asiatica*, *Carthamus tinctorius*, and the weight loss supplement 'HerbaLife' (that previously contained GTE and ephedra), as the most common causes[47]. They also found weight loss to be the most common reason for supplement use, which was also the most common indication reported by Bessone *et al*[18] Interestingly, while *Garcinia cambogia* (*G. cambogia*) is the third most frequent cause of liver injury in Latin America, as reported by Bessone *et al*[18], it was not present in the Spanish DILI registry. The authors suspected this was due to native cultural influences and surrounding geography, as well as the growing potential of different regions.

Malaysia

In Malaysia, a national centralized database of hepatic adverse drug reactions sponsored by the Ministry of Health was used to collect cases retrospectively and Lee

et al[47] presented data from 2000 to 2017. They presented 2090 cases of DILI, 11.24% of which were attributable to HDS. Causality was determined using WHO-Uppsala Monitoring Center criteria employed by physician and pharmacist members of the Malaysian Adverse Drug Reactions Advisory Committee (MADRAC). Of note, only 27.1% of products causing liver injury in this study were registered with the Ministry of Health, meaning the vast majority were unregulated. This highlights the similar regulatory challenges faced by authorities in Asia and in the West.

China

There are no significant updates to the China registry since Shen *et al*[13] extrapolated data from the National Health and Family Planning Commission to conduct the first nationwide study on HILI in mainland China, published in 2019. However, given the surge in literature investigating the impact of COVID-19 on liver injury, a potential confounder, we expect updates to Chinese HILI and DILI registries will be forthcoming.

LiverTox

Livertox is a database founded and maintained by the National Institute of Health. At present, it lists 1095 drugs, including 66 herbal and dietary supplements, and their potential for hepatotoxicity[48]. Likelihood scores are attributed to each herbal or supplement, ranging A-E, as designed by the United States DILIN to determine causality. In the LiverTox compendium, 24 (36.4%) of listed herbs or supplements have an A, B, or C rating, meaning a drug has "well known or more than 50 cases described", "known or highly likely or 12-50 cases described", or "probable or less than 12 cases described" to cause liver injury, respectively, based on published reports. In 2020, entries for 11 (16.6%) herbal and dietary supplements were updated on the website (Table 1).

NEWLY DESCRIBED HEPATOTOXINS

Peumus boldus

Peumus boldus (*P. boldus*) has been implicated as a cause of hepatotoxicity when consumed orally as an infusion, like a tea, especially in elderly patients[49]. The compound Epigallocatechin gallate (EGCG) has been identified as the underlying cause of hepatotoxicity[49]. Oliveira *et al*[49] describe a case report of an 87-year-old male patient who presented with weakness, anorexia, and jaundice. He was found to have a hepatocellular injury pattern. It was later discovered that the patient had been orally ingesting infusions of *P. boldus* over the past month as a treatment for dyspepsia. After exclusion of other causes of liver injury, the authors determined *P. boldus* was the probable cause of HILI, although they did not include a causality assessment score[49]. The patient's liver tests returned to baseline with conservative management.

Skyfruit

Skyfruit, also known as Xiang-tian-guo, is used to treat diabetes and hypertension, and was first reported to be hepatotoxic in 2018[50]. Since then, fewer than five case reports are documented in the literature. A 67-year-old woman with skyfruit exposure for six months and presenting with jaundice received a RUCAM score of 7, indicating 'probable' causality, described by Shao *et al*[51]. Xia *et al*[52] describe another case of a 63-year-old woman with a three day history of skyfruit use, who developed epigastric pain, nausea, and fever, and was given a RUCAM score of 10, indicating 'highly probable' causality. As diabetes and hypertension are common afflictions and clinicians become more aware of skyfruit's hepatotoxic potential, the incidence of skyfruit-induced liver injury may increase.

Ashwagandha

Ashwagandha, from the roots of *Withania somnifera,* is an Ayurvedic medication used to treat anxiety, depression, and erectile dysfunction. Björnsson *et al*[42] published a case series, drawing from an Icelandic registry and the United States DILIN, of five patients with Ashwagandha-induced liver injury. The authors used DILI expert opinion to determine causality in these patients who developed jaundice and pruritus after a latency period ranging from two to twelve weeks. The pattern of liver injury was cholestatic or mixed and liver enzyme abnormalities self-resolved within one to

Table 1 Herbs or supplements with an A, B, or C rating as listed in LiverTox

Herbal or supplement	Likelihood score	Last updated	Most recent citation
Aloe vera	B	2016	2015
Ashwagandha	C	2020	2019
Black cohosh	A	2020	2019
Butterbur	C	2019	2018
Polygonum multiflorum	A	2020	2020
Sho Saiko to and Dai Saiko to	B	2020	2019
Eugenol	C	2019	2018
Flavocoxid	C	2018	2013
Garcinia cambogia	C	2018	2013
Germander	A	2018	2017
Green tea	A	2020	2020
Kava	A	2018	2017
Kratom	B	2020	2020
Margosa oil	C	2020	2019
Noni	C	2020	2017
Pennyroyal oil	B	2020	2017
Red yeast rice	C	2018	2017
Skullcap	B	2020	2019
Usnic acid	B	2018	2017
Valerian	C	2020	2018
Move free	C	2020	2018
OxyELITE pro	C	2020	2018

five months in four of the five patients; the fifth patient was lost to follow up. Prior to this paper, only one case report had been published on the topic.

'Thermo gun'

Ferreira *et al*[53] described a case of a 36-year-old male who presented to the hospital with jaundice one week after taking the dietary supplement 'Thermo gun'. The authors reported no previous reports of a HILI association, but noted that oxilofrine, white willow, and caffeine could all play a possible role. Laboratory exams showed a cholestatic liver injury pattern. The drug was discontinued but the patient's liver function continued to deteriorate and he eventually developed acute liver failure. He successfully underwent liver transplantation and continued to do well at long term follow up. The authors assigned this case a RUCAM score of 7, indicating 'probable' causality by 'Thermo gun'.

UPDATES ON KNOWN HEPATOTOXINS

Kratom

Kratom is a controversial herbal compound derived from *Mitragyona speciosa* and originating in Southeast Asia. It has dominated headlines in recent years because of its popularity as a stimulant and the associated legal ramifications of its use to reduce opiate withdrawal symptoms[54,55]. The active components are believed to be Mitragyona and 7-Hydroxymitragynine (7-HMG)[56]. Kratom has been used as a stimulant at lower doses or to treat pain and precipitate euphoria at higher doses. At even higher doses, it acts as a sedative. Although it has found use in people who suffer from opioid addiction to prevent withdrawal, at present there are no medical

indications for kratom use in the United States, and the FDA has labeled it a 'drug of concern'. Despite being banned in countries including Thailand and Malaysia, it remains widely available in the United States over the internet - although it is banned in Alabama, Arkansas, Indiana, Rhode Island, Vermont, and Wisconsin.

Despite these admonitions, it is becoming more mainstream with over $207 million in annual sales[57]. Of note, in 2021, Schimmel *et al*[58] published the first national survey of kratom use in the United States. Using data from the Non-Medical Use of Prescription Drugs (NMURx) Program, these investigators conducted a cross-sectional study of kratom users in the United States from 2018 to 2019, and found kratom users were more likely to be young, male, and have more severe substance abuse profiles, as measured using DAST-10, than cannabis, alcohol, or cigarette users[58]. They also estimated a prevalence of kratom use of 0.8%.

Cultural differences may influence the use of kratom, and subsequently its adverse effects. Ramanathan and McCurdy argue that kratom has been more harmful in the west as compared to Southeast Asia. These authors propose this is because western users are more likely to ingest kratom recreationally[59]. To further delineate the motivations for using kratom in their Malaysian cohort, they found that current opioid users were more likely to use kratom to ameliorate withdrawal symptoms as compared to former opioid users, who used kratom recreationally (OR 1.9, $P < 0.035$) [60].

Current legal status

In 2016, the Drug Enforcement Agency (DEA) attempted to classify kratom as a Schedule I drug, meaning it has no medical indication and high potential for abuse, alongside heroin, lysergic acid diethylamide, and methylenedioxymethamphetamine (ecstasy). However, this effort was met with pushback from lobbying groups, members of congress, and the public. A bipartisan group of senators, including Bernie Sanders and Orrin Hatch, signed a letter protesting the FDA's immediate scheduling of kratom, and encouraged a lengthier investigation into the safety of kratom given its long history of use in other countries and growing popularity in the United States[54]. Moreover, some researchers believe that restricting kratom as a Schedule I drug would prevent advancement of research because of increased bureaucratic processes previously illustrated by studies on marijuana and psychedelic-assisted therapies[61]. Thus, kratom remains legal at the federal level, despite its known hepatotoxic potential.

Polypharmacy

Polypharmacy plays a significant role in kratom's potential for hepatotoxicity. Mitragynine inhibits glucuronidation by UDP-glucuronosyltransferases (UGT), which may explain kratom's increased toxicity when co-administered with other substances, such as UGT substrates including buprenorphine and ketamine[56]. Polysubstance abuse with kratom furthermore increases rates of death. The CDC collects data on death from substance abuse in the State Unintentional Drug Overdose Reporting System (SUDORS), and has investigated kratom, most recently publishing updated data in 2019[62]. Of the 27338 deaths due to overdose reported to SUDORS from July 2016 to December 2017, kratom was implicated in 152 (0.56%) cases. Among the 152 cases, medical examiners determined kratom to be a cause of death in 91 (59.9%), with kratom identified as the only substance in seven cases. Eggleston *et al*[63] conducted a retrospective review using kratom exposures reported to the National Poison Data System and New York State's county medical examiner's office records, and found 2312 cases of kratom exposure, of which 935 reported kratom as the only substance used.

Product contamination

The potential lethality of kratom is heightened by issues with product contamination, with both heavy metals and organisms that may cause illness. Most recently, in 2018, the FDA/DEA completed an investigation of kratom products contaminated with salmonella resulting in an outbreak affecting 199 individuals across 41 states[64]. Contaminants in kratom products were most recently found in a survey of kratom use in a Chicago suburb, which also revealed the presence of heavy metals, fungi, and bacteria[65,66].

New reports of kratom liver injury

Despite the safety concerns surrounding kratom, its popularity is continuing to rise. According to data from the System to Retrieve Information from Drug Evidence/ST-

ARLiMS, the DEA's registry for seized drugs, reports of kratom increased to 589 in 2018 from one in 2010[67]. A PubMed query for "kratom" revealed 101 articles published in 2020 compared to just 11 in 2010. The United States DILIN reported 11 cases of kratom-induced hepatotoxicity in the United States from 2003 to 2019, and causality was determined by expert consensus opinion[17].

Schimmel and Dart published a review of 85 kratom cases that nicely summarizes its clinical signature with respect to liver injury[68]. Using published case reports and abstracts, cases in the United States DILIN, FDA databases, and online user forum, they found most patients presented with abdominal discomfort, jaundice, pruritus, and dark urine. While liver tests revealed a mixed injury pattern, histology often showed cholestasis. The authors were only able to calculate a RUCAM score for 20 cases, with a median modified RUCAM score of 5 and mean of 4.5 (range 1-8), indicating 'possible' causality.

A newly reported form of kratom-induced injury is cholestasis resembling primary biliary cholangitis. A case report by Gandhi *et al*[69] from India, is only the second in the literature, reported. Causality in this case was determined by clinical judgment using symptoms of nausea, decreased appetite, fatigue, and jaundice with associated elevated bilirubin levels in the setting of kratom use two weeks prior to presentation. Cholestatic liver injury consistent with primary biliary cholangitis, was confirmed by histology revealing centrilobular cholestasis, moderate chronic portal tract inflammation, and brisk lymphocytic-predominant bile duct injury. Symptoms resolved with supportive care and steroids.

GTE

Green tea is one of the most widely consumed drinks worldwide, and is not considered a hepatotoxin[70]. In contrast, GTE has gained significant popularity for its weight loss enhancing potential and can be found in over a 100 herbal preparations in varying concentrations[70]. and have been associated with the potential for hepatotoxicity[71]. A systematic review of GTE performed by the United States Pharmacopeial Convention (USP) in 2008 and revisited in 2019 urged the use of cautionary labels to warn the general public of such causal relationships[71].

The USP reviewed both human case reports and animal studies to establish the role of GTE in hepatotoxicity. EGCG, a highly bioactive phytochemical, is felt to be the main compound implicated in liver injury and is seen in approximately 10% of GTE formulations at varying concentrations[70-72]. Indeed, the concentration of EGCG has been directly correlated to risk of liver injury[71]. The review conducted by the USP of human cases determined the median intake of 720 mg/d of EGCG for at least two weeks was related to liver injury[71]. Notably, the average over-the-counter GTE supplement contains an EGCG concentration from 45-1575 mg/d[71]. In addition, the bioavailability of EGCG increases in a fasting state, increasing serum concentrations at lower consumed dosages[71].

GTE-related hepatotoxicity almost always presents as an acute hepatitis with a hepatocellular injury pattern[70,71]. While the exact pathogenesis of injury is unclear, proposed mechanisms include the interaction of cytochrome P450 and EGCG, direct mitochondrial toxicity from reactive oxygen species produced by EGCG, or possibly, bactericidal effects of EGCG causing endotoxic induced liver injury[72,73]. Additionally, there is believed to be an idiosyncratic, dose-independent cause in genetically susceptible individuals related to individual HLA phenotype[41,72].

Hoofnagle *et al*[41] performed a retrospective review of 1414 cases of drug induced liver injury, of which 40 were attributed to GTE. 95% of these patients had the typically hepatocellular injury pattern with 3 ultimately requiring liver transplant. Notably, an HLA analysis on these 40 patients found that 72% had HLA-B*35:01[41]. There have been reports of other drugs causing idiosyncratic liver injury related to HLA-B *35:01, including trimethoprim-sulfamethoxazole and *P. multiflorum*[74,75]. This pharmacogenetic association suggests there may be a possible immunologic susceptibility in GTE-related HILI.

G. cambogia

G. cambogia is derived from the fruit of the Malabar tamarind tree found in South East Asia[76-78]. This herb continues to be an increasingly popular over the counter herbal supplement for its potential for enhancing weight loss[78,79]. Its weight loss potential stems from the active agent within *G. cambogia*, hydroxyl citric acid (HCA). HCA is thought to be an appetite suppressant which has demonstrated weight loss in rat models[78,80]. Additionally, HCA prohibits cholesterol and fatty acid synthesis in tissue through inhibition of adenosine triphosphate-dependent citrate lyase enzyme

helping in weight reduction[78,81]. Although it is an OTC supplement, caution must be taken as there have been rare, but serious cases of serotonin syndrome, rhabdomyolysis and hepatotoxicity[78].

It has been estimated that approximately 1 in 10000 individuals using *G. cambogia* experience significant liver-related injury[76,78]. Onset of injury generally occurs over one week to a few months after initiation[77]. The pattern of liver injury is typically hepatocellular. This year, cases of *G. cambogia* -induced liver injury with a pattern similar to autoimmune hepatitis appeared[76-79]. Injury and subsequent recovery is frequently managed with abstinence from offending supplements and supportive care [77-79]. However, there have been instances of individuals requiring liver transplant or even death related to such liver injury[78]. Although the pathogenesis of liver injury is unclear, proposed mechanisms through rat models include excessive production of reactive oxygen radicals from lipid peroxidation resulting in increased oxidative stress and cytoplasmic vacuolization signaling hepatocyte injury[77,79]. Nonetheless, there is thought to be two broader mechanisms; a dose-dependent mechanism through HCA consumption and an idiosyncratic, dose-independent etiology[78].

One of the most well-known examples of *G. cambogia* associated hepatotoxicity was seen in the weight loss supplement, "Hydroxycut™"[81]. This product was recalled in 2009 after the FDA issued a warning of its potential hepatotoxic effects based on numerous case reports reporting severe hepatotoxicity[81,82]. Andueza *et al*[82] summarized 21 cases of *G. cambogia* related liver injury of which seven were attributed to the use of Hydroxycut. RUCAM was utilized in two of seven cases and was deemed 'highly probable' in both cases with a score of 9. Hydroxycut™ has been newly formulated in the absence of *G. cambogia* and continues to be marketed. Despite the new formulations however, new cases of Hydroxycut-related liver injury continue to be reported. Yousaf *et al*[77] described a tabulated summary of eight reported cases of non-*G. cambogia* containing Hydroxycut induced hepatotoxicity cases from found in 2010-2018. Of the eight reported cases, RUCAM was used in six, with scores ≥ 6[77]. An additional case associated with the use of "Proclinical Hydroxycut™" over a twelve weeks period presented with tremor, progressive fatigue, chest pain and hepatocellular liver injury on laboratory tests. RUCAM was 9, indicating a 'highly probable' causality with this new formulation of Hydroxycut[81].

In addition to the cases of liver injury from Hydroxycut, there have been other notable cases of *G. cambogia*-induced liver injury from other *G. cambogia* containing products this year[77,80,83]. Three recent cases described liver injury related to GC-containing products occurring four weeks to seven months after ingestion[77,80]. Both were in young patients and presented with hepatocellular injury patterns[77,80,83]. It is important to note, however, that the patient presenting seven months after ingestion was also taking GTE[80]. Two of the three patients ultimately required liver transplant due to failed conservative management[80,83]. RUCAM scoring was used in one of three cases, who did not require liver transplant and recovered with conservative management. The RUCAM score in this case was 9, deeming causality 'highly probable'[77].

An additional noteworthy case was the first presentation of *G. cambogia*-induced liver injury with a pattern of AIH. A 39-year-old female presented with jaundice, hepatomegaly and fatigue five weeks after using "slimming tea" containing *G. cambogia*[76]. Liver tests demonstrated a hepatocellular injury pattern with positive ANA and anti-smooth muscle antibodies. A liver biopsy was suggestive of DILI with superimposed AIH[76]. Given these findings, the patient was treated with high-dose prednisone but relapsed after a steroid taper, and was eventually transitioned to chronic immunosuppressive agents. No causality score was presented for this patient.

PES

The use of PES has become a billion dollar industry[84]. Usage of multiple different PES is commonplace, confounding the ability to determine causality in many cases of liver injury[84].

SARMs have become increasingly popular outside the fields of bodybuilding and professional athletics[85]. Their selective tissue effects on muscle and bone allow for the benefit of building muscle mass without unwanted side effects[86,87]. SARMs act intracellularly through the binding of androgen receptors that subsequently regulate the production of androgen genes within the cell's nucleus[87]. Due to these effects, SARMs are being actively investigated in the management of sarcopenia, osteoporosis and profound nutritional deficiency. However, they are not approved by the United States FDA for such uses[87].

In fact, the FDA warns users of such supplements due to their hepatotoxic effects [87]. Several recent reports have described both SARM-induced cholestatic as well as hepatocellular injury, all starting within two weeks to four months after ingestion[86-89]. The SARMs described in these cases were Ligandrol (Alpha Elite), RAD-140 (Alpha Bolic) and enobosarm[86-89]. Liver enzymes improved with conservative management in all cases. In the cases described by Flores *et al*[89], liver injury was related to Ligandrol and RAD-140, presenting five weeks and four months respectively after initial ingestion. Laboratory findings were consistent with hepatocellular and hepatocellular-cholestatic injury respectively. RUCAM scoring deemed both cases as 'probable'. RUCAM was not used in the other cases, with causality determined simply by ruling out viral, autoimmune and possible other medication-induced liver injuries[86-88].

Stimulant workout supplements have also been implicated in DSLI[90-92]. These mixtures may vary in concentrations of ingredients or contain undeclared active ingredients that can result in harm[90]. Eiswerth *et al*[91] described a case of hepatocellular liver injury in a previously healthy 38-year-old male after using a popular pre-workout brand "Bucked Up." It is thought the component "deer antler extract," which contains insulin-like growth factor, was the culprit for such injury[8]. Liver enzymes were shown to downtrend with supportive care[90]. A RUCAM causality score was deemed 'probable' with a score of 7[91].

Two additional cases of pre-workout PES-induced liver injury were reported in previously healthy young adults[90,92]. In one case, the patient was found to have a cholestatic injury pattern[92]. He admitted to taking creatine, whey protein powder and "Mr. Hyde" pre-workout, containing the ingredient theacrine which was thought to be the cause of liver injury[92]. Indeed, rats exposed to theacrine in high concentrations demonstrated centrilobular hepatocellular necrosis[92]. Additionally, the co-ingestion of caffeine, which is also found in "Mr. Hyde" pre-workout, has been shown to increase the bioavailability of theacrine, potentially raising serum concentrations to hepatotoxic levels[92]. The other case described hepatocellular liver injury after ingesting of "Dust V2" pre-workout consistently for four months [90]. While the patient's liver enzymes declined with conservative management, his clinical course was further complicated by severe aplastic anemia two months after the initial presentation requiring hematopoietic stem cell transplant[90]. RUCAM was not used to assess causality in either of these two cases.

Additional brief reports of DSLI noted on our literature review included usage of creatine and glutamine powder[84,93].

TCM

TCM aims to establish and maintain balance in patients through acupuncture, massage, tai chi, and herbals, and its influence continues to grow[94]. In 2019, TCM was officially recognized by the World Health Organization[95]. TCM is included in Chapter 26 of the 11th ICD, set to roll out in 2022, which will broaden its reach worldwide[96]. However, controversy exists over this decision, as some clinicians argue it is dangerous to perpetuate practices that are not evidence based[97].

Our search yielded 264 results on TCM published during 2020. The interested reader is referred to a comprehensive review by Pan *et al*[98] emphasizing the complexity of TCM agents and their mechanisms for hepatotoxicity. We will highlight a few examples of TCM liver injury.

P. multiflorum

P. multiflorum is a commonly used and widely researched herbal within TCM, with its major active ingredients being stilbene glucosides and anthroquinones[99]. Although believed to have therapeutic effects on the liver, it is also a known hepatotoxin and is the only TCM listed on LiverTox with a likelihood score of A[49]. Much of the current literature on *P. multiflorum* induced liver injury is focused on its mechanism of toxicity. Li *et al*[99] argue that *P. multiflorum* liver toxicity is idiosyncratic and immune-mediated, rather than direct as previously proposed in the literature. Zhang *et al*[100] conducted a prospective study using metabolomics to examine serum samples, and identified 25 metabolites that could distinguish between groups susceptible to or tolerant of *P. multiflorum* induced liver injury. In another study investigating risk factors for *P. multiflorum*-induced hepatotoxicity, Yang *et al*[101] identified HLA-B35:01 as a potential susceptibility factor.

San-Qi and G. japonica

San-Qi is a TCM that is used for hemostasis and to treat trauma and ischemic

cardiovascular disease, with the main component being *Panax notoginseng*[102]. Two other herbals, both called Tu-San-Qi, one of which contains the pyrrolizydine alkaloid (PA)-producing *G. Japonica* and the other is *Sedum aizoon* (*S. Aizoon*), which does not produce PAs[102]. *G. Japonica* and *S. Aizoon* are also known to induce blood flow and detumescence as well as treat pain. The similarity of the names has led to confusion with regard to usage which has led to cases of liver injury, as PAs are known hepatotoxic agents, specifically causing hepatic sinusoidal obstruction syndrome (HSOS). A review by Zhu *et al*[102] identified 2156 incidences of Tu-San-Qi induced HSOS. While the authors used the 'Nanjing Criteria', developed by the Hepatobiliary Diseases Committee of the Chinese Society of Gastroenterology, to evaluate PA-induced HSOS, it is unclear how causality was determined for the patients identified in this study. Furthermore, the authors conceded that in many of the cases, they did not specify which agent was included in the specific formulation of Tu-San-Qi.

Bu Gu Zhi and Psoralea corylifolia

Bu Gu Zhi (BGZ) is a TCM used to treat osteoporosis, and the main ingredient is *Psoralea corylifolia* (*P. corylifolia*). In a retrospective review conducted by Wang *et al* [103], 40 cases of BGZ-induced liver injury were identified at a single hospital in Beijing. Causality was determined using presence of clinical symptoms, namely decreased appetite, dark urine, and fatigue, as well as liver enzyme abnormalities, 92% of which were consistent with hepatocellular injury. Zero patients died or required liver transplantation. This is the first study of this size examining BGZ-induced liver injury.

Rhubarb

Rhubarb, also known as dahuang in TCM, possesses anti-inflammatory properties through its anthraquinones, specifically rhein, emodin, aloe-emodin[104]. Zhuang *et al* [104] reviewed the literature on the dual protective and toxic properties of rhubarb on the liver, and concluded rhubarb's hepatotoxicity increases with higher doses and less processing of the product. More studies are required to make definitive conclusions regarding rhubarb's effect on the liver.

Ayurveda

Ayurveda is another form of ancient medicine, and while not as mainstream in the United States, interest in the field is growing. The practice of Ayurveda comes from India and is based on balancing the five elements to optimize bodily humors[105].

Assessing HILI due to Ayurvedic medication is difficult because labeling of ingredients is often incomplete or incorrect. In one case series, a woman is described to have developed acute liver injury after consumption of a combination powder medication called "puriyas" prescribed by a local healer[106]. When Ayurvedic ingredients are identified, the literature commonly describes ashwagandha, brahmi/gotu kola, turmeric, guggul, bakuchi, Indian senna, aloe vera, Indian mulberry, pyrrolizidine alkaloids[107].

In their case series, Karousatos *et al*[108] present three patients with HILI from three different Ayurvedic preparations. The medications presented were Giloy kwarth containing the hepatotoxic *Tinospora cordifolia*, followed by a combination of Manjishthadi kwatham and Aragwadhi kwatham, containing 52 and 10 individual plant extracts with 23 and nine known hepatotoxins, respectively, and finally Kanchnar guggulu, comprised of 10 individual plant extracts of which nine are known hepatotoxins. The individual RUCAM scores for each product ranged from 7 to 8, indicating 'probable' HILI. The complexity of these preparations highlights the need for clinician awareness with regard to HILI from Ayurveda.

Turmeric

Turmeric has been suggested to have hepatotoxic effects through its active ingredient of curcumin. Lombardi *et al*[109] published a series of cases of acute liver injury in Tuscany following ingestion of turmeric, using RUCAM to establish a causal relationship that was supported by a positive de-challenge response in six of seven 'possible' and 'probable cases, although the actual RUCAM scores were not provided. A systematic review identified 23 cases of 'possible' to 'probable' turmeric-induced liver injury, but the majority of patients had a concomitant exposure to another medication. A case reported by Lee *et al*[110] described a patient who developed AIH following turmeric ingestion, established using a RUCAM of score 9, indicating turmeric was 'highly probable'. This patient also was using piperine, and the authors propose the combined use of turmeric and piperine increased the absorption of

turmeric and increased the risk for liver injury.

Ayurveda and autoimmune hepatitis

Ayurvedic medicine has also been shown to worsen liver injury in patients with existing liver disease. In their single-center case-control study, Philips *et al*[111] found that in patients diagnosed with AIH who are treated with Ayurvedic and herbal medicines, defined in this study broadly as complementary alternative medicine, had significantly worse biomarkers and changes on pathology, leading to reduced short-term survival compared to those who were treated with conventional medicine. Specifically, patients treated with polyherbal Ayurvedic compounds, which comprised the majority of complementary and alternative medicine (CAM) therapy employed, displayed significantly higher Child-Pugh, chronic liver failure, and discriminant function scores. When comparing the two groups at the end of one-, three-, and six-month follow-up periods, authors found a significantly higher mortality among CAM patients, with sepsis the most common cause of death in both groups. Authors also identified the contamination of the CAM compounds with heavy metals, antibiotics, chemotherapy agents, nonsteroidal anti-inflammatory drugs, alcohols, antidepressants, anxiolytics, and recreational drugs.

OTHER HERBAL AND DIETARY SUPPLEMENTS

Khat

Khat is an herbal stimulant originating in Ethiopia and used in Eastern Africa, Somalia, and Yemen that can be chewed, ingested, or smoked[112]. The main active components are cathine and cathinone. A number of case reports depicting khat-induced liver injury have been published, but Argueta *et al*[113] present the first case of hepatotoxicity due to khat in the United States. The patient was a 28-year-old man from Yemen who presented with hepatotoxicity in the setting of regular recreational khat use until one week prior to presentation. The authors identified abnormal liver enzymes consistent with hepatocellular injury. Cessation of Khat resulted in clinical improvement, indicated a positive de-challenge response which was the basis of causality as RUCAM scoring was not mentioned. Of note, American clinicians are likely unfamiliar with the presentation of Khat, as it is illegal in the United States.

Skullcap

Skullcap comes from the root of *Scutellaria baicalensis* and is commonly used in TCM. There are previously published case reports of skullcap causing liver injury through the active ingredient wogonin. Skullcap has a designated LiverTox likelihood score of B[114]. Puri *et al*[115] imply that these case reports may have overstated the hepatotoxic potential of skullcap, as patients were all concurrently at least one other HDS with established association with hepatotoxicity, and conducted their own prospective study to test their hypothesis. They found that skullcap ingestion did not result in significant liver enzyme abnormalities or hepatic dysfunction.

Black cohosh and arborvitae

Black cohosh, from *Cimicifuga racemose*, is a well-established hepatotoxin with greater than 50 cases reported cases[116]. It is native to North America and is used to treat menopausal symptoms[117]. Recent studies have investigated the effect of adding black cohosh to clomiphene to treat infertility[118,119]. Black cohosh's main active ingredients are glycoside and terpene. Arborvitae or white cedar, from *Thuja occidentalis (T. occidentalis)*, is a tree native to North America and is used to treat respiratory infections, uterine malignancy, amenorrhea[120]. Unlike black cohosh, arborvitae has not been described in the literature as a hepatotoxin. Arborvitae's main active ingredient is thujone. Caruntu *et al*[120] present a case of a 40-year-old female from Bangladesh and living in the United States who concomitantly used black cohosh and arborvitae to increase her fertility. The combination of these herbal supplements was given a RUCAM score of 6, indicating 'probable' HILI. Both agents were discontinued at the same time, neither were re-challenged, and the patient showed clinical improvement. Thus, it is impossible to determine if the liver injury was caused entirely by black cohosh or if arborvitae also contributed. As such, clinicians should remain aware of the possibility of hepatotoxicity from arborvitae use.

HERBAL HEPATOCYTOPROTECTION

A significant number of review articles were identified in the past year dealing with the potential protective effects of herbals on the liver. The majority of reports found however, were conducted either using *in vitro* or *in vivo* rat models. In order to provide the most relevant information to clinical practice we focused only of those herbals utilized in human studies, in particular, silibinin (milk thistle) and N-acetylcysteine (NAC) to prevent anti-tuberculosis medication liver injury and vitamin E to protect against methotrexate DILI.

Mycobacterium tuberculosis continues to be the leading cause of infection related mortality amongst adults worldwide[1]. The mainstay of treatment consists of quadruple therapy [isoniazid (INH), pyrazinamide (PZA), ethambutol and rifampin] for two months followed by rifampin and isoniazid for the remaining four months [121]. Despite adjustments in duration of treatment, the hepatotoxic effects of PZA, INH and rifampin limit their use, leading to therapy discontinuation in approximately 11% of patients[122,123]. The mechanism of hepatotoxicity of PZA and INH is thought to stem from oxidative injury and production of toxic metabolites[122]. Rifampin upregulates hepatic microsomal enzymes accelerating INH metabolism, increasing toxic metabolites thus increasing risk of liver injury[123].

Silibinin (milk thistle) is a TCM flavonoid derived from the extract of the plant *Silybum marianum*[123]. Goh *et al*[122] investigated silibinin's hepatoprotective role against INH, PZA and combination regimen with *in vitro* assays as a prophylactic agent (prior to anti-TB treatment), rescue agent (given with anti-TB treatment), and as a salvage agent (given after onset of hepatotoxicity). They found that silibinin was most effective as a rescue agent by way of reducing intracellular levels of oxidative stress and oxidative damage to intracellular targets and mitochondria, leading to decreased apoptotic activity[122]. Silibinin was not effective as a prophylactic or salvage agent. Additionally, it was found that silibinin was more protective against INH alone compared to PZA or combination regimens suggesting that silibinin does not protect against PZA-induced hepatotoxicity[122].

Additionally, Singh *et al*[2] performed a systematic review of randomized control trials of chemoprophylaxis in the setting of four-drug regimen anti-tuberculosis treatment. They identified four trials utilizing silymarin/silibinin and three trials utilizing NAC[2]. Only one of four trials demonstrated clinically significant cytoprotection. The study in question, however, was shown to have insufficient power and was stopped prematurely for safety concerns[2]. These findings are concordant with the study performed by Goh *et al*[122] in which silibinin showed protection against INH, but not PZA. NAC however, showed clinically significant cytoprotection in all three studies reviewed. Its hepatoprotective effect is thought to stem from the increase in glutathione, protecting the liver against oxidative stress[2].

Sanjay *et al*[123] studied gallic acid, an Ayurvedic herbal medicine that is present in various fruits and vegetables in the setting of INH and rifampin DILI in Wistar rat models. Gallic acid was co-administered with INH and rifampin and was compared to both negative control and positive control (silymarin treated) models[123]. Gallic acid demonstrated a hepatic protective effect with co-administration and was comparable to the protective effect of the silymarin treated group[123]. Mechanism of action was attributed to gallic acid's antioxidant properties by increasing expression and activation of Nrf2[123].

Vitamin E and methotrexate

Methotrexate is one of the main treatments used in rheumatoid arthritis[124]. However, long term use has been associated with the development of fatty liver disease, fibrosis and cirrhosis[125]. As a result, it is often discontinued when aminotransferases reach 3× upper limit of normal (ULN) or remain persistently above 2× ULN[124]. Vitamin E has been studied for its beneficial effects in patients with non-alcoholic fatty liver disease (NAFLD), and a systematic review and meta-analysis performed by Amanullah *et al*[126] looked at five randomized controlled trials of adult patients with NAFLD treated with vitamin E that demonstrated biochemical and histological improvement.

Vaidya *et al*[124] performed a prospective open-label case-control study over a six month span evaluating the hepatoprotective effects of vitamin E in the setting of methotrexate use. Prior animal studies have demonstrated vitamin E hepatoprotection against methotrexate[124]. The groups were randomized such that each consisted of their individualized methotrexate regimen, folate 1mg/daily along with dietary and exercise advice to minimize lifestyle induced fatty liver disease. The treatment group received vitamin E 400 mg twice a day while the control group did not. This study also

included a crossover design in which the control group individuals that were shown to have ≥ 1-fold but less than 3-fold rise in aminotransferase levels at the three month follow up visit were then treated with vitamin E. The study found that the vitamin E treated group had a statistically significant reduction in AST/ALT levels compared to controls. Additionally, those individuals who were crossed over to receive vitamin E also demonstrated a statistically significant decrease in AST/ALT. The authors concluded that vitamin E attenuates methotrexate-induced liver injury. A limitation of this study is not knowing if these patients had underlying fatty liver disease prior to methotrexate initiation.

Numerous additional studies were identified that investigated the hepatoprotective effects of many other herbal medications for their antioxidant, anti-inflammatory and anti-apoptotic roles. These studies were largely conducted through *in vitro* or *in vivo* rat models as mentioned above. The individual studies that may be of interest to the reader include polyphenols and acetaminophen (APAP)[127,128], Gamisou-san and APAP[129], Lycopene and tamoxifen[130], and licorice and cisplatin[131]. Additional herbal agents were identified as cytoprotective after induction of liver injury by carbon tetrachloride or APAP[132-142].

HILI MISCELLANY

Psoralen and APAP-induced toxicity

Psoralen, an organic compound found in the seeds of *P. corylifolia*, is known for its photosynthesizing properties used to treat psoriasis and vitiligo. Unfortunately, it has also been implicated in hepatotoxicity and is one of the key ingredients responsible for liver injury in the popular TCM, buguzhi. Britza *et al*[143] conducted an *in vitro* study using a line of liver carcinoma cells and showed that psoralen exacerbates APAP hepatotoxicity. Interestingly, when non-toxic doses of psoralen and APAP were concurrently applied to the cell cultures, they synergistically induced liver injury. These findings have yet to be applied to *in vivo* animal models.

Selenium

Selenium is a trace element abundant in brazil nuts and fish and believed to protect against oxidative stress and infection[144]. In a cross-sectional study conducted by Aktary *et al*[145], a negative association was observed between selenium intake and the presence of NAFLD in a Canadian cohort. Similar findings suggesting selenium's hepatoprotective effect was seen in multiple rat models[146,147]. However, in a population-based study in China, Wu *et al*[148] found a significant association between dietary selenium intake and the presence of NAFLD, consistent with a dose-response relationship. Our understanding of selenium's effect on the liver therefore remains inconclusive.

Usnic acid

Usnic acid derived from lichens is a well-documented agent of liver injury with first reported cases dating to 2000 in relation to the dietary supplement, LipoKinetix[149]. Approximately 21 cases of LipoKinetix-induced liver toxicity were reported leading to one death and one liver transplant[149]. This dietary supplement has since been removed from the market[150]. Usnic acid's known mechanism of liver injury is a dose- and time-dependent manner through decoupling oxidative phosphorylation along with inducing oxidative stress through glutathione depletion[149].

Contaminants

Herbal products are not subjected to the same quality control measures as prescription drugs and such can lead to contaminations and subsequent liver injury. Quan *et al*[4] describes contaminates of herbal products as nonphyto-hepatoxins. These contaminates can be divided into heavy metals, biologic factors, pesticide and herbicidal residue[4]. Of the heavy metal arsenic, mercury, cadmium, nickel and lead are most commonly detected[4]. A study performed by Abualhasan *et al*[151] analyzed 18 green and herbal tea samples. Seven of 18 samples were detected to contain chromium and lead at concentrations above set limits set by WHO. In this study microbial contamination were also detected in six of these seven metal containing samples[151]. These microbial contaminations have been shown to be hepatotoxic through decreasing antioxidation, increasing lipid peroxidation and upregulating apoptotic genes.[4] Additionally, the use of pesticides and herbicides have been shown to cause hepato-

toxicity through hepatic mitochondrial toxicity and obstructive cholestasis[4].

CONCLUSION

HILI continues to be a growing concern for clinicians both in the United States and worldwide. While currently considered a subtype of DILI, differences in composition, application, and outcomes of HDS compared to conventional medications indicate that HILI may deserve to be considered independently.

The lack of HDS regulation in the United States limits our understanding of their potential for hepatotoxicity. Even an accurate estimate of the incidence of HILI is difficult to ascertain, and the frequencies that are reported using registries, single center hospitals, and population-based cohorts, make them difficult to compare.

Moreover, the diagnosis of HILI remains a challenge, and while assessment tools are valuable in determining causality, even the widely applied RUCAM scale - designed to evaluate DILI - falls short of adequately evaluating HILI[31]. Complete data are required for proper utilization of RUCAM, thus highlighting the importance of prospective registries. While imperfect, the RUCAM scale is currently the most widely used tool available, and until a better alternative is developed, we encourage its continued use and refinement to help identify verifiable HILI cases[31]. Development of prospective HILI/DSLI registries in Asia would also improve the overall utility of RUCAM and provide a more reliable and standardized causality scoring system. Future studies in HILI should examine (1) causality assessment scores; (2) clinical significance of using multiple herbal ingredients simultaneously; and (3) prospective studies to better understand incidence in Western countries. By improving assessment tools and expanding the data, advocates may be able to make stronger arguments to regulatory boards in support of consumer protection laws with regard to HDS.

The use of pharmacogenetics has identified susceptibility factors to HILI in the case of GTE and HLA-B *35:01. The search for other associations showing a strong correlation to idiosyncratic HILI is ongoing[41].

The use of herbals in hepato-protection continue to show promising outcomes in preventing and/or attenuating DILI from anti-TB liver injury. Further human clinical trials are still required in order to assess the true therapeutic benefit of cytoprotective herbals in other settings.

Kratom and its legal status will undoubtedly remain a hotly debated topic in coming years, as the opioid epidemic continues. At present, kratom is legal at the federal level, but banned in several states and countries. The literature indicates kratom is potentially lethal, not only through overdose but also by contaminated products, and some degree of regulation certainly seems warranted[63-65,152]. However, it has yet to be determined which end of the spectrum, through a ban or legalization, would best serve consumers.

The highlights of the updated literature over the past year indicate interest in HILI that we expect will continue to increase as the multi-billion-dollar supplement industry in the United States grows.

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MINIREVIEWS

Challenges in the discontinuation of chronic hepatitis B antiviral agents

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Abstract

Long-term antiviral treatment of chronic hepatitis B patients has been proven to be beneficial in reducing liver-related complications. However, lengthy periods of daily administration of medication have some inevitable drawbacks, including decreased medication adherence, increased cost of treatment, and possible long-term side effects. Currently, discontinuation of antiviral agent has become the strategy of interest to many hepatologists, as it might alleviate the aforementioned drawbacks and increase the probability of achieving functional cure. This review focuses on the current evidence of the outcomes following stopping antiviral treatment and the factors associated with subsequent hepatitis B virus relapse, hepatitis B surface antigen clearance, and unmet needs.

Key Words: Viral hepatitis B; Relapse; Retreatment; SCALE-B; Stop treatment strategy; Nucleoside analogs

Core Tip: Stop strategy is one of the options to get closer to functional cure with a finite duration of treatment in chronic hepatitis B patients. Virological relapse and clinical relapse are common after stopping antiviral agent. Half the patients with clinical relapse require retreatment. Novel biomarkers and the SCALE-B score predict clinical relapse and hepatitis B surface antigen clearance. Knowing when to restart treatment and novel sensitive biomarkers are unmet needs.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem globally; approximately 292 million people are affected by this virus[1]. Patients with chronic hepatitis B (CHB) infection are at risk of developing long-term liver-related complications, *e.g.*, cirrhosis, decompensation, and malignant liver tumors[2]. Although the prevalence of CHB infection has declined as a result of immunization programs, the majority of Southeast Asian countries are still categorized as intermediately to highly endemic areas[3]. HBV replication occurs through the formation of covalently closed circular DNA (cccDNA), and the persistence of intrahepatic cccDNA is the major reason for disease chronicity and a major obstacle for the eradication of HBV[4]. However, the measurement of intrahepatic cccDNA is not practical in clinical practice as it can only be done through liver biopsy.

Long-term nucleos(t)ide analogs (NA) inhibit the reverse transcriptase activity of viral polymerase and effectively inhibit HBV replication, reverse liver fibrosis, and reduce the risk of hepatocellular carcinoma (HCC)[5,6]. However, NA have no direct effect on intrahepatic cccDNA or virus transcription in the liver. Therefore, because functional cure, defined as hepatitis B surface antigen (HBsAg) clearance with or without anti-HBs seroconversion, is not often achieved, and most patients need long-term or even lifelong NA therapy[7].

Currently, the best time to stop NA therapy before HBsAg clearance is still uncertain because of the high rates of nontreatment recurrence. For instance, the pooled analysis of a systematic review showed a virological relapse (VR) rate of about 50% to 60% within 12 to 36 mo after drug withdrawal[8]. Although recent clinical guidelines suggest that some patients may stop taking NA before achieving HBsAg serum clearance[9-11], sensitive and reliable biomarkers for identifying patients with low recurrence risk have not yet been established[12,13]. This review focuses on both benefits and risks of discontinuing antiviral agents, as well as the current recommendations, factors, and novel biomarkers for predicting outcomes following NA cessation, and unfulfilled demands.

ADVANTAGES *VS* DISADVANTAGES OF ANTIVIRAL AGENT DISCONTINUATION

Benefit and risk concerns of CHB antiviral cessation are summarized in Figure 1.

Advantages

Increased HBsAg loss: The ultimate goal of CHB treatment is clearance of intrahepatic cccDNA. Nonetheless, this endpoint seems to be unrealistic with the current treatment options[9-11]. A more pragmatic endpoint is HBsAg loss with undetectable HBV DNA or a so called "functional cure," yet HBsAg loss is rarely achieved with long-term NA therapy. In a French study of 18 CHB patients with NA treatment, the annual decrease of HBsAg levels was only 0.084 $\log_{10}$ IU/mL[14], with a study-derived model predicting that HBsAg loss after continuous treatment with NA would be achieved in 52.2 years.

On the other hand, cessation of NA therapy may increase HBsAg clearance. An initial study by Hadziyannis *et al*[15] showed a high rate of HBsAg loss of 39.4% at 6 years after stopped adefovir (ADV) in hepatitis B e-antigen (HBeAg) negative CHB patients. That study was followed by a peak of interest in NA discontinuation[15]. A recent systematic review including 1085 patients reported a rate of HBsAg loss of approximately 8%[8]. In contrast, a subsequent study reported HBsAg loss in a minority of patients on continuous NA therapy, approximately 2.1% after 10 years of follow-up[16].

Finite duration: Generally, long-term treatment with NA is required, in contrast to the definable duration of interferon-based therapy, 12 mo in HBeAg-negative, and 6-12 mo in HBeAg-positive patients[17]. Even though the side effects after several years of medication are very few, they can be problematic in real-life practice. An attempt to define a limited duration of NA therapy was first proposed in the Asian Pacific Association for the Study of the Liver (APASL) 2008 guidelines[18]. Finite duration may increase drug adherence, lower the chances of developing side effects from the drug, and reduce costs[19].

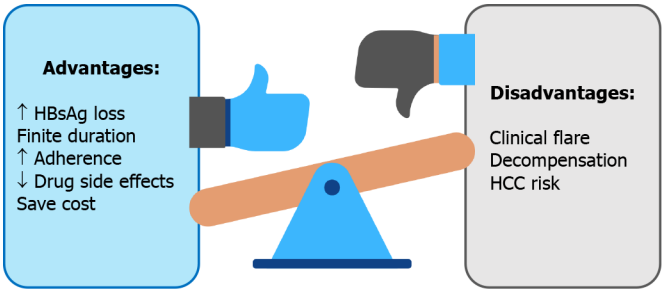

Figure 1 Advantages *vs* disadvantages of antiviral agent discontinuation in chronic hepatitis B. HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma.

Increased adherence: Longer use of NA treatment is associated with lower medication compliance. Drug adherence is of concern in real-life practice[20]. Poor antiviral agent compliance is associated with emerging resistance, particularly in agents with a low genetic barrier[21]. A large retrospective study that included 11,100 CHB patients in the United States found a rate of adherence of 87%[20]. Moreover, a systematic review and meta-analysis included of 30 studies reported that the long-term adherence rate was only 74.7% after a median follow-up of 16 mo[22]. Notably, it was suboptimal compared with a good adherence rate of 95% defined in previous studies[20,23-25]. Compliance to antiviral agent use may improve with finite duration of treatment.

Decreased side effects: A recent systematic review indicated adverse events associated with NA were not common. However, some events were fatal, especially mitochondrial toxicity[26]. Long-term treatment with NA potentiates renal and bone side effects, particularly in tenofovir disoproxil fumarate (TDF) and ADV users. In addition to the well-known side effects of tubular dysfunction and Fanconi syndrome associated with TDF and ADV, the real-world data also found that the estimated glomerular filtration rate (eGFR) declined more quickly in TDF and ADV users than in untreated CHB patients[27]. Despite the observation in recent registration trials that tenofovir alafenamide (TAF) had significantly lower rates of bone mineral density and eGFR reduction compared with TDF[28,29], making it is safer for long-term use, the reported safety data were from follow-up of no longer than 96 wk[30]. Therefore, whether TAF is truly safe for extended treatment is yet to be confirmed. Nonetheless, as shorter time of exposure to NA would decrease the risk of side effects.

Cost savings: As mentioned above, hepatitis B treatment with NA might be a long-term therapy. According to a survey in Singapore, fewer than half the patients preferred lifelong treatment[31]. One of the most concerns of lifelong therapy is the cost of treatment. Moreover, only about a quarter of the patients were willing to pay for lifelong therapy, with an acceptable daily cost of 8 United States dollars.

Disadvantages

Clinical flare and decompensation following off-therapy: The concerning issue after NA discontinuation is HBV flare, especially clinical relapse (CR). Most studies defined CR as an off-therapy HBV DNA > 2000 IU/mL plus an alanine aminotransferase (ALT) level > 2 times the upper limit of normal (ULN)[8,32]. The overall CR rate from a pooled data analysis with a follow-up ranging from 12-69 mo duration after NA discontinuation was 34.6% in which CR was higher in HBeAg-negative patients (43.7%) than in HBeAg-positive (23.8%)[8]. CR, particularly severe CR, may lead to jaundice, prolonged prothrombin time (PT), or eventually liver failure. In our study in Thai patients, two noncirrhotic HBeAg-negative patients developed jaundice (classified as severe CR) 3 mo after NA discontinuation[12]. Jaundice and hepatitis resolved in both patients after retreatment. Clinical decompensation and death following NA discontinuation has been reported in Asian studies; decompensation and fatality were observed in 0%-1.58% and 0%-0.19% in noncirrhotic patients at 1-3 years of follow-up, while there was a limited number of studies in cirrhotic patients [33-35]. The annual incidence of liver decompensation and death were recently reported to be 2.95% and 1%, respectively in cirrhotic patients who stopped NA[33]. Of interest, ENUMERATE study of the patients in the United States reported hepatic decompensation in five of 61 entecavir-treated patients (8.2%) after a median follow-up of 4 years[36]. Although not from a head-to-head comparison, the data are of

concern because liver decompensation in cirrhotic patients who stopped NA therapy seems to be numerically higher than in those who continued treatment. Thus, the current evidence indicates a need for vigilance after NA discontinuation in cirrhotic patients.

HCC risk: There are several well-known benefits of NA treatment in CHB patients[9-11]. Antiviral therapy with NA results in viral suppression, fibrosis improvement, and lower risk of HCC development[37]. Whether patients who stop NA will experience an increased occurrence of HCC in the future than those with continuous treatment is not clear. Nevertheless, to date, HCC development in patients who discontinued NA is not significantly higher than in those who continued NA treatment[33].

GUIDELINE RECOMMENDATIONS

Currently, international practice guidelines for CHB management suggest that patients who had consecutive findings of undetectable HBV DNA for a certain duration can stop NA[9-11]. The expert consensus from the APASL first mentioned treatment discontinuation in 2008, advocating that NA therapy can be stopped in selected patients because of drug resistance concerns in long-term NA treatment[18]. The latest recommendations from international hepatology societies for considering stopping NA therapy are shown in Table 1.

In HBeAg-positive CHB patients, all guidelines allow NA discontinuation in patients who develop HBeAg seroconversion with persistent normal ALT levels and undetectable HBV DNA following consolidation therapy after e-seroconversion for at least 12 mo[38] or preferably 3 years in the APASL guidelines[11]. For patients who are HBeAg-negative, the APASL guideline states that NA can be withdrawn in noncirrhotic patients after treatment for at least 2 years, with an undetectable HBV DNA documented on three consecutive visits, 6 mo apart, or until HBsAg loss with or without development of anti-HBs[11]. Likewise, the European Association for the Study of the Liver (EASL) allows stopping NA in highly selected patients with 3 years of continuously suppression of HBV DNA in noncirrhotic patients[9]. On the contrary, the American Association for the Study of Liver Diseases (AASLD) recommend continuing NA treatment indefinitely unless HBsAg loss is achieved[10]. In patients with liver cirrhosis, the APASL recommends that the discontinuation of NA might be considered, but only with close monitoring[11].

HBV RELAPSE AND PREDICTIVE FACTORS

HBV relapse is a common event after NA discontinuation and can be simply categorized into virological relapse (VR) and CR. Most of the studies defined HBV DNA greater than 2000 IU/mL as the definition of VR, and when in combination with an ALT of at least two times the ULN, CR is recognized. A systematic review by Papatheodoridis *et al*[8], reported VR rates of 51.4% and 38.2% at 1- and 3-year, respectively, after NA discontinuation. The occurrence of VR was higher in HBeAg-negative patients than in HBeAg-positive patients. The rates were 56.3% *vs* 37.5% and 69.9% *vs* 48.5% at 1- and 3-year, respectively. VR commonly occurred when NA was stopped, but VR alone might not have a clinically significant impact. In some patients, VR may be transient, with a spontaneous decline of viral replication resulting from an immune response. In contrast, a CR may require initiation of retreatment, or more importantly lead to severe flare, and hepatic failure. A randomized controlled study by Liem *et al*[39] reported that half the patients developed CR after NA discontinuation[39]. Consequently, three-quarters of the patients required retreatment. A summary of the studies reporting the occurrence of VR, CR, and HBsAg loss after NA discontinuation is shown in Table 2.

Various baseline and on-treatment factors are associated with VR off-therapy patients. At pretreatment, the baseline characteristics of increasing age and male sex have been associated with an increased relapse rate[40]. During treatment, extension of consolidation treatment duration by more than 1 to 3 years reduces the risk of VR in both HBeAg-positive and HBeAg-negative patients[38]. For that reason, the international guidelines recommend at least 1 year of consolidation therapy, and preferably 3 years in the APASL guidelines[11], after HBeAg seroconversion before considering NA discontinuation in HBeAg-positive patients. Moreover, the end of treatment (EOT) HBsAg level is highly predictive of HBV relapse, a higher level is correlated with a

Table 1 Guidelines for stopping nucleos(t)ide analog therapy

Guidelines	HBeAg-positive CHB	HBeAg-negative CHB
APASL 2015 [11]	HBeAg seroconversion: + undetectable HBV DNA + normal ALT for ≥ 12 mo (or preferably 3 yr). Cirrhotic patients may be stopped with careful monitoring	Undetectable HBV DNA at least 2 yr with documented on three separate occasions, 6 mo apart: Or HBsAg clearance either at least for 1 yr; Or until anti-HBs seroconversion. Cirrhotic patients may be stopped with careful monitoring
AASLD 2018 [10]	HBeAg seroconversion + undetectable DNA + normal ALT for ≥ 12 mo. Not recommended in cirrhosis	HBsAg clearance. Not recommended in cirrhosis
EASL 2017[9]	HBeAg seroconversion + undetectable DNA for ≥ 12 mo. Not recommended in cirrhosis	HBsAg clearance. Or selected noncirrhotic with undetectable HBV DNA ≥ 3 yr. Not recommended in cirrhosis

AASLD: American Association for the Study of the Liver; ALT: Alanine aminotransferase; APASL: Asian Pacific Association for the Study of the Liver; CHB: Chronic hepatitis B; EASL: European Association for the Study of the Liver; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

higher HBV relapse rate[40,41].

From our point of view, the CR is more clinically important than VR, as it may be followed by liver-related complications. A study in a Thai cohort demonstrated that EOT hepatitis B core-related antigen (HBcrAg) and HBV RNA level were independent risk factors for the subsequent development of CR[12]. A recent meta-analysis including 1573 patients indicated that the higher pretreatment HBsAg levels were associated with shorter consolidation duration and the higher EOT HBsAg levels, especially those > 1000 IU/mL, were independently associated with CR[33]. Many studies attempted to find factors associated with VR and CR, and the reported results are summarized in Table 3.

HBsAg CLEARANCE AND PREDICTIVE FACTORS

HBsAg clearance is the desired goal of hepatitis B treatment. Nonetheless, as mentioned above, even if possible, it seldom occurs while on NA treatment, and stopping NA may be a strategy to increase the chance of HBsAg loss. A pivotal Greece study with 33 genotype D, HBeAg-negative patients who stopped ADV, HBsAg loss occurred in 13 of 33 patients (39.4%) after a 6-year follow-up[15]. In addition, the first small randomized controlled trial (RCT) from Germany reported HBsAg clearance in 4 of 21 HBeAg-negative CHB patients after 3-year of off-therapy[42]. However, another RCT conducted by Liem *et al*[39], in which the majority of the patients were Asian, HBsAg loss occurred in only one patient 1.5 years after NA cessation[39]. Ethnicity and HBV genotype may affect the rate of HBsAg loss.

A large retrospective Taiwanese study that included 691 patients, demonstrated a shorter time to undetectable HBV DNA (especially if assayed less than 12 wk after NA initiation), on-treatment reduction of HBsAg level of > 1 $\log_{10}$ IU/mL, and an EOT HBsAg level of < 100 IU/mL were independently associated with an increase in the likelihood of off-therapy HBsAg loss[33]. Furthermore, lower pretreatment ALT and HBV DNA levels, lower EOT HBsAg level, and longer treatment duration predicted HBsAg loss in another study[40]. The predictive factors for HBsAg loss in off-therapy patients are summarized in Table 4.

NOVEL BIOMARKERS TO PREDICT HBV RELAPSE AND HBsAg CLEARANCE

HBsAg quantification

Quantitative serum HBsAg (qHBsAg) has been around in the management of CHB for a while. In untreated patients, serum HBsAg quantification can help to define disease stage, predict spontaneous HBsAg clearance, and predict long-term liver-related complications[43]. As qHBsAg has been used in the clinical practice nowadays, commercial assay kits are widely available. There is increasing evidence of qHBsAg as a marker to aid physicians in deciding whether to discontinue NA. A Taiwanese study by Chen *et al*[40] found that a cutoff level of < 120 IU/mL predicted HBsAg clearance in HBeAg-negative patients and < 300 IU/mL in HBeAg-positive, respectively[40]. A systematic review by Liu *et al*[41] indicated that an EOT HBsAg level < 100 IU/mL

Table 2 Off-therapy virological relapse, clinical relapse, and hepatitis B surface antigen loss in chronic hepatitis B patients

Ref.	Country	*n* (%)	HBeAg-negative, *n* (%)	Follow-up time (mo)	Virological relapse rate (%)	Clinical relapse rate (%)	HBsAg loss, *n* (%)
Fung *et al*[67], 2004	Canada	27	27	18	44.4	25.9	NR[1]
Enomoto *et al*[68], 2008	Japan	22	22	48	68.7	68.7	NR
Yeh *et al*[69], 2009	Taiwan	71	0	15	26.8	26.8	0
Fung *et al*[70], 2009	Hongkong	22	0	20	63.6	31.8	NR
Wang *et al*[71], 2010	China	125	125	24	30.4	NR	NR
Kuo *et al*[72], 2010	Taiwan	124	0	> 12	66.1	66.1	NR
Cai *et al*[73], 2010	China	11	0	22	42.8	0	NR
Liu *et al*[74], 2011	China	61	61	15	50.8	45.9	8/61
Jung *et al*[75], 2011	South Korea	19	9	12	31.6	21	0
Chan *et al*[76], 2011	Hongkong	53	53	47	69.8	NR	9/53
Liang *et al*[77], 2011	Hongkong	84	43		44	14.3	NR
Chaung *et al*[78], 2012	United States	39	0	14	89.7	38.5	0
Hadziyannis *et al*[15], 2012	Greece	33	33	69	45.4	45.4	13/33
Ha *et al*[79], 2012	China	145	145	16	65.5	64.1	NR
Song *et al*[80], 2012	South Korea	48	0	18	41.6	NR	NR
He *et al*[81], 2013	China	66	66	17	28.8	NR	2/66
Kim *et al*[82], 2013	Korea	45	45	26	73.3	53.3	NR
Jeng *et al*[83], 2013	Taiwan	95	95	> 12	57.9	45.3	0/95
Kwon *et al*[84], 2013	South Korea	16	NR	32	25	25	2/16
Ridruejo *et al*[85], 2014	Argentina	35	0	15	25.7	NR	18/35
Sohn *et al*[86], 2014	South Korea	95	54	22	83.1	NR	0/95
Patwardhan *et al*[87], 2014	United States	33	33	36	63.6	48.5	0/33
He *et al*[88], 2014	China	97	0	32	8.2	1	11/97
Chen *et al*[40], 2014	Taiwan	188	105	49	66.5	NR	33/185
Jiang *et al*[89], 2015	China	72	39	13	65.3	41.7	NR
Seto *et al*[90], 2015	Hongkong	184	184	12	91.8	22.8	0
Peng *et al*[91], 2015	China	65	21	12	43.1	27.7	1/65
Jeng *et al*[92], 2016	Taiwan	85	85	155	69	52	2/85
Qiu *et al*[93], 2016	China	112	0	52	48.2	NR	1/112
Yao *et al*[94], 2017	Taiwan	119	119	6 yr	25.2	12.7	44/119[2]
Cao *et al*[95], 2017	China	82	22	91	70.7	34.1	5/82
Chen *et al*[96], 2018	Taiwan	143	104	104	67.1	48.9	7/143
Hung *et al*[97], 2017	Taiwan	73	73	6 yr	54.8	6.8	20/73
Berg *et al*[42], 2017	German	21	21	144	52	23	4/21
Jeng *et al*[33], 2018	Taiwan	691	691	6 yr	79.2	60.6	42/691
Liem *et al*[39], 2019	Canada	45	27	72	71	13	1/45
Kaewdech *et al*[12], 2020	Thailand	92	70	48	63	33.7	2/92

[1]Not reported.
[2]All patients had hepatitis B surface antigen level < 200 IU/mL at the end of treatment.
EOT: End of treatment; HBsAg: Hepatitis B surface antigen; NR: Not reported.

Table 3 Factors predictive of hepatitis B virus relapse

Baseline at pretreatment	On-treatment	End of treatment
Virological relapse		
High age[40,44]	Short consolidation duration[38]	High HBsAg level[40,41]
Male sex[40]		High HBcrAg level[12]
High HBsAg level[44]		High HBV RNA level[12]
Clinical relapse		
High HBsAg level[44]	Short consolidation duration[44]	High HBsAg level[13,40,41]
		High HBcrAg level[12,13,52]
		High HBV RNA level[12,52]

HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

Table 4 Factors predictive of hepatitis B surface antigen clearance

Baseline at pretreatment	On-treatment	End of treatment
Low ALT level[40]	Long treatment duration[40]	Low HBsAg level especially < 100 IU/mL[41]
Low HBV DNA level[40]	HBsAg level reduction > 1 $\log_{10}$ IU/mL[33]	Low HBcrAg level[13]

ALT: Alanine aminotransferase; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

was the optimal cutoff[41] to predict low rates of HBV relapse and a high chance of HBsAg loss. A meta-analysis involving 1573 patients found that the same EOT HBsAg level (> 100 IU/mL) was associated with an increased risk of VR and CR, however, it is not predictive of CR in a subgroup of Asian patients[44]. The finding is consistent with our study in Thai patients in which the HBsAg level was not associated with the development of CR. A recent multicenter study by Sonneveld *et al*[13] found that a cutoff level of < 50 IU/mL was the best for predicting a sustained response and HBsAg loss[13]. In conclusion, HBsAg level is a good predictor of HBsAg loss after NA cessation, but its use as a biomarker to predict CR, especially in Asian patients, is still not clear.

HBcrAg level

Serum HBcrAg has emerged as a novel biomarker in CHB patients. Serum HBcrAg measurement is the combined assay of hepatitis B core antigen, HBeAg, and p22 protein, and it has been shown to be a potential surrogate marker of intrahepatic cccDNA[45,46]. In previous Japanese reports, an increased HBcrAg level was associated with an increase in the rate of off-therapy relapse in NA-treated patients[47]. In addition, a multicenter cohort of Taiwanese patients showed that HBcrAg and HBsAg measured at the time of NA discontinuation were predictive of off-therapy relapse [48]. Moreover, data from CREATE project, a multicenter study including both Asian and Caucasian patients, confirmed the utility of serum HBcrAg. The low cutoff of < 2 $\log_{10}$ U/mL was associated with sustained response and HBsAg clearance regardless of HBeAg status and ethnicity[13]. A compilation of the clinical applications of HBcrAg in the cessation of NA is shown in Table 5.

HBV RNA level

Serum HBV RNA is closely associated with the transcriptional activity of intrahepatic cccDNA and can be quantified by polymerase chain reaction-based techniques[31]. Moreover, this novel marker is potentially valuable in monitoring for relapse after NA

Table 5 Hepatitis B core-related antigen level and clinical application

Ref.	*n* (%)	End of treatment HBcrAg level (log_{10} U/mL)	Clinical application
Shinkai *et al*[98], 2006	22	< 3.4	Predictive factor for absence of the off-therapy relapse
Matsumoto *et al*[47], 2007	34	< 3.2	Predictive factor for absence of the off-therapy relapse
Jung *et al*[99], 2016	113	≤ 3.7	Virological relapse within 1 yr of NA cessation
Hsu *et al*[48], 2019	135	NR	Predictive factors of HBsAg loss and lower clinical relapse
Kaewdech *et al*[12], 2020	92	< 3	Low risk of off-therapy relapse
Papatheodoridi *et al*[54], 2020	57	< 2	Predictive factor of HBsAg loss, not required retreatment
Sonneveld *et al*[13], 2020	572	< 2	Higher risk of sustained response and HBsAg loss

ALT: Alanine aminotransferase; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; NA: Nucleos(t)ide analog; NR: Not reported.

discontinuation[49]. A study by Wang *et al*[49] reported that viral rebound occurred in 100% of patients who had detectable HBV RNA at EOT[49]. A recent study in HBeAg-positive patients found that positive serum HBV RNA at EOT was associated with the development of off-therapy CR[50].

The combination of biomarkers

Together, the data suggest that serum qHBsAg, HBcrAg, and HBV RNA, especially at EOT, are predictive of the outcomes following NA cessation. A few studies have explored the usefulness of combining the biomarkers to select the best candidates for stopping NA[12,48,51,52]. A post-hoc analysis from China included 130 CHB patients who discontinued NA and serial followed-up HBV DNA, qHBsAg and HBV RNA[50] found that the combination of negative HBV DNA and HBV RNA at EOT correlated with lower a CR rate and had an excellent 92% negative predictive value (NPV). Another study, combining qHBsAg, and HBcrAg reported that lower qHBsAg, and HBcrAg levels were associated with lower CR and increased HBsAg clearance[48]. Furthermore, a combination of the two biomarkers before stopping NA showed that no patients with negative HBV RNA, and HBcrAg < 4 log_{10} U/mL at EOT developed CR[52]. The result is consistent with that observed in our study of the combination of the three biomarkers, *i.e.* qHBsAg, HBcrAg, and HBV RNA in the prediction of CR after cessation of NA. We found that HBcrAg of < 3 log_{10} U/mL and HBV RNA of < 2 log_{10} U/mL had 100% NPV for CR[12]. Nonetheless, when combining all three biomarkers, the prediction of CR was not better than that with HBcrAg plus HBV RNA [12].

SCORING SYSTEMS TO PREDICT HBV RELAPSE AND HBsAg CLEARANCE

Apart from using only biomarkers, previous studies illustrated that other clinical and laboratory parameters were significantly associated with post off-treatment outcomes. Therefore, the development of scoring systems utilizing various variables to predict HBV relapse and HBsAg clearance is foreseeable. The first score to predict CR after NA discontinuation is the Japan society of hepatology (JSH) score that consisted of the HBsAg level and HBcrAg level at the time of cessation. The JSH scores are divided into low, moderate, and high-risk groups for HBV relapse after NA cessation[53]. However, this predictive score is not widely used outside the country of origin.

The SCALE-B scoring system was developed using data from 135 Taiwanese CHB patients[48]. The score is comprised of the HBsAg level (S), HBcrAg (C), age (A), ALT (L), and tenofovir (E) for HBV (B) and is calculated as HBsAg (log_{10}IU/mL) + 20 × HBcrAg (log_{10}U/mL) + 2 × age (yr) + ALT (U/L) + 40 for the use of tenofovir. The scores are divided into three strata, low (< 260 points), intermediate (260-320 points), and high (> 320 points) risk of CR. A score of < 260 points was associated with a subsequent HBsAg loss in 27.1% of the patients at 3 years[48]. The SCALE-B score has

been validated in a Caucasian population in which it predicted HBsAg clearance, but not relapse[54]. Recently, the CREATE study, which included a large number of Asian as well as Caucasian patients reported that the SCALE-B score predicted CR and HBsAg loss regardless of HBeAg status or ethnicity[13].

IMMUNE SYSTEM EFFECTS AFTER DISCONTINUATION OF ANTIVIRAL AGENTS

T cells contribute to the control of HBV infection by killing infected hepatocytes[55]. However, chronic HBV infection can exhaust immune activity, particularly T cell function[55], as the longer time of HBV infection is associated with the length of exposure to high antigenicity[56]. With NA therapy, T cell function decreases over time. With discontinuation of NA, T cell function may recover with the increase in the number of active T cells and less exhausted phenotypes[57,58].

After the cessation of NA treatment, the HBV DNA usually becomes detectable and often triggers ALT flares that reflect the immune response. Increased numbers of HBV-specific T cells were observed in patients in virological remission after NA discontinuation[59]. A study by Rinker *et al*[58] that high function of HBV-specific T cells was observed after NA cessation in patients with subsequent HBsAg loss, especially HBV-specific CD4* T cells[58]. In addition, T cell function increased after programmed death-ligand 1 blockage. More recently, a study by a Spanish group[60] reported that an HBsAg level of ≤ 1000 IU/mL, lower cccDNA transcriptional activity, and a higher HBV-specific T cell response were associated with the development of HBsAg loss.

A new concept of the immune response after NA cessation, beneficial flare *vs* bad flare is of interest, and was introduced by a Taiwanese group[61]. HBsAg kinetics may be useful in predicting whether patients will require retreatment after CR. Initiation of retreatment is considered in patients who have an increase in HBsAg level before or during ALT flare, which reflects an ineffective immune response. On the other hand, patients in whom a reduction on the HBsAg level was observed before or during ALT flare may not need retreatment, and spontaneous HBsAg clearance may eventually occur[62].

MONITORING /RESTARTING THERAPY AFTER STOPPING ANTIVIRAL AGENT THERAPY

At present, there is no consensus on how to monitor and when to restart NA therapy. Previous studies reported that most HBV relapses occurred within 1 year after the discontinuation of antiviral agents. Most studies recommend careful monitoring, with physical examinations, liver function tests, and serum HBV DNA assays every 1-2 mo for the first 3 mo, every 3 mo for 1 year, and every 6 mo thereafter[12-14,63]. If the patient experiences ALT flare, then close follow-up every week with liver function tests and PT are mandatory for deciding whether prompt retreatment is needed.

Currently, retreatment criteria differ among the studies summarized in Table 6[12,13,39,63]. Most suggested that retreatment should be initiated in patients with an ALT level > 10 times above the ULN regardless of bilirubin level, with an ALT level > 5 times above ULN plus a bilirubin > 1.5-2 mg/dL, persistent of ALT level > 5 times the ULN for 4 wk, or an ALT elevation with either a prolonged PT > 2 sec or a bilirubin level >1.5-2 mg/dL. The retreatment strategy is challenging as CR may reflect the immune restoration and reintroduction of NA might alleviate the effect. However, delayed initiation of retreatment can cause severe ALT flare, and eventually liver decompensation. The biomarkers or tools to aid justification of the optimal timing of retreatment are unmet needs.

PERSPECTIVE OF NA DISCONTINUATION IN EASTERN AND WESTERN COUNTRIES

In Asian and Caucasian populations, there are differences in rates of HBsAg clearance and HBV relapse. Caucasians have a higher probability of achieving a functional cure after NA cessation[13]. HBsAg clearance has been observed in 19%-29% of Caucasians at 2 years[42,64] whereas it had been found in only 1.78%/year in Asians. This

Table 6 Summary of follow-up interval and retreatment criteria

Ref.	Follow-up interval	Criteria of retreatment
Berg *et al*[42], 2017	Every 2 wk in the first 3 mo, every 4 wk until week 48, and every 12 wk thereafter until week 144	Two consecutive total bilirubin > 1.5 mg/dL plus ALT > ULN
		Two consecutive PT ≥ 2.0 seconds (INR ≥ 0.5) prolonged from baseline with adequate vitamin K therapy plus ALT > ULN
		Two consecutive ALT > 10 × ULN
		ALT > 2 × but ≤ 5 × ULN persisting for ≥ 12 wk plus HBV DNA > 20000 copies/mL
		ALT 5 × but ≤ 10 × ULN persisting for ≥ 4 wk
Papatheodoridi *et al*[63], 2018	Every mo in the first 3 mo then at least every 3 mo until month 12	Greece cohort: (1) ALT > 10 × ULN; (2) ALT > 5 × ULN plus total bilirubin > 2 mg/dL; (3) ALT > 3 × ULN plus HBV DNA > 100000 IU/mL; and (4) ALT > ULN plus HBV DNA > 2000 IU/mL on three sequential occasions
		Taiwanese cohort: (1) ALT > 2 × ULN twice 3 mo apart plus HBV DNA > 2000 IU/mL; (2) Total bilirubin > 2 mg/dL; and (3) PT ≥ 3 seconds of control range
Liem *et al*[39], 2019	Wk 4, 6, 12, 18, 24, 36, 48, 60, and 72	HBeAg seroreversion
		HBV DNA > 2000 IU/mL plus ALT > 600 IU/mL
		HBV DNA > 2000 IU/mL plus ALT > 5 × ULN (40 IU/mL) on two consecutive visits
		HBV DNA > 2000 IU/mL plus ALT > 200 IU/mL but < 600 IU/mL for > 6–8 wk
		HBV DNA > 20000 IU/mL on two consecutive visits at least 4 wk apart
García-López *et al*[60], 2020	Monthly in the first 6 mo then every 3-4 mo until 24 mo	Two consecutive ALT > 10 × ULN regardless of HBV DNA level
		ALT > 5-10 × ULN and HBV DNA > 2000 IU/mL persisting for ≥ 4 wk
		ALT > 2-5 × ULN and HBV DNA > 2000 IU/mL persisting for ≥ 6 mo
		Need for immunosuppressive treatment

ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen; HBV: hepatitis B virus; INR: International normalized ratio; PT: Prothrombin time; ULN: Upper limit of normal.

phenomenon might be explained by the difference of HBV genotypes between Asians and Caucasians, and the duration of infectivity. In Asians, the most common genotypes are B and C, in contrast to the Caucasians in which genotype D is more common[65]. Regarding the duration of infection, most Asian CHB patients are infected perinatally, resulting in a longer extent of chronic infection than in Caucasian patients[66]. Therefore, apart from the chance of patients to have drug-free periods, lower long-term side effects and costs, the ultimate benefit of achieving a functional cure after NA cessation is lower in Asians than in Caucasians.

Another discrepancy between East and West is the consideration of stopping NA in cirrhotic patients. The APASL recommends that in highly selected cirrhotic patients, NA discontinuation may be considered according to the stopping criteria and safety results of previous Asian studies[11,33]. On the contrary, the AASLD and EASL do not recommend NA cessation in cirrhotic patients because safety concerns[9,10].

CONCLUSION

From our perspective, the stop strategy is optimal in highly selected noncirrhotic CHB patients. At present, we propose the ideal candidates for NA discontinuation in CHB patients as shown in Figure 2. The major benefit of this strategy is it enhances the chance of achieving a functional cure faster than continuous long-term NA therapy. However, there are some caveats, including severe CR, liver decompensation, or HCC development to be considered. The current unmet needs for NA discontinuation strategy in CHB patients are the better prediction of the patients who are good candidates for stopping, emerging and more widely available noninvasive biomarkers, and the identification of the best timing to consider retreatment initiation, balancing the chance of achieving functional cure and liver decompensation.

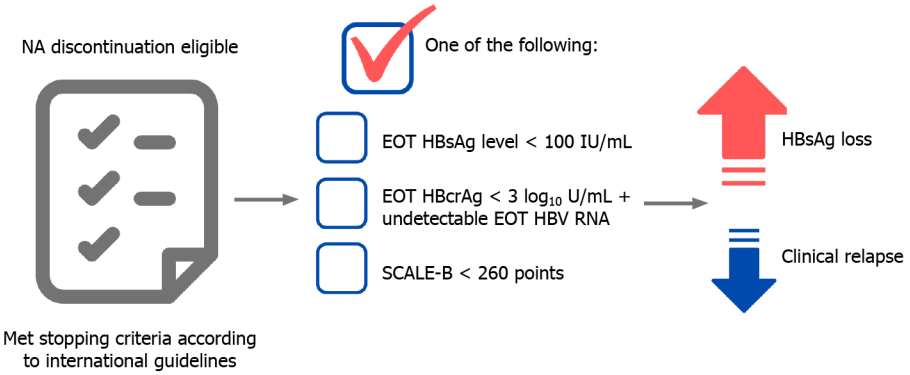

Figure 2 Proposed ideal candidates to for stopping the use of antiviral agents in chronic hepatitis B patients. EOT: End of treatment; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

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MINIREVIEWS

Liver kidney crosstalk: Hepatorenal syndrome

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Abstract

The dying liver causes the suffocation of the kidneys, which is a simplified way of describing the pathophysiology of hepatorenal syndrome (HRS). HRS is characterized by reversible functional renal impairment due to reduced blood supply and glomerular filtration rate, secondary to increased vasodilators. Over the years, HRS has gained much attention and focus among hepatologists and nephrologists. HRS is a diagnosis of exclusion, and in some cases, it carries a poor prognosis. Different classifications have emerged to better understand, diagnose, and promptly treat this condition. This targeted review aims to provide substantial insight into the epidemiology, pathophysiology, diagnosis, and management of HRS, shed light on the various milestones of this condition, and add to our current understanding.

Key Words: Hepatorenal syndrome; Liver; Kidney; Crosstalk; Acute kidney injury

Core Tip: The dying liver causes the suffocation of the kidneys, a simplified way of describing the pathophysiology of hepatorenal syndrome (HRS). This targeted review aims to provide substantial insight into the epidemiology, pathophysiology, diagnosis,

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INTRODUCTION

Incidence and prevalence

Hepatorenal syndrome (HRS) has been a challenge for clinicians and patients for many years. It is imperative to have a proper understanding of risk factors, patient populations involved, and possible preventive measures to be taken to minimize the progression of this complicated clinical state.

Older and more recent studies have revealed that acute kidney injury (AKI) is diagnosed in almost 50% of hospitalized cirrhotic patients, and HRS-AKI represents 11% to 20% of those cases[1]. HRS occurs in approximately 10% to 40% of patients with ascites and advanced liver cirrhosis[2,3], with the one-year probability of developing HRS estimated to be 18% and the five-year probability 39%[4]. Fortunately, the prevalence of the syndrome is not elevated when no precipitating factors are detected. The most common precipitating events contributing to the development of HRS are infections, gastrointestinal hemorrhage, and large-volume paracentesis (LVP)[4,5].

AKI-HRS is associated with a 30% increase in the risk of mortality during hospital stays. A comprehensive meta-analysis revealed mortality rates of 58% at 1 mo and 63% at one year[3]. Broader knowledge is needed to identify the potential predictors of HRS and stratify the individual risk score. To this end, three independent predictors have been implicated in multivariate analysis: No evidence of enlarged liver, elevated plasma renin activity, and hyponatremia[5].

PATHOPHYSIOLOGY AND PROGRESSION OF HRS

HRS is a reversible functional renal impairment seen in hepatic cirrhosis with portal vein hypertension and is caused by multiple pathophysiological changes[6]. Renal dysfunction commonly occurs in cirrhotic patients and is associated with high morbidity and mortality[5].

Historically, there were two types of HRS. Type 1 was defined as a fast deterioration of renal function over two weeks with a serum creatinine level > 2.5 mg/dL, while type 2 was described as a subtle impairment over months. According to the more recent definition proposed by the Acute Kidney Injury Network in 2007 and supported by the International Club of Ascites (ICA) and Acute Dialysis Quality Initiative in 2011, HRS was divided into subgroups based on the underlying pathologic process[1]: HRS-AKI and non-HRS AKI. The distinction between these is that HRS-AKI is a functional renal impairment that is reversible with liver transplantation, whereas non-HRS AKI is a structural pathology of the renal parenchyma caused by various injuries. ICA specific criteria for HRS-AKI were defined as an increase in serum creatinine of ≥ 0.3 mg/dL or ≥ 1.5 times the baseline creatinine or a 50% increase within 48 h from baseline, no response to diuretic discontinuation, the presence of cirrhosis with ascites, no evidence of shock, no history of administering nephrotoxic medications, and no signs of organic renal disease[3,5].

Several mechanisms are involved in the pathophysiology of HRS, such as circulatory dysfunction and splanchnic arterial vasodilation, increased vasoconstrictor effects on renal vasculature, cardiac impairment, systemic inflammation, and adrenal insufficiency[1]. Portal hypertension in cirrhosis causes a structural strain on the endothelium, leading to the release of endogenous vasodilators, such as nitric oxide, prostacyclin, carbon monoxide, and endocannabinoids[5,7]. Gut bacterial translocation in the mesenteric lymph nodes and then into the bloodstream, along with nitric oxide

and other vasodilators, also contributes to intense splanchnic vasodilation and pooling of large plasma volume into the splanchnic vascular bed[2,4]. This creates low effective circulatory volume, which stimulates the baroreceptors in the carotid body and aortic arch. As a result, counterregulatory systemic vasoconstrictor pathways, such as the sympathetic nervous system, renin-angiotensin-aldosterone system (RAAS), and the non-osmotic release vasopressin, are triggered[6,8]. Consequently, hyperdynamic circulation occurs with increases in cardiac output, heart rate, sodium and water retention, and renal vasoconstriction, leading to the development of ascites and subsequent renal dysfunction. RAAS and vasopressin act on sodium and exacerbate free water retention, further worsening the developing ascites and aggravating renal impairment[1].

In the incipient stages, the kidneys maintain an adequate glomerular filtration rate (GFR) due to renal prostaglandins, which keep the afferent arterioles dilatated. However, cirrhosis progression intensifies both splanchnic and systemic vasodilation and contributes to decreased mean arterial pressure, prolonged renal vasoconstriction with reduced renal blood flow, and GFR[5]. Overall, a state of renal hypoperfusion occurs. Therefore, HRS is a prerenal type of renal failure, which is not responsive to fluids.

Cardiac dysfunction in HRS is caused by the diseased liver itself and less commonly by the same etiologic factor of cirrhosis (*e.g.*, alcohol). Myocardial impairment is complex and has several contributory mechanisms: Increased neurohumoral activity leading to myocardial hypertrophy and fibrosis with affected relaxation and inhibitory effects of the cytokines on the ventricular function[6]. Generally, inotropic and chronotropic functions become altered in hepatocardiorenal syndrome[9].

Non-infectious systemic inflammatory response syndrome was identified in almost half of the patients with AKI-HRS[5]. On the other hand, HRS is often preceded by bacterial infections. Inflammation in cirrhosis is induced by macrophage activation, oxidative stress, and inflammatory molecules[9]. Pathogen-associated molecular patterns emerge from the translocation of gut bacteria and damage-associated molecular patterns from the damaged hepatocytes. In turn, these inflammatory molecules activate cytokine release, leading to increased vasodilator production, with the result being reduced systemic arterial resistance and mean arterial pressure[6].

Relative adrenal insufficiency (RAI) is observed in less than half of the patients with advanced cirrhosis and may develop into HRS. The mechanisms are not well established; however, depletion of the substrates for cortisol production and dysfunction of the hypothalamus-pituitary axis by the pro-inflammatory cytokines have been implied[6]. Other mechanisms have been theorized to contribute to the HRS, mainly the hepatorenal reflex. The hepatorenal reflex is thought to be the result of abnormal hepatic blood flow directly affecting kidney hemodynamics. Evidence to support this theory is reinforced by the transjugular intrahepatic portosystemic shunt placement, which leads to the HRS's amelioration by reducing portal hypertension[8].

Reduction in GFR and decreases in renal blood flow progress along with the degree of cirrhosis. The following objective evidence indicates that renal impairment in cirrhotic patients is functional: No evidence of morphological changes and largely preserved tubular function on kidney biopsy, resolution of AKI-HRS following liver transplant, and successful cadaveric transplantation of kidneys from patients with HRS[1] (Figure 1).

HRS DIAGNOSIS

The diagnostic criteria for the HRS were first developed in 1994, and since then, it has undergone multiple modifications[10]. In the previous years, AKI in cirrhotic patients was defined as a serum creatinine level of ≥ 1.5 mg/dL[11]. The latest guidelines of the ICA reveal that the definition of AKI in this population has changed based on modifications of the Kidney Disease Improving Global Outcomes (KDIGO) criteria[12]. The removal of this static value has led to the earlier identification of this condition in patients with chronic liver disease (CLD)[12].

AKI is now defined as an increase of serum creatinine of ≥ 0.3 mg/dL within 48 h and/or increase of ≥ 50% from the patient's baseline within 7 d (or within the past 3 mo before admission, if a value within the previous week is not available). Furthermore, the ICA classifies AKI in three stages based on serum creatinine levels. Stage 1 is when there is an increase of ≥ 0.3 mg/dL or an increase of ≥ 1.5-fold to 2-fold from the baseline; stage 2 is when there is an increase of > 2-fold to 3-fold from the baseline; stage 3 is when there is an increase of > 3-fold from the baseline or serum

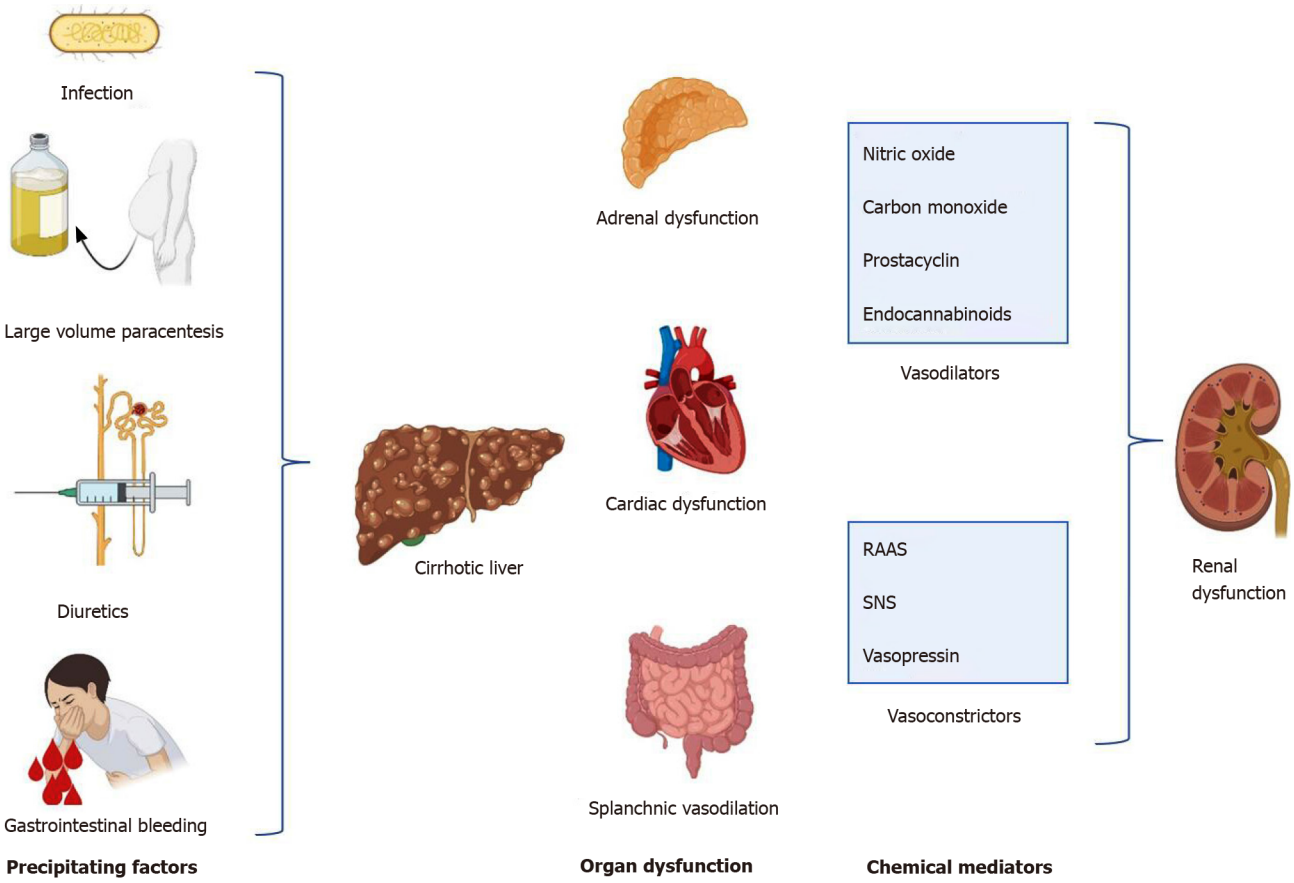

Figure 1 Pathophysiology of hepatorenal syndrome. Figure created with BioRender.com. RAAS: Renin-angiotensin-aldosterone system; SNS: Sympathetic nervous system.

creatinine is ≥ 4.0 mg/dL with an acute increase of ≥ 0.3 mg/dL or the initiation of renal replacement therapy (RRT)[11].

The use of urine output as a criterion for AKI in CLD was subsequently removed [11]. Despite this, in a retrospective study, Amathieu *et al*[13] found that the addition of urine output as a criterion, along with serum creatinine for identification of AKI in patients with CLD, showed increased sensitivity for the identification of this pathology and that the presence of transient oliguria was associated with an increase in mortality rates[13]. Therefore, in this population, an acute decrease in urine output should be considered, particularly in patients with transient oliguria.

HRS is diagnosed when a patient with cirrhosis and ascites has stage ≥ 2 AKI per the ICA guidelines, has no response to diuretic withdrawal or a trial of treatment with albumin for volume expansion (1 g/kg per day with a maximum of 100 g/d) for a total of 2 d, and has no evidence of other etiologies causing kidney injury (*i.e.* absence of shock, no recent use of nephrotoxic drugs, no macroscopic signs of structural kidney injury, such as the presence of proteinuria, microhematuria, or abnormal findings on renal ultrasonography)[10,12,14,15].

HRS was previously classified as HRS type 1 and HRS type 2, based on the acuity of kidney function deterioration. HRS type 1 was defined as a doubling of serum creatinine above 2.5 mg/dL within 2 wk, and type 2 was defined as a slower increase in serum creatinine to a value > 1.5 mg/dL. These definitions have been renamed from HRS type 1 to HRS-AKI and HRS type 2 to HRS-chronic kidney disease[12].

New biomarkers have been identified for HRS diagnosis, including the urinary neutrophil gelatinase-associated lipocalin (NGAL) and the serum cystatin C. The use of these biomarkers has been shown to help diagnose HRS early and prognostic assessment in patients with decompensated cirrhosis[16]. A systematic review by Puthumana *et al*[17] revealed that both interleukin (IL) 18 and NGAL might be useful in the differentiation between AKI due to acute tubular necrosis (ATN) and HRS. These and other markers have not been included in the diagnostic criteria at the time of this review but might be considered in the future.

HRS PREVENTION

A proper understanding of HRS's underlying pathophysiology is crucial in preventative strategies used in today's clinical practice. Discussed below are some strategies found beneficial for the prevention of HRS. The focus of all strategies is on reversing the poor perfusion to the kidney due to a combination of renal vessels' constriction and decreased renal blood flow in response to systemic vasodilation.

Role of diuretics

Diuretic therapy may cause intravascular volume contraction and result in compensatory vasoconstriction, further worsening an already impaired renal function. In severely decompensated patients, diuretic therapy may induce HRS. The current recommendation for patients with ascites is to receive spironolactone treatment not exceeding 400 mg daily and divided doses of furosemide not exceeding 160 mg daily. In hospitalized patients, the addition of albumin to diuretic regimens may prevent diuretic-induced changes in creatinine and BUN[18].

Large-volume paracentesis can lead to the deterioration of kidney function. Plasma renin activity, baseline creatinine measurements, and daily monitoring should be performed, which helps identify patients deemed to be at high risk of developing post-paracentesis HRS. Such patients should receive supplementation with albumin, with the recommended dosing 6–8 gm/L of ascitic fluid removed[19].

Spontaneous bacterial peritonitis (SBP)

It is a known fact that SBP is a common precipitant of HRS. Prompt recognition and treatment of SBP and managing the patient in a monitored setting are crucial in preventing HRS development. For patients with impaired renal function and bilirubin levels of > 4 mg/dL, IV albumin infusion at 1.5 mg/kg should be initiated[20].

Rifaximin

In a study by Ibrahim *et al*[21], published in the European Journal of Gastroenterology and Hepatology, prolonged therapy with Rifaximin showed benefits due to decreased cirrhosis-related complications, SBP, and recurrent hepatic encephalopathy, along with hemodynamic and renal improvement in patients with alcoholic hepatitis. Furthermore, patients on Rifaximin therapy for 12 weeks showed more stable renal function than placebo[21].

Finally, another study by Dong *et al*[22] reported a lower incidence of acute renal injury in patients treated with Rifaximin for at least 90 d.

DIFFERENTIAL DIAGNOSES

HRS-AKI is considered a diagnosis of exclusion, and the ICA defines it as AKI (an increase in serum creatinine of 0.3 mg/dL or more within 48 h) in the setting of cirrhosis and ascites, with failure to improve after 48 h of diuretic withdrawal and volume expansion with albumin, in the absence of shock, nephrotoxic drugs, and signs of structural kidney injury (proteinuria > 500 mg/d, microhematuria > 50 RBC/HPF, or abnormal renal imaging)[23-25].

AKI is reported in about 20–30% of hospitalized cirrhotic patients[24,26], with six-fold higher mortality[26], and although HRS is unique to cirrhosis, AKI in cirrhotic patients can be due to other causes, including prerenal azotemia and ATN[23,24]. Other causes such as glomerulonephritis and post-renal AKI should also be considered [24]. As these causes differ markedly in their treatment options and prognosis, early differentiation is key to improving outcomes[23,24,27].

In studies involving cirrhotic patients, hypovolemic AKI was reported as the most common cause of AKI stage IA (stage I with sCr < 1.5 mg/dL), which has better survival (90 d survival rate of 82%) than AKI stage IB (stage I with sCr ≥ 1.5 mg/dL) (90 d survival rate of 55%), where HRS and ATN were more frequent[23]. It was also reported that acute, chronic liver failure was more likely with AKI stage IB[24].

Prerenal AKI: Renal hypoperfusion without tubular or glomerular lesion usually occurs in GI bleeding, dehydration, and/or diuretic use[28]. It is differentiated from the other causes of AKI by improvement after volume replacement with albumin and/or fluids and diuretics withdrawal[23,29].

ATN: Tubular cell necrosis is usually the result of an ischemic (in the setting of shock) or toxic (*e.g.*, nephrotoxic drugs) insult[28]. As with HRS, there is no improvement with withdrawing diuretics and giving albumin[29]. Intrinsic AKI is

excluded using the ICA-HRS criteria[29].

The use of UNa and FeNa to differentiate causes of AKI is deemed less useful in cirrhosis: Prerenal AKI and HRS have urinary Na excretion < 20 mEq/L and FeNa < 1%, whereas ATN classically has UNa > 40 mEq/L and FeNa > 1%[28].

Limitations to this rule are that patients with cirrhosis can be on diuretics, which will falsely increase UNa[28]. Additionally, as cirrhosis is a sodium acid state, some ATN cases were reported to have FeNa < 1%[24,28].

The presence of urinary casts may not be helpful either in cirrhosis, as granular and epithelial cell casts (classically seen in ATN) can be present as nonspecific findings in cirrhosis due to hyperbilirubinemia[28].

The use of urinary biomarkers to differentiate the various AKI etiologies in cirrhotic patients is promising: NGAL (a glycoprotein that is overexpressed by injured kidney tubular epithelia) is the most studied, but other urinary markers such as IL-8, albumin, and liver fatty acid-binding protein have also been investigated and show similar performance[24]. Higher levels were found in intrinsic AKI/ATN, compared to HRS. Meanwhile, prerenal had the lowest levels[23,24]. One study done on 94 patients with decompensated cirrhosis showed a median urine neutrophil gelatinase-associated lipocalin (uNGAL) of 1217.50 in ATN, 465.00 in HRS, and 95.50 in prerenal AKI (P < 0.001)[23]. It also determined the optimal cutoffs for the various diagnoses: ATN is likely with uNGAL more than 650 ng/mL (100% sensitivity, 83.78% specificity), HRS is likely with uNGAL between 299-650 ng/mL (87.9% sensitivity, 96.3% specificity), while prerenal is likely with uNGAL less than 299 ng/mL[23].

uNGAL and IL-8 were also found to predict prognosis, where the higher the biomarker levels, the higher is the short-term mortality[23,24].

It is to be noted that leucocytes can also produce uNGAL. Hence, levels of uNGAL can be elevated in the setting of urinary tract infection and should be cautiously interpreted in these settings[24].

HRS treatment

The definitive treatment for HRS is liver transplantation[30]. The goal of therapy is to maintain adequate kidney function before the patient undergoes a liver transplant, which can be achieved by optimizing the mean arterial blood pressure and cardiac output[31,32]. Patients should be screened thoroughly for signs of infection, and if necessary, empiric antibiotic therapy should be started[33]. Therapy for the treatment of viral hepatitis, if present, should be continued. Diuretic therapy should be stopped, as these have been identified to be a provoking factor for HRS development.

Patients should receive volume expansion with albumin, as it has shown to significantly reduce mortality in this population, which has not been seen with other volume expanders. The protective effects of albumin are thought to be also driven by its anti-inflammatory and antioxidative effects[5]. Several vasodilators have been studied in the past as potential treatment options for HRS, including dopamine, prostaglandins, and endothelin receptor blockers, which have not been effective in improving kidney function[34,35]. The use of vasoconstrictors, such as terlipressin, norepinephrine, or a combination of midodrine, octreotide, and albumin showed improved renal function and are considered the first line of therapy for HRS[36,37]. The rationale behind its use is the reversal of splanchnic vasodilatation thought to cause renal impairment in this population. The choice of therapy depends on different factors, including which drugs are available at the time of treatment, if the patient is admitted to the intensive care unit or medical floors, and if they qualify for a liver transplant[32].

In patients who have no response to pharmacological alternatives, non-pharmacological approaches should be considered. This includes transjugular intrahepatic portosystemic shunt, RRT, and molecular adsorbent recirculating system (MARS)[33].

Vasoconstrictive therapy

Terlipressin and albumin: Terlipressin (a vasopressin analog) and albumin are the most effective medical therapy for HRS[30]. It has been associated with reducing mortality and increased renal function in patients with type 1 HRS (HRS-AKI as per new definition)[38]. It is the most commonly used combination of vasoconstrictive agents (however, not available worldwide) with its efficacy ranging between 25% and 75%[36]. Several studies have compared the efficacy of albumin alone *vs* albumin combined with terlipressin, demonstrating that their combination is significatively more efficacious[39].

Terlipressin should be administered either by intravenous bolus (0.5–1 mg every 4–6 h) or continuous infusion with an initial dose of 2 mg/d. If there is no appropriate response to therapy (defined as a decrease of at least 25% of creatinine levels), the

intravenous bolus dose may be increased up to 2 mg every 4 h or the continuous infusion increased to a maximum of 12 mg/d. Albumin should be administered by intravenous bolus for the first 2 d, with doses of 1 g/kg (with a maximum dose of 100 g/d) and later continued with 25-50 g/d until the terlipressin therapy is stopped[32,36].

Terlipressin has been associated with an increased risk of cardiovascular events and ischemia induction[32,36,38,40]. However, it has a relatively good safety profile, as adverse events are reported in < 1% of patients[41]. Factors that help determine response to therapy are increased mean arterial pressure of ≥ 5 mmHg and decreased serum bilirubin levels to < 10 mg/dL on day 3 of therapy[42]. In a recent phase 3 trial conducted by Wong *et al*[43], the combination of terlipressin and albumin was reported to be significantly more effective when compared to placebo. However, its use was associated with significant adverse events, including respiratory failure.

Norepinephrine and albumin: Norepinephrine is an acceptable alternative to terlipressin[30]. It is used as a continuous intravenous infusion rate of 0.5–3 mg/h[30]. Its use is limited as the patient needs a central venous catheter for its administration; therefore, it is usually administered in the intensive care setting[33]. Terlipressin is superior to norepinephrine at decreasing RRT's need and increasing survival in this population[44].

Midodrine, octreotide, and albumin: The combination of midodrine, octreotide, and albumin improves hemodynamics, leading to increased GFR and decreased mortality [45,46]. Midodrine is dosed at 7.5 mg every 8 h and can be increased to a maximum dose of 15 mg every 8 h. Octreotide can be given as a continuous infusion at a rate of 50 μg/h or subcutaneously at doses of 100 μg to 200 μg every 8 h. Albumin is added to an intravenous bolus, with doses of 1 g/kg[32]. In a study by Wang *et al*[47], terlipressin was reported to be superior to octreotide for improved kidney function but did not show superiority to norepinephrine. This combination is usually used in countries where terlipressin is not yet available[36]. The use of this combination is acceptable in non-intensive care settings, such as inpatient medical floors[30].

Non-vasoconstrictive therapy

Transjugular intrahepatic portosystemic shunts: Transjugular intrahepatic portosystemic shunts (TIPS) have been shown to improve renal function in patients with HRS [48]. However, its use is limited, mainly due to its complications, including a higher incidence of hepatic encephalopathy[49]. A study by Song *et al*[50], in which 128 patients with HRS were treated with TIPS, revealed a pooled rate of hepatic encephalopathy of 49%, with a pooled rate of renal function improvement of 93% and 83% in HRS type 1 and 2, respectively (HRS-AKI and HRS-CKD per the new definitions).

Renal replacement therapy: The indications for RRT in patients with HRS are the same as those without it[10]. RRT may be effective until liver transplantation is available[36,51]. In a retrospective study by Sourianarayanane *et al*[52], where 380 patients were reviewed, there was no significant improvement in the survival rates of patients undergoing RRT who did not receive liver transplantation. Other studies suggest that mechanical ventilation might play a role as an independent risk factor for worse outcomes at the time of initiation of RRT. Furthermore, RRT initiation in these patients might be futile, compared to those who are not mechanically ventilated[53].

Molecular adsorbent recirculating system (MARS): Albumin dialysis with MARS has been shown to decrease creatinine levels in patients with HRS. However, there have been no significant changes in survival rates among patients receiving this treatment [36,51,54].

Emerging therapies: Serelaxin, a recombinant human relaxin 2, is a molecule that acts on renal vasculature, increasing perfusion. It has been suggested that Serelaxin could be used for the treatment of HRS, given that in animal models, it has been shown to exert renal vasodilatation[5].

Pentoxifylline is a phosphodiesterase inhibitor that has also been suggested as a potential therapeutic option. A small study showed that it is safe to use along with albumin, midodrine, and octreotide[55]. However, further studies are needed to evaluate the effectiveness of these therapies.

MORTALITY/COMORBIDITY OF HRS

HRS is a significant illness linked to poor prognosis in patients with cirrhosis[56]. Patients with Type I HRS have a mortality rate of 80% 2 wk after the confirmation of the disease, increasing to a 100% within months. Patients with type II HRS have a median survival of 3–6 mo after presentation[57]. In 24-47% of patients with chronic ascites and liver disease, RAI is observed, influencing HRS progression[58].

CONCLUSION

Prognosis after intervention for HRS

The most crucial objectives in HRS treatment are to reverse AKI and enable additional medications to be provided to the patients before orthotopic liver transplant (OLT). A recently published study reported that patients with HRS who received treatment before OLT had a significantly higher three-year survival rate, lower incidence of renal dysfunction and serious and acute infections, and lower number of days in the ICU and the hospital, as compared to patients who received transplants without HRS and had normal renal function[59]. HRS is closely linked to hyponatremia, and when serum sodium levels fall below 130 mmol/L, the incidence of HRS due to hyponatremia increases[60]. Raising serum sodium levels leads to hemodynamic recovery. OLT is the best treatment strategy for HRS[61]. Most clinicians use the Model for End-Stage Liver Disease-Sodium (MELDNa) score to determine the prognosis of CLD, especially in cirrhosis. In patients with cirrhosis, the MELDNa score was superior to the MELD score for predicting postoperative 90 d mortality[62].

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MINIREVIEWS

Hepatitis C virus treatment failure: Clinical utility for testing resistance-associated substitutions

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Abstract

The hepatitis C virus has a high mutation capacity that leads to the emergence of resistance-associated substitutions (RAS). However, the consequence of resistance selection during new direct-acting antiviral drug (DAA) treatment is not necessarily the therapeutic failure. In fact, DAA treatment has shown a high rate (> 95%) of sustained virological response even when high baseline RAS prevalence has been reported. In the context of RAS emergence and high rates of sustained viral response, the clinical relevance of variants harboring RAS is still controversial. Therefore, in order to summarize the data available in international guidelines, we have reviewed the clinical utility of testing RAS in the era of new pangenotypic DAA drugs.

Key Words: Hepatitis C virus; Treatment failure; Resistance; Direct-acting antiviral

Core Tip: The presence of resistance-associated substitutions (RAS) to hepatitis C virus (HCV) treatment is a frequent event. Direct-acting antiviral (DAA) treatment repre-

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sents a milestone in the antiviral therapy of chronic HCV infection. The role of RAS in sustained virological response remains controversial. We herein discuss the clinical utility of testing RAS in the era of pangenotypic DAA drugs.

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INTRODUCTION

For years, the only available treatment for chronic hepatitis C virus (HCV) infection was pegylated interferon and its combination with ribavirin (PEG-IFN/RBV) therapy. However, the sustained viral response (SVR) to treatment of infected patients was limited, varying between 42% and 46% for HCV genotype 1, about 60% for HCV genotype 4, and 76% to 80% for HCV genotype 2 or 3[1-5]. The outcomes were troublesome in patients coinfected with human immunodeficiency virus /HCV, whose SVR rates were even lower[6-9]. Fortunately, treatment against HCV infection has improved significantly in the last decade, changing from a nonspecific immunomodulatory therapy with multiple and severe side effects, such as PEG-IFN/RBV, to specific viral target options such as direct-acting antiviral (DAA) drugs against NS3, NS5A, and NS5B proteins. Thus, since the development of the latest generation of DAA drugs, the SVR is achieved in 95% to 99% of treated patients[10]. Although this scenario is very encouraging, the 1% to 5% of patients who do not achieve SVR are the pitfall of DAA therapy. Therefore, the current complex challenge is to rescue patients who fail to one or more DAA schemes.

Response to treatment with PEG-IFN/RBV was associated with viral variants and single nucleotide polymorphisms[11-17]. The introduction of DAA drugs implied a higher specific and targeted pressure on the virus, which favor the selection of resistance-associated substitutions (RAS) to different antiviral agents. In this context, virological failure was associated with RAS that may be present either from the beginning (baseline RAS) of treatment or acquired during it[18].

Naturally, HCV produces approximately 10^{12} viral particles per day[19]. In addition, the viral replication complex lacks proofreading activity, resulting in a large amount of viral variants in each infected individual. Although, in theory, all possible mutants can be produced in just 1 day, not all of them are able to remain in the population. That is because some viral genome regions have constraints and most mutations generate variants that impair viral fitness and, therefore, do not proliferate. As a result, a large mutant spectrum known as quasispecies is generated[20]. The quasispecies, that represent the lowest level of viral diversity, drives virus adaptability and constitute the greatest challenge to treatment resistance[20].

DAA drug administration inhibits wild-type HCV variants allowing the selection of reduced susceptibility variants, which present a better fitness to this environment. Although initially they do so inefficiently, over time they develop compensatory amino acid substitutions that have a higher fitness and increase the frequency of resistant variants in the quasispecies spectrum (Figure 1). Additionally, each antiviral drug has a different genetic barrier that is characterized by a threshold above which DAA resistance develops. The threshold is determined by several factors including the number of required nucleotide mutations, the level of resistance, and the viral variant fitness. Therefore, even when a viral variant with a RAS emerges, it does not mean that it is sufficient to lead to therapeutic failure. In that way, therapeutic outcome will depend on a finely poised and complex balance between the DAA genetic barrier and viral-resistant variant fitness. Consequently, a highly resistant strain with a low replication capacity will be clinically less relevant than a less resistant one that replicates more efficiently. Fortunately, more powerful DAA drugs with greater genetic barriers have been developed in the last few years[21].

In preclinical and in real-life studies, the reported prevalence rate of baseline RAS is around 5% to 40%, raising concern of the effect on reducing SVR[22-28]. Eventually, the adverse impact of baseline RAS could be minimized by extending treatment

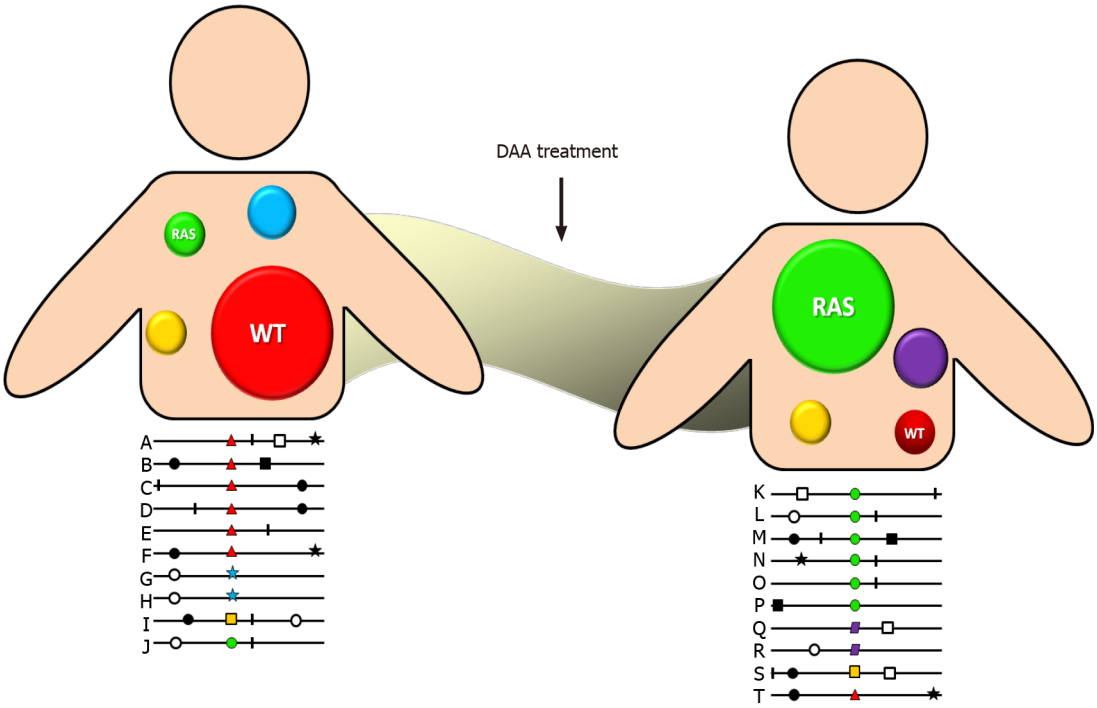

Figure 1 Quasispecies distribution. Simplified representation of quasispecies infecting an individual. Each genome is identified with a letter. The mutation highlighted by a red triangle in the wild-type (WT) confers a selective advantage that results in dominance of that mutation after a given number of replication rounds in an untreated patient. After the pressure generated by direct-acting antiviral (DAA) treatment, a modification of the consensus sequence is observed, where a green circle confers resistance to treatment and becomes dominant. In the upper example, mutant classes are represented as circles of sizes proportional to the number of genomes in each class. Red circles represent the WT, green circles represent a variant with resistance-associated substitutions (RAS). Yellow, light blue, and purple circles are variants with changes of the WT that are not associated with treatment response.

duration or optimizing DAA regimens. However, that is not always clinically possible, as a considerable proportion of treatment failures are caused by RAS acquired during it[29,30]. Table 1 shows the most relevant RAS reported for the currently most used DAA drugs.

RAS DETERMINATION

Unfortunately, the lack of a large market of standardized commercial assays for RAS determination has led to developing in-house RAS assays, which has created a great disparity the techniques that are used, the determined RAS, and their interpretation. Two main techniques for RAS detection have been applied. One is direct sequencing (Sanger) with sensitivity that allows detecting viral species present in between 15% and 25% within quasispecies, and the second is next generation sequencing (NGS), which allows the detection of variants present in less than 1%[31,32]. NGS is thus a more sensitive technique, but it is also much more expensive. It is therefore very likely that direct sequencing will continue to be the technique of choice because of its cost/benefit in the context of the high SVR rates of currently used DAA regimens.

Since the implementation of DAA agent, the main question that has been asked is the extent to which the RAS frequency impacts the outcome of treatment. It has been reported that the presence of a low proportion of viral variants carrying RAS within the quasispecies of an infected patient would have a lesser impact on SVR rates. In fact, some studies have reported a 15% cutoff of the viral population harboring RAS from in which a drop in the virological response rate was observed. Ikeda *et al*[33] (2017) reported that the SVR rates to daclatasvir (DCV)/asunaprevir (ASV) in HCV-infected patients with Y93H ratios of < 1%, 1%–25%, 26%-75%, and > 76% were 99%, 100%, 71%, and 23%, respectively[33]. Similarly, using a 15% NS5A pretreatment cutoff of ledipasvir (LDV)-specific RASs, Zeuzem *et al*[23] (2017) reported significant differences in SVR rates in patients treated with sofosbuvir (SOF)/LDV[23]. Overall, it has been established that SVR decreases as the proportion of RAS in the quasispecies infecting a patient increases. The second question was whether there was a differential impact of RAS depending on whether the patients were treatment naïve or previously

Table 1 Hepatitis C virus resistance-associated substitutions to currently used direct-acting antiviral drugs

Drug family	Drug	Licensed for genotype	RAS
NS3 inhibitors	Glecaprevir (GLE)	1, 2, 3, 4, 5, 6	36M, 56H, 156G/V, 168A/K/L/R
	Grazoprevir (GZR)	1, 4	36A/L/M, 56H/F, 155G/K/L/Q/T/S, 156T/V, 168any
	Paritaprevir (PTV)	1, 4	36A/M, 43L, 155C/K/Q/H, 156T/V, 168any
	Voxilaprevir (VOX)	1, 2, 3, 4, 5, 6	36A/G/L/M, 41K/R/S/V, 43L/S7V, 54S, 55A/I, 56H/F, 80K/L, 122D/G/N, 155G/K/N/K/T/W, 156L/S/T/V, 168any
NS5A inhibitors	Daclatasvir (DCV)	1, 3, 4	24H, 28A/M/S/T, 30D/E/G/H/K/N/Q/R/S/T, 31I/F/M/V, 32L/del, 58A/D/N/S, 62L, 93C/H/N/R/S/W
	Elbasvir (EBR)	1, 3, 4	28G/T, 30G/H/K/R/V/Y, 31F/M/V, 58D, 93C/H/N
	Ledipasvir (LDV)	1, 3, 4, 5, 6	24N/G, 28A/M/T, 30E/G/H/K/N/R/S/T/Y, 31I/M/V, 32L/del, 38F, 58D, 92K/T, 93C/H/N/R/S/T/W
	Ombitasvir (OBV)	1, 4	28M/S/T/V, 30E/Q/R/Y, 31I/F/V, 32del, 58D, 92T, 93C/H/N/S
	Pibrentasvir (PIB)	1, 2, 3, 4, 5, 6	24R, 28G/K/S, 30K/R, 31I/M, 32del, 58C/D, 93H/N
	Velpatasvir (VEL	1, 2, 3, 4, 5, 6	28V, 30E/H/K, 31M/V, 32L, 93H/N/R/S/W
NS5B nucleoside analogs inhibitors	Dasabuvir (DSV)	1	316Y, 368T, 395G, 411S, 414T, 444K, 445F, 448C/H, 451S, 553T/V, 554S, 556G/N/R, 557R,558R, 559G/N, 561H, 565F
NS5B non-nucleoside analogs inhibitors	Sofosbuvir (SOF)	1, 2, 3, 4, 5, 6	159F, 282R/T, 289L, 320I/V, 321A

RAS: Resistance-associated substitutions.

treated. That question will be discussed in more detail below.

CLINICAL UTILITY OF RAS DETECTION

The clinical impact of RAS depends particularly on both the HCV genotype/subtype and the administered DAA regimen, which varies in efficacy according to the type of RAS as well as the treatment experience and presence of cirrhosis.

Naïve patients

In naïve patients, the prevalence of RAS that significantly affect the response to treatment is estimated to be approximately 5%. In that case, the SVR rates of patients with RAS would be 91%, while for patients without RAS it would be approximately 99%[23,34,35]. In summary, RAS assessment prior to the beginning of treatment is not recommended for naïve patients. In previously treated patients, the situation is more complex and refers to subjects who have failed to respond to treatment with a DAA compound. In that case, the presence of post-failure RAS is more than 75%, and SVR rates are more affected. In fact, it has been reported that SVR rates are between 75% and 85% in patients with RAS, while for patients without RAS they continue to be remarkably high (> 95%)[23,34,35].

Identifying the HCV genotype/subtype before starting therapy in naïve patients, in the pangenotypic treatment era, remains useful and may be necessary when drug availability or lack of affordability require genotype-specific treatment or optimal treatment regimens. In that sense, HCV genotyping and subtyping should be performed by nucleotide sequence analysis of some coding regions, generally the core, NS3, or the NS5B coding regions, which accurately discriminates HCV subtypes[36,37]. Furthermore, the use of the NS3 or NS5B regions to determine the viral genotype and subtype also allows the detection of the baseline RAS[36]. On the other hand, as HCV subtypes, including 1l, 3b, 3g, 4r, 6u, 6v, among others, harbor a high frequency of baseline RAS, knowing the HCV subtype before treatment in regions or countries

where these subtypes are prevalent (*i.e.* China, South-East Asia, and sub-Saharan Africa) is strongly recommended in order to optimize treatment[38-41]. Indeed, infrequent subtypes harboring RAS that confer resistance to NS5A inhibitors should be considered for treatment with the fixed-dose combinations SOF/velpatasvir (VEL)/voxilaprevir (VOX) for 12 wk.

HCV-1 is the most prevalent genotype worldwide (46.2%), and one third of the HCV-1 that infects patients belongs to subtype 1a[42]. Several studies have reported that DAA-naïve individuals infected with HCV-1a are more difficult to treat than those infected with HCV-1b[23,43-45]. In fact, it has been observed that in the presence of cirrhosis, high baseline viral load, or failure of previous treatment with PEG-IFN/RBV, the SVR rates of patients treated with elbasvir (EBR)/grazoprevir (GZR), or SOF/LDV were significantly lower for HCV-1a compare with HCV-1b infected individuals[23,43-45]. In EBR/GZR phase III clinical studies, the SVR rate was as low as 58% in HCV-1a treatment-naïve infected patients who harbored baseline NS5A RAS [46]. On the contrary, SVR rates were high (> 97%) in HCV-1b infected patients[46]. Nevertheless, the effect of RAS in HCV-1a infected patients can be overcome by extending treatment to 16 wk and adding RBV to patients with baseline NS5A RAS [44]. Therefore, NS5A resistance testing at baseline is recommended for HCV-1a infected patients with a viral load above 800.000 IU/mL if 12 wk treatment duration is intended.

In addition, pretreatment genotyping is recommended if cirrhotic patients will be treated with SOF/VEL, as baseline RAS reduce SVR rates in HCV-3 cirrhotic patients treated with that regimen. Moreover, a recent study analyzing 539 HCV-3 infected patients showed that patients with baseline Y93H and/or A30K RAS had an SVR rate of 72.2%, while HCV-3 infected patients without NS5A RASs achieved an SVR rate of 95.7% (P = 0.002)[47]. Accordingly, a large meta-analysis that included more than 6500 subjects with chronic HCV infection reported reduced effectiveness of GLE/PIB in HCV-3 infected patients with baseline RAS like A30K, Y93H, and P53del, and recommended, in order to improve prognosis of treatment outcome and selection of therapy, testing of RAS in such patients[48].

According to the American Association for the Study of Liver Diseases guidelines, pretreatment RAS testing is recommended in cirrhotic HCV-3 infected patients because those without a baseline Y93H RAS in NS5A are eligible for 12 wk of SOF/VEL therapy. On the other hand, cirrhotic HCV-3 infected patients with baseline Y93H RAS should be treated with SOF/VEL plus RBV or SOF/VEL/VOX for 12 wk[49]. However, since HCV-3 infections are frequent in developing countries, the benefit of pretreatment screening for RAS should be weighed. On the contrary, the European Association for the Study of the Liver (EASL) guidelines recommend the same therapeutic regime for all compensated cirrhotic patients regardless of viral genotype[50].

Retreatment for DAA failures

Even in the context of a low treatment failure rate (< 5%), the number of patients requiring retreatment is quite high because of the large number of patients with chronic HCV infection who are treated with DAA worldwide[22-24,29-30]. Currently, the main international treatment guidelines do not recommend massive testing of RAS before starting DAA treatment, although there are exceptions[49,50].

Treatment with SOF/VEL/VOX for 12 wk is one of the most promising pangenotypic regimens for rescuing patients who have failed treatment. Two phase III trials, POLARIS-1 and POLARIS-4, assessed the safety and efficacy of the SOF/VEL/VOX regimen for 12 wk in patients who failed treatment with NS3 and/or NS5A inhibitors [51]. In the POLARIS-1 study, which included 263 patients with NS5A inhibitor failure, the overall retreatment SVR rate was 96% (one breakthrough and six relapses). As expected, cirrhotic patients, who constituted 46% of the study population, had lower SVR than noncirrhotic patients (93% *vs* 99%, respectively). It is important to highlight that neither the HCV genotype nor the RAS profile at the beginning of retreatment influenced SVR[51,52]. Unlike POLARIS-1, the POLARIS-4 study included previously treated patients without NS5A inhibitors. Cirrhotic patients were equally represented in both studies (46%). In POLARIS-4, the overall SVR rate of retreatment with SOF/VEL/VOX for 12 wk was 98% (178/182; one relapse) compared with 90% (136/151; one breakthrough and 12 relapses) in patients retreated with SOF/VEL for 12 wk[51,52]. Regardless of patient gender, body mass index, HCV genotype, and baseline HCV-RNA levels, several real-life studies have confirmed the high SVR rates achieved with the SOF/VEL/VOX scheme in randomized clinical trials[53-56].

The other available pangenotypic option for the treatment of patients with resistant variants is GLE/PIB. However, the combination did not have a suitable genetic barrier to achieve optimal SVR rates in patients failing previous DAA treatment[57]. In the

MAGELLAN-1 Part 2 study, GLE/PIB was used for the retreatment of previous DAA failures. SVR12 was achieved by 89% and 91% of HCV-1 and HCV-4 infected patients who received 12 wk and 16 wk of treatment, respectively. Previous treatment with one inhibitor class (protease or NS5A) had no impact on SVR12, whereas past treatment with both classes of inhibitors was associated with lower SVR12 rates[57]. Another study adds support of the efficacy of the 16 wk regimen for retreatment of HCV-1 infected patients with a history of sofosbuvir/NS5A inhibitor treatment failure[58]. Consequently, treatment with GLE/PIB is recommended as an alternative regimen for the retreatment of patients who failed to a prior DAA regimen including a, NS5A or NS3 inhibitor. It is not recommended for patients who have failed treatment with the combination of both inhibitors[50]. Therefore, at present, the SOF/VEL/VOX combination is the regimen of choice for the retreatment of patients who did not achieve SVR after a course of DAA treatment. RAS determination is not necessary before initiating treatment[49,50].

Currently, the most challenging scenario is represented by patients who failed combinations containing the latest generation of pangenotypic DAA agents GLE/PIB and SOF/VEL/VOX. Thus, such patients who are very difficult to cure, the combinations of SOF/VEL/VOX or SOF/GLE/PIB with RBV for 12 wk, or without RBV for 16-24 wk, are the recommended options. In a previous study, 31 patients who failed GLE/PIB were retreated with SOF/VEL/VOX achieved an SVR of 94% despite the presence of NS5A RAS in 90% of the cases[59]. On the other hand, in the ongoing MAGELLAN-3 study, 23 patients who failed GLE/PIB and received treatment with SOF/GLE/PIB combined with RBV achieved an SVR of 96%, despite the presence of RAS in the NS5A region in 91% of them[60].

Recently, failure to SOF/VEL/VOX has been reported in 40 patients[61]. RAS testing after SOF/VEL/VOX failure showed that all HCV-1a had either NS3 or NS5A RAS. On the contrary, in HCV-1b, individual NS3 RAS were rather rare (11%), and the overall frequency of NS5A RAS was moderate (33%). Finally, for HCV-3, RAS in NS5A (56%) and in NS3 plus NS5A (28%) were relatively frequent. In 22 of the cases, rescue treatment with SOF/GLE/PIB, with or without RBV, for 12-24 wk achieved an SVR rate of 79%. Unfortunately, as all types of DAA drugs have been used in most developing countries; failure is a real possibility. Therefore, surveillance of circulating viral variants is imperative. From a practical point of view, if DAA treatment fails, there are two possibilities: (1) To determine RAS and adjust the new DAA regime according to the result; and (2) to administer empirical DAA treatment following clinical practice guidelines.

The EASL currently recommends first line therapy regimens that do not require pretreatment RAS detection. The 2020 EASL Recommendations on Treatment of Hepatitis C state that in areas where the regimens are not available or not reimbursed, physicians who have access to reliable resistance tests can use the results to guide their decisions, according to[50]. Thus, the selected retreatment option depends on the availability of RAS testing, the actual access to the DAA agent indicated in the event of the failure, and the preference of the treating physician.

CONCLUSION

In the current clinical setting, there is no need for baseline detection of RAS before DAA therapy initiation in naïve patients. The use of adequate pangenotypic regimes may overcome the effect of RAS in the first treatment. After treatment failure, RAS may be determined when available. Otherwise, SOF/VEL/VOX for 12 wk is the regimen of choice, as it has shown the highest SVR rates. GLE/PIB for 16 wk is an alternative regime and it may be used in patients who have failed NS5A or NS3 inhibitors, but not a combination of both. Failure to treatment with multiple DAA regimens may be the clearest clinical scenario for RAS detection. In such cases, rescue treatment can be guided based on the results. If after many failures, RAS detection is not available, treatment should be evaluated by multidisciplinary teams. SOF/VEL/VOX or SOF/GLE/PIB with RBV for 12 wk or without RBV for 16-24 wk are the regimens of choice as they have shown effectiveness in curing these difficult-to-treat patients.

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MINIREVIEWS

Indeterminate liver lesions on gadoxetic acid-enhanced magnetic resonance imaging of the liver: Case-based radiologic-pathologic review

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Abstract

Different histopathological manifestations of focal liver lesions show varying common and uncommon imaging findings and some pathologies may show similar appearance despite of different histopathology. It is necessary to characterise focal liver lesions accurately as not only benign and malignant lesions are managed differently, but also certain benign lesions have differing management. These lesions are increasingly being detected due to rapid growth of use of cross-sectional imaging as well as improvement in image quality and new imaging techniques. Contrast enhanced magnetic resonance imaging (MRI) is considered the gold standard technique in characterising focal liver lesions. Addition of gadoxetic acid has been shown to significantly increase diagnostic accuracy in the detection and characterization of liver abnormalities. Classic imaging characteristics of common liver lesions, including their behaviour on gadoxetic acid enhanced MRI, have been described in literature over recent years. It is important to be familiar with the typical aspects of these lesions as well as know the uncommon and overlapping imaging features to reach an accurate diagnosis. In this article, we will review the well-described characteristic imaging findings of common and rare focal liver lesions and present several challenging cases encountered in the clinical setting, namely hepatocellular adenoma, focal nodular hyperplasia, hepatic angiomyolipoma, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, neuroendocrine tumours as well as a pleomorphic liposarcoma of the liver.

Key Words: Indeterminate liver lesions; Magnetic resonance imaging; Gadoxetic acid; Hepatobiliary phase; Hepatocellular carcinoma

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Core Tip: Being familiar with the typical magnetic resonance imaging aspects of focal liver lesions as well as knowing the uncommon and overlapping imaging features can help reach an accurate diagnosis without the need for further interventions. Gadoxetic acid has been shown to significantly increase diagnostic accuracy in the detection and characterization of liver abnormalities, although in certain challenging cases it may be prudent to seek histological confirmation.

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INTRODUCTION

Recent years have seen a rapid growth of the use of cross-sectional imaging as well as an increase in image quality and new imaging techniques. This has led to a rise in the detection of a variety of benign and malignant focal liver lesions. It is necessary to characterise focal liver lesions accurately as not only benign and malignant lesions are managed differently, but also certain benign lesions have differing management. The ability to accurately identify various liver lesions on imaging also saves the patient from biopsy or other invasive interventions needed to reach a diagnosis, which carry associated complications such as bleeding, abdominal pain, or even mortality[1,2].

Contrast enhanced magnetic resonance imaging (MRI) is considered the gold standard technique in characterising focal liver lesions because it provides superior tissue contrast resolution, safe contrast agent profile and is ionising radiation free. Gadoxetate disodium (Primovist, Bayer Schering Pharma), also known as gadoxetic acid, in particular, has been shown to significantly increase diagnostic accuracy in the detection and characterisation of focal liver lesions[3,4]. It provides dynamic vascular phases [arterial phase (AP), portal venous phase (PVP) and equilibrium phases] and due to its progressive distribution into functional hepatocytes and bile ducts also a hepatobiliary phase (HBP). Gadoxetic acid has been demonstrated to be invaluable in detecting hepatocellular carcinoma (HCC) in the cirrhotic liver and distinguishing between focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA)[4-6].

Different histopathological manifestations of focal liver lesions show varying common and uncommon imaging findings and some pathologies may show similar appearance despite different histopathology. Classic imaging characteristics of common liver lesions, including their behaviour on gadoxetic acid enhanced MRI, have been described in literature over recent years. It is important to be familiar with the typical aspects of these lesions as well as know the uncommon and overlapping imaging features to reach an accurate diagnosis. In this article, we will review the well-described characteristic imaging findings of focal liver lesions and present several challenging cases encountered in the clinical setting.

BENIGN LESIONS

HCA

HCA is a rare benign liver tumour which occurs predominantly in young and middle-aged women and is associated with the use of oral contraceptives or other steroid medications. In contrast to other benign liver tumours, an HCA may be complicated by malignant transformation or bleeding[7]. As such, because of its serious clinical consequences, an HCA is often treated with surgical resection while FNH is managed conservatively in the majority of cases, without the need for surgical intervention. Therefore, accurate diagnosis is important. The use of MRI with a hepato-specific contrast agent, specifically gadoxetic acid, makes the diagnosis relatively easy to reach [5,8,9].

Generally, typical MRI findings seen in HCA include mild to moderate high signal intensity on T2 weighted imaging (T2-WI), sometimes with small cystic areas or

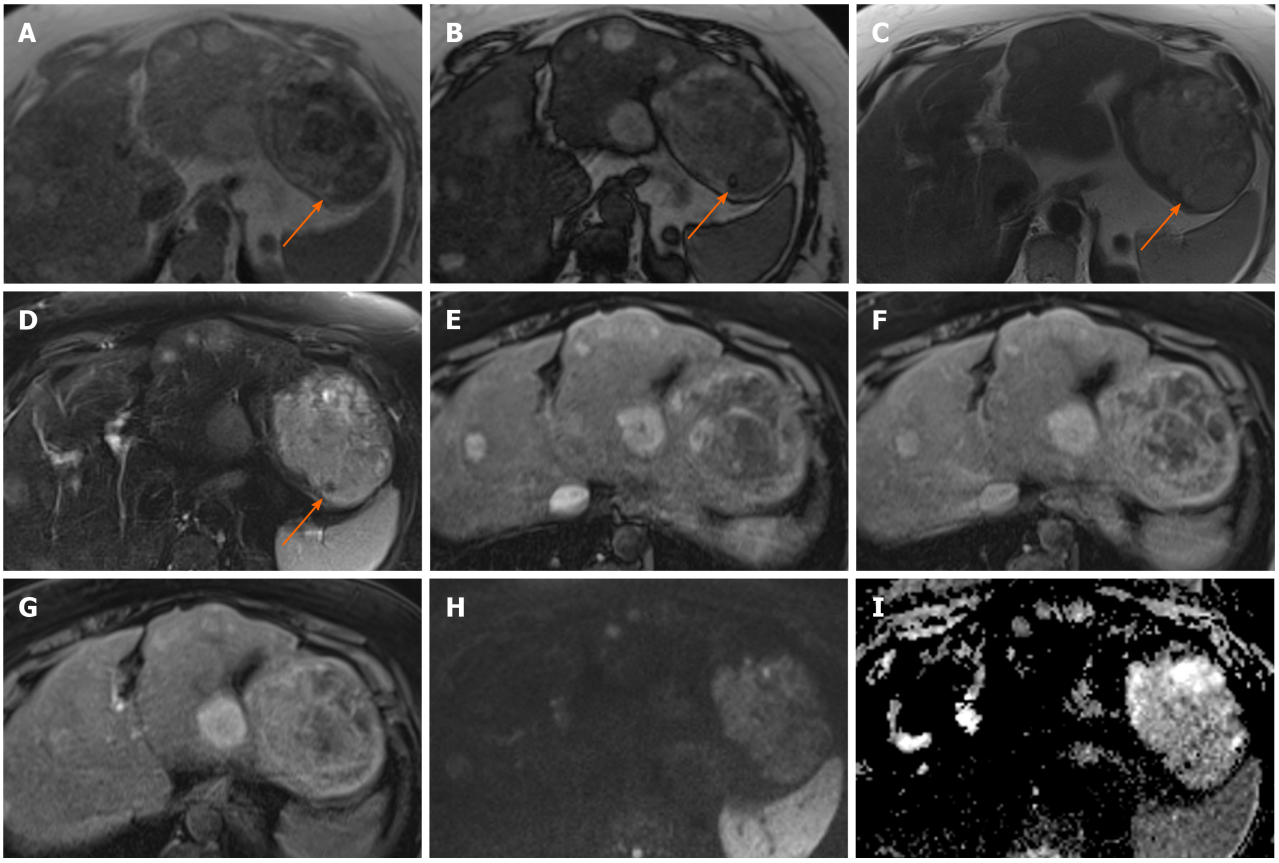

Figure 1 Hepatocellular adenoma. A 42-year-old lady with congenital absence of portal vein and history of use of oral contraceptive medication presented with worsening jaundice. She underwent computed tomography that demonstrated multiple liver lesions that could not be characterised and subsequent magnetic resonance with gadoxetic acid was performed. This demonstrates multiple small lesions showing characteristics those of focal nodular hyperplasia. There is a further exophytic large lesion arising from the left liver lobe. The lesion is well-defined, T2 hyperintense and shows intratumoral fat (arrowed). A: In phase T1; B: Out-of-phase T1; C: T2-weighted imaging (T2-WI); D: Fat suppressed T2-WI; E-G: The arterial (E) and equilibrium (F) phase sequences demonstrates heterogenous enhancement with progressive filling in and there is contrast retention on hepatobiliary phase (G); H and I: Diffusion-weighted imaging (H) and apparent diffusion coefficient (I) sequences show no restricted diffusion. Due to atypical appearances this was resected and histology revealed this to be an adenoma with background steatotic liver.

diffuse homogeneous steatosis of the lesion and it may show internal bleeding or atoll sign. FNH classically shows the presence of a T2-weighted (T2-W) hyperintense central scar. Both lesions show enhancement on the AP imaging and tend to be isointense in the PVP[10]. In particular, when compared with background liver parenchyma, on the HBP image an HCA is hypointense in the majority of cases whereas FNH is hyper- or isointense. FNH is composed of functional hepatocytes with abnormal biliary ductules and is therefore expected to accumulate hepatobiliary specific contrast agents, while HCA traditionally has been thought of as not having bile ductules and would often be expected to not retain such contrast[8].

The diagnostic conundrums are usually encountered when differentiating between HCA and malignant entities and characterising different molecular types of HCA (Figures 1 and 2). HCAs are classified into few major molecular subtypes: HNF1α inactivated HCA (H-HCA), inflammatory HCA (IHCA), β-catenin activated HCA (β-HCA) and β-catenin activated inflammatory HCA (β-IHCA) and sonic hedgehog HCA. The term Unclassified HCA is applied to those HCAs in which no specific mutation is identified[11]. The highest risk of malignant transformation was shown in mixed β-catenin-activated and inflammatory and β-catenin-activated forms[11]. Hepatobiliary contrast agent retention in the HBP can be seen in 83% of β-HCAs, 29% of IHCAs and not been demonstrated in H-HCA and unclassified HCAs[12]. Hyperintensity on HBP of HCAs could potentially help identify HCAs at high risk of malignancy[13]. However, this feature of high-risk HCAs makes it harder to differentiate radiologically from FNH which is hyperintense on HBP. Other MRI features may be helpful such as the presence of a central scar, the heterogeneous "periseptal" uptake of FNH on HBP, or other MR phases features. In addition, β-HCA typically demonstrates a subtle heterogenous hyperintense signal on T2-WI MRI, unlike FNH[12]. It is suggested that in patients with inflammatory HCA risk factors (such as obesity, metabolic syndrome,

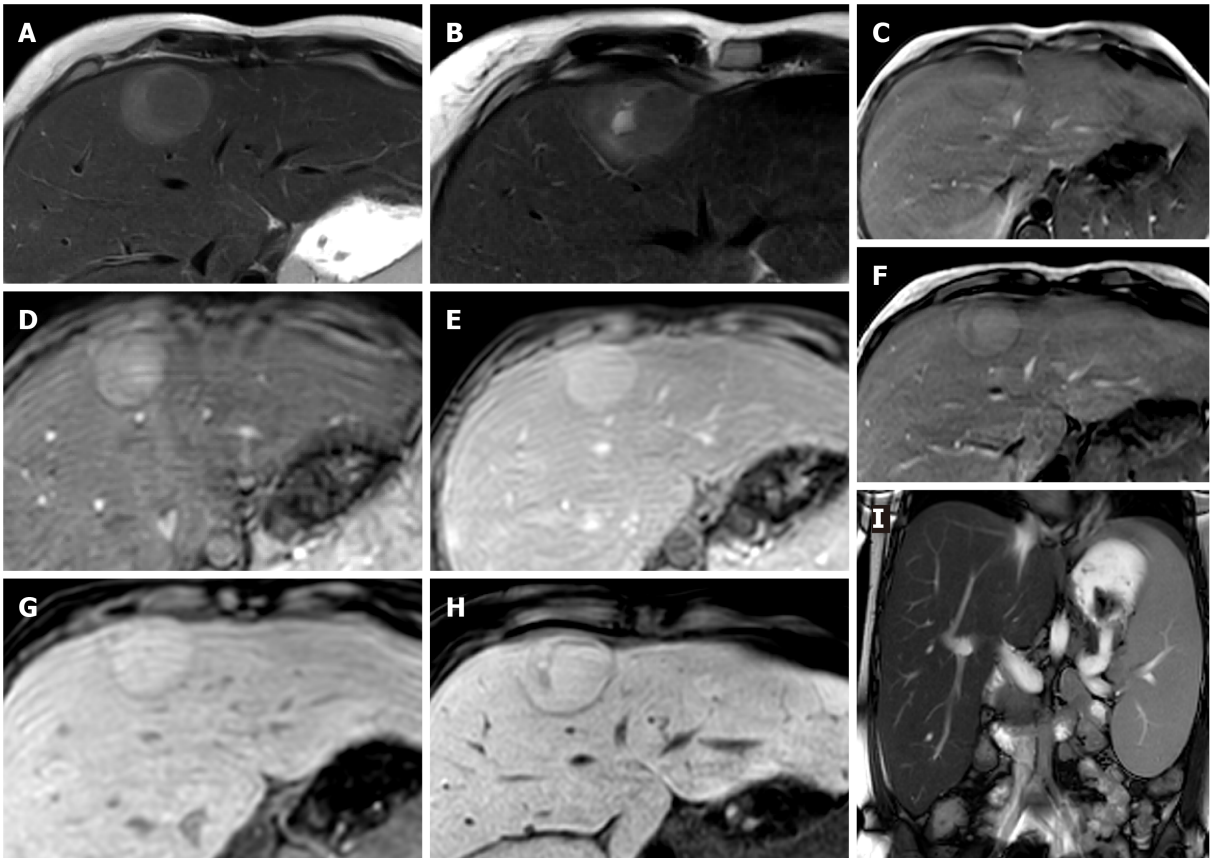

Figure 2 Hepatocellular adenoma. A 27-year-old lady with background of glycogen storage type 1 disease. A and B: Segment IVA liver lesion demonstrating mild T2 hyperintensity with atoll sign (A) and cystic foci (B); C and F: No signal drop out on out-of-phase (F) when compared to in-phase (C) T1-weighted sequence; D, E and G: There is quite homogenous hyperenhancement on arterial phase (D) with no washout on portal venous (E) and delayed (G) phases; H: Hepatobiliary phase shows contrast retention within the lesion; I: Coronal T2-weighted shows hepatosplenomegaly as features of glycogen storage disease type I. The lesion has increased in size and therefore was resected, histology revealed an inflammatory subtype hepatocellular adenoma.

and alcohol use), relying on MRI features alone to differentiate FNH from inflammatory HCA may not be appropriate[8]. Histopathological analysis may be required in certain cases still, in order to achieve the final diagnosis.

FNH

FNH is the second most frequent benign hepatic tumour (haemangioma being the most common). It is found most typically in women in their 3rd-5th decades of life. FNH is rarely symptomatic and usually found incidentally[14], unless very large in which case it can cause vague abdominal pain. There is some debate whether FNH is caused by or associated with use of oral contraceptives, but it may promote the growth of FNH. An FNH, contrary to HCA, has no malignant potential or life-threatening complications, and as such a surgical resection or further evaluation is not required if a diagnosis can be made confidently on imaging.

FNH is believed to represent a local hyperplastic response of hepatocytes to a congenital vascular anomaly. It is a proliferation of normal, non-neoplastic hepatocytes that are abnormally arranged. Normal portal venous structures are not present, but most lesions contain thick-walled arterial vessels that provide outstanding arterial supply; therefore haemorrhage, infarction and necrosis would be extremely rare[14]. Although the lesions have well-demarcated margins, they do not have a true capsule, which is consistent with their hyperplastic rather than neoplastic nature.

Typical MR features of FNH are iso- or mild hypointensity on T1-weighted imaging (T1-WI) and an iso- or slightly hyperintense lesion on T2-W sequences. FNH is known to have a classic central stellate fibrovascular scar, which is only seen in about 50% of cases and when present usually shows a high signal intensity on T2-WI. FNH is homogeneously and strongly enhanced on AP except for the central scar. It becomes isointense to the liver parenchyma during portal phase, with the central scar remaining relatively hypointense. The central scar typically shows enhancement in delayed phase. On the HBP FNH becomes iso- to hyperintense compared to surrounding liver without or with hypointense central scar[10]. Size of > 5 cm,

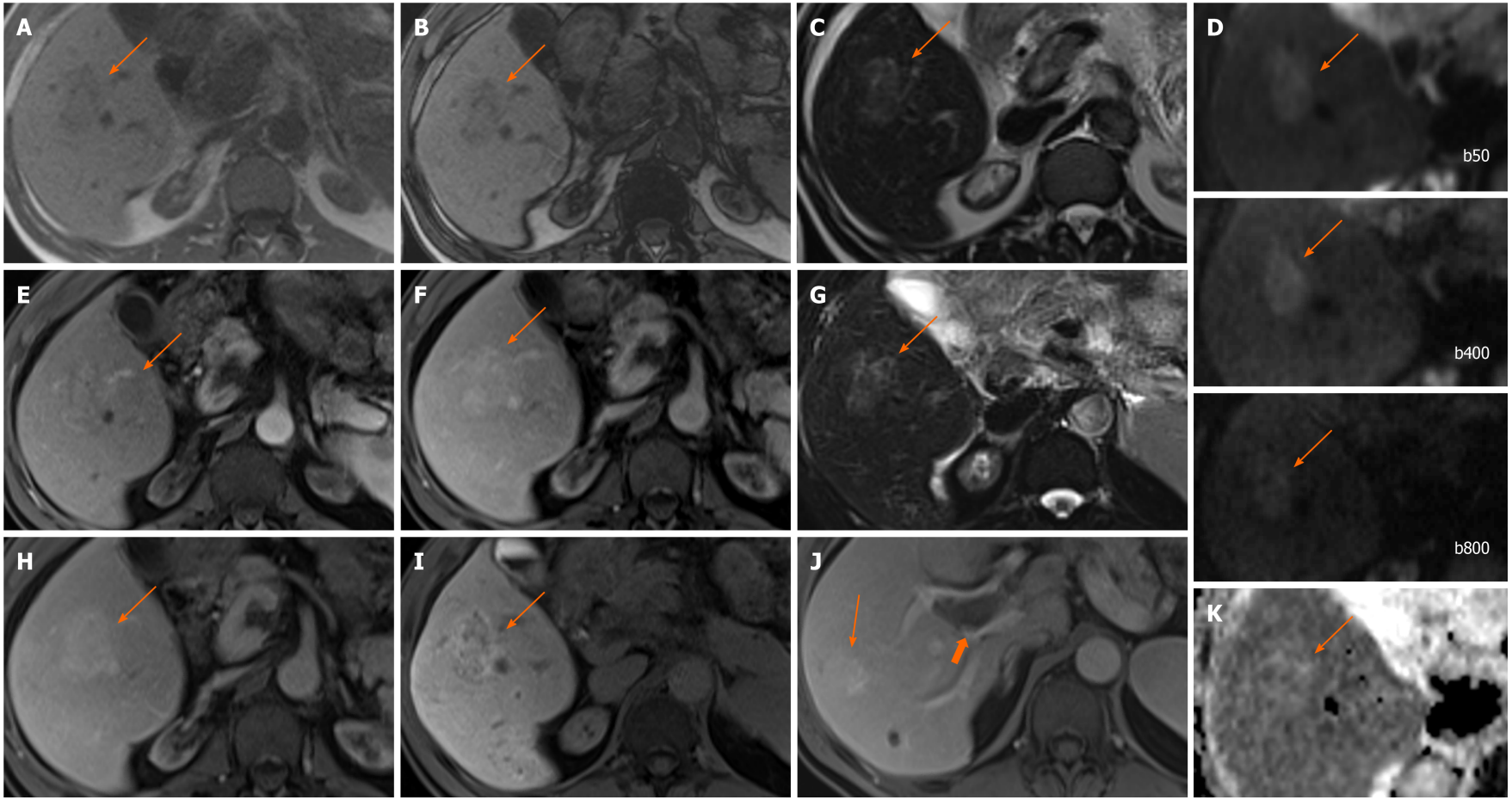

Figure 3 Focal nodular hyperplasia. A 53-year-old woman with background of renal failure with renal transplant and history of autoimmune hepatitis since childhood. She underwent ultrasound (US) of the abdomen after an episode of pancreatitis which identified portal vein thrombosis. Subsequent unenhanced computed tomography (due to poor renal function) demonstrated a liver lesion in segment 5. Initially contrast US was attempted due to renal failure, which showed liver lesions to be multiple, but the lesions were indeterminate and subsequent magnetic resonance with gadoxetic acid was performed. Largest lesion in segment 5 selected as example. A and B: In-(A) and out-(B) of phase imaging shows some signal loss and mildly hypointense T1-weighted signal of the ill-defined right lobe lesion; C and G: T2-weighted without (C) and with fat suppression (G) show mildly hyperintense T2 signal; D and K: Diffusion-weighted imaging (D) and apparent diffusion coefficient (K) images show no diffusion restriction. E, F, and H: There is heterogenous enhancement on arterial phase (E) with no washout and slightly more homogenous contrast enhancement on portal venous (F) and delayed (H) phases; I and J: Heterogenous contrast uptake persists on hepatobiliary phase (I), which is mostly rim-like. Further similar lesion demonstrated on portal venous phase (J) in segment 7 (long arrow) and the known portal vein thrombus (short arrow). Initial radiological diagnosis favoured hepatocellular carcinoma. Liver function tests were normal. Initial non targeted liver biopsy was inconclusive for underlying cirrhosis. Second targeted lesion biopsy was performed. Both specimens were further reviewed in a national liver centre. Histology of the lesion was consistent with focal nodular hyperplasia and background liver demonstrated no cirrhosis, but signs consistent with nodular regenerative hyperplasia.

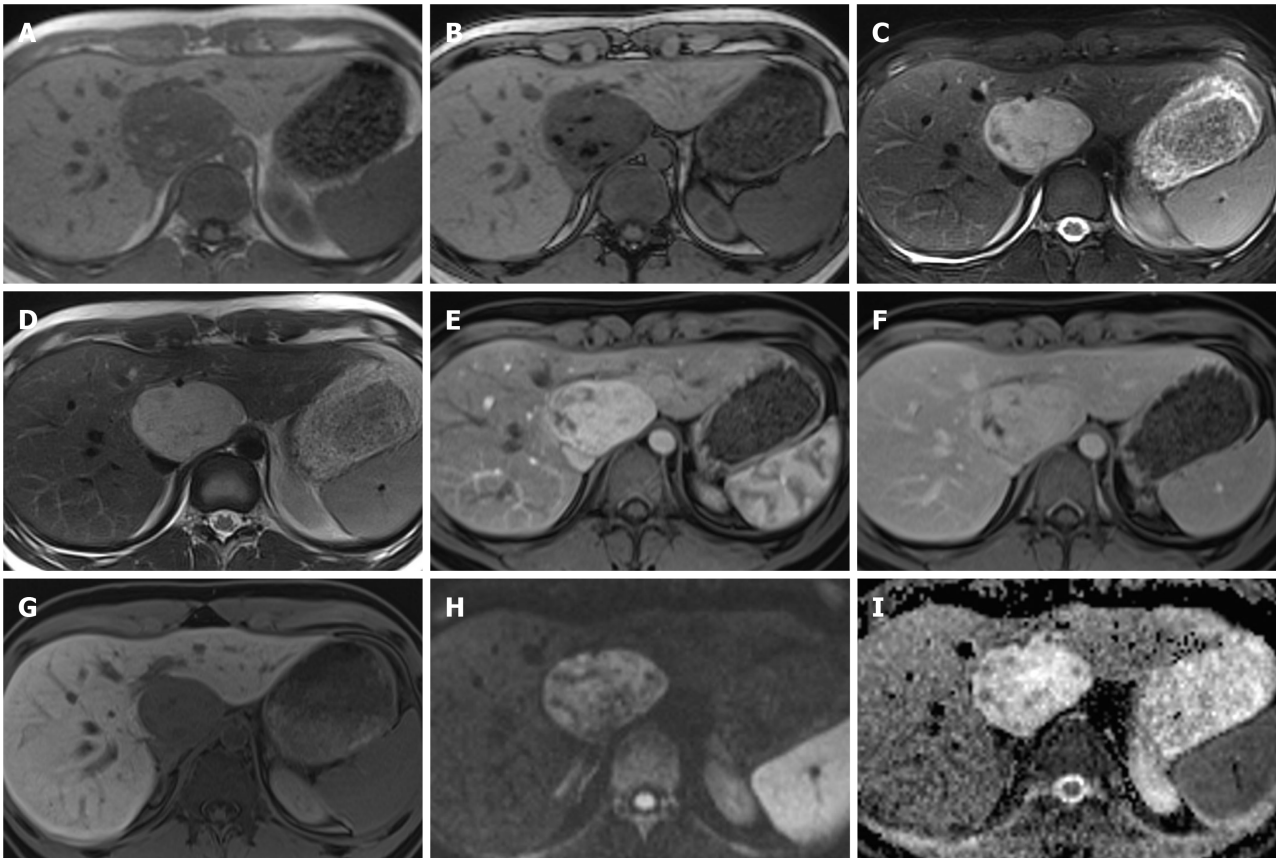

Figure 4 Hepatic angiomyolipoma. A 21-year-old man referred by general practitioner for ultrasound of liver due to 6-mo history of intermittent abdominal pain and isolated raised bilirubin, treated as Gilbert's syndrome. The patient had no prior medical history, no use of drugs or steroids and was not a heavy drinker. Incidental liver lesion was found and patient underwent subsequent magnetic resonance (MR) with gadoxetic acid to characterise this further. This was initially described as adenoma, but as the lesion increased in size on follow up imaging it was resected. Histology showed this to be an angiomyolipoma. A and B: MR demonstrates well-defined lesion with high signal foci on T1 in-phase (A) showing loss of signal on out-of-phase imaging (B); C and D: There are also hypointense foci on fat suppressed T2-weighted (C) when compared to T2-weighted imaging without fat suppression (D); E and F: The lesion shows enhancement on arterial phase (E) with no washout on equilibrium phase (F) and no pseudocapsule; G: There is no contrast uptake on hepatobiliary phase; H and I: No diffusion restriction as seen on diffusion-weighted imaging (H) and apparent diffusion coefficient (I) sequences.

presence of multiple lesions and evidence of haemorrhage and necrosis are considered atypical[15]. Rarely FNH may contain fat. Cases mimicking HCC, for example complete perfusion defect on HBP[16], and various enhancement patterns (Figure 3), such as a peripheral ring-like enhancement without a visible central scar, have also been described[16,17].

Hepatic angiomyolipoma

Hepatic angiomyolipoma (HAML) is a rare, hepatic mesenchymal neoplasm which more frequently occurs in the kidneys, with the liver representing the second most common site of involvement[18]. It is found in both males and females, and in a majority of cases is asymptomatic. The tumour consists of 3 components: fat, vascular and smooth muscle. These components can vary significantly within each lesion and it is this heterogeneity that proves the preoperative diagnosis by imaging difficult (Figure 4).

The presence of fatty areas and solid tissue components is considered typical, however due to a significant overlap of the imaging features, most HAMLs are misdiagnosed as HCC with fatty metamorphosis. Both of these lesions show comparable dynamic enhancement patterns during the AP, followed by low signal intensity on PVP or late dynamic phases[19,20]. Generally, HAMLs are lacking hepatocytes, whereas HCCs contain hepatocytes with various degrees of malignant change, which in turn leads to a more homogeneous hypointensity on HBP compared with that of the spleen and sharper margins in HAML, compared to heterogeneous signal intensity and the ill-defined margin of HCCs at the HBP[19].

In a study by Wang *et al*[21], absence of a pseudo capsule, presence of an early draining vein and tumour vessels, and a higher apparent diffusion coefficient (ADC) in the hypervascular hepatic tumour on the MRI were helpful to distinguish a HAML

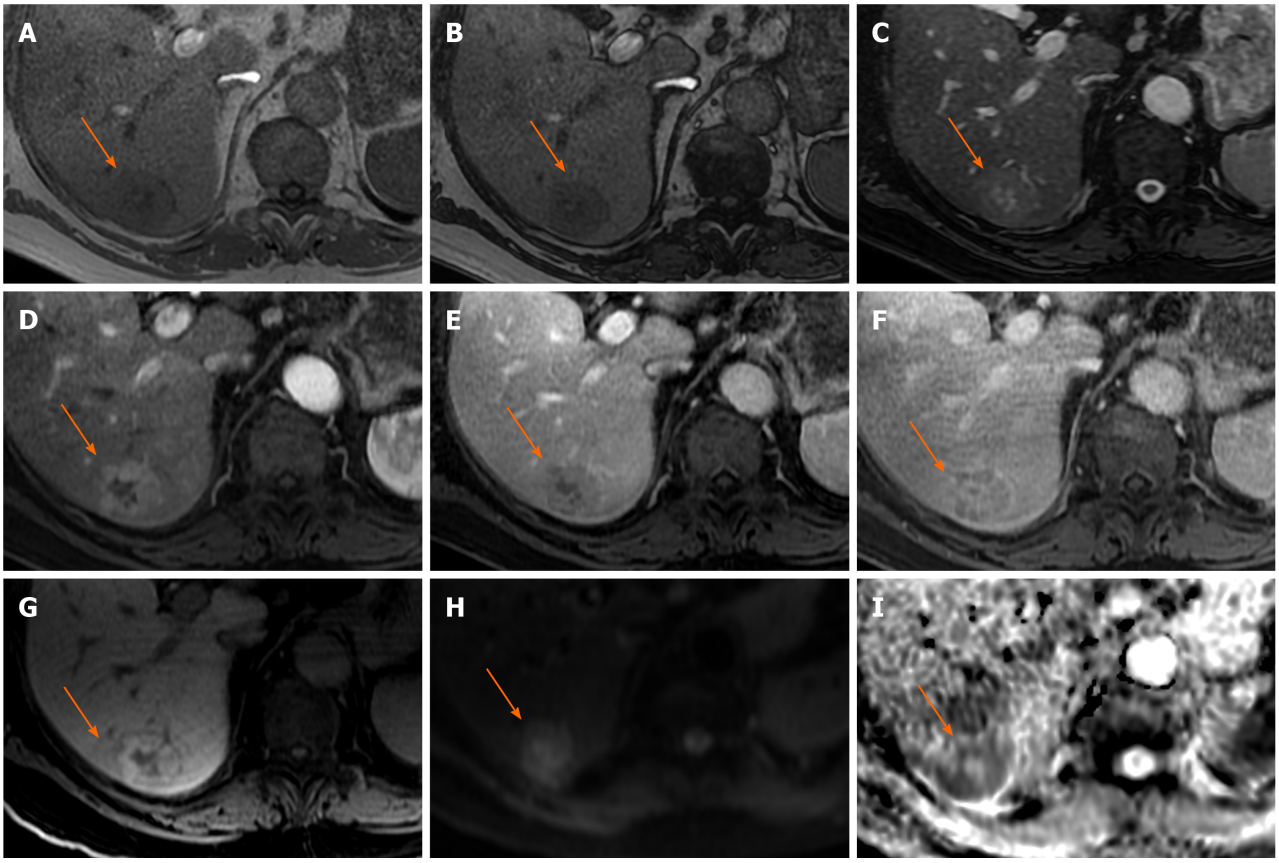

Figure 5 Hepatocellular carcinoma. A 74-year-old man presented with incidental liver lesion found on routine computed tomography colonography. He had normal liver function and alpha-fetoprotein levels. The lesion had undergone further characterisation with magnetic resonance. A and B: There is no evidence of intralesional fat on T1-weighted in-phase (A) and out-of-phase (B) sequences; C: On T2-weighted images, the lesion is nearly isointense to the background liver and shows a hyperintense central scar, which can sometimes be seen in focal nodular hyperplasia; D-F: The lesion then demonstrates enhancement on the arterial phase (D) with evidence of washout as compared to background liver parenchyma on the portal venous (E) and delayed phases (F); there is also subtle peripheral enhancement on the delayed phase, likely representing a capsule, but the central scar remains largely unenhanced throughout; G: Hepatobiliary phase sequence demonstrates uptake of contrast in the majority of the lesion, with no uptake in the central scar and rim; H and I: diffusion-weighted imaging 500 (H) and low apparent diffusion coefficient (I) images suggest areas of diffusion restriction. Due to patient's age, gender and indeterminate contrast characteristic, the lesion was resected. Histology showed the lesion was a well to moderately differentiated hepatocellular carcinoma. There was no background cirrhosis, but evidence of mild steatosis.

from fat-containing HCC. The presence of an early draining vein is considered a conspicuous dilated or non-dilated vessel originating from the tumour with draining to the portal vein, hepatic vein, or inferior vena cava. A tumour pseudo capsule is defined as a thin hyperintense rind in the equilibrium phase.

Although historically HAML is considered a benign lesion, few case reports have discovered a potential for malignant transformation with evidence of recurrence[20,22,23]. As such, the potential risk of malignant changes of HAML needs to be recognised and some authors suggest that these lesions should be followed up after surgery.

MALIGNANT LESIONS

HCC

HCC is the commonest primary hepatic malignancy, showing an increasing worldwide prevalence[24,25]. Cirrhosis constitutes a crucial risk factor for the development of HCC with the estimated prevalence of cirrhosis among patients with HCC of 80%-90%[26]. Having an underlying liver disease impacts the management and therapeutic options. Due to high rates of intrahepatic recurrence, the prognosis for patients with advanced HCC remains poor[27], however when diagnosed at an early stage, curative treatments such as surgical resection, liver transplantation, and radiofrequency ablation are possible. Hence, precise imaging diagnosis in patients with early-stage HCC is crucial.

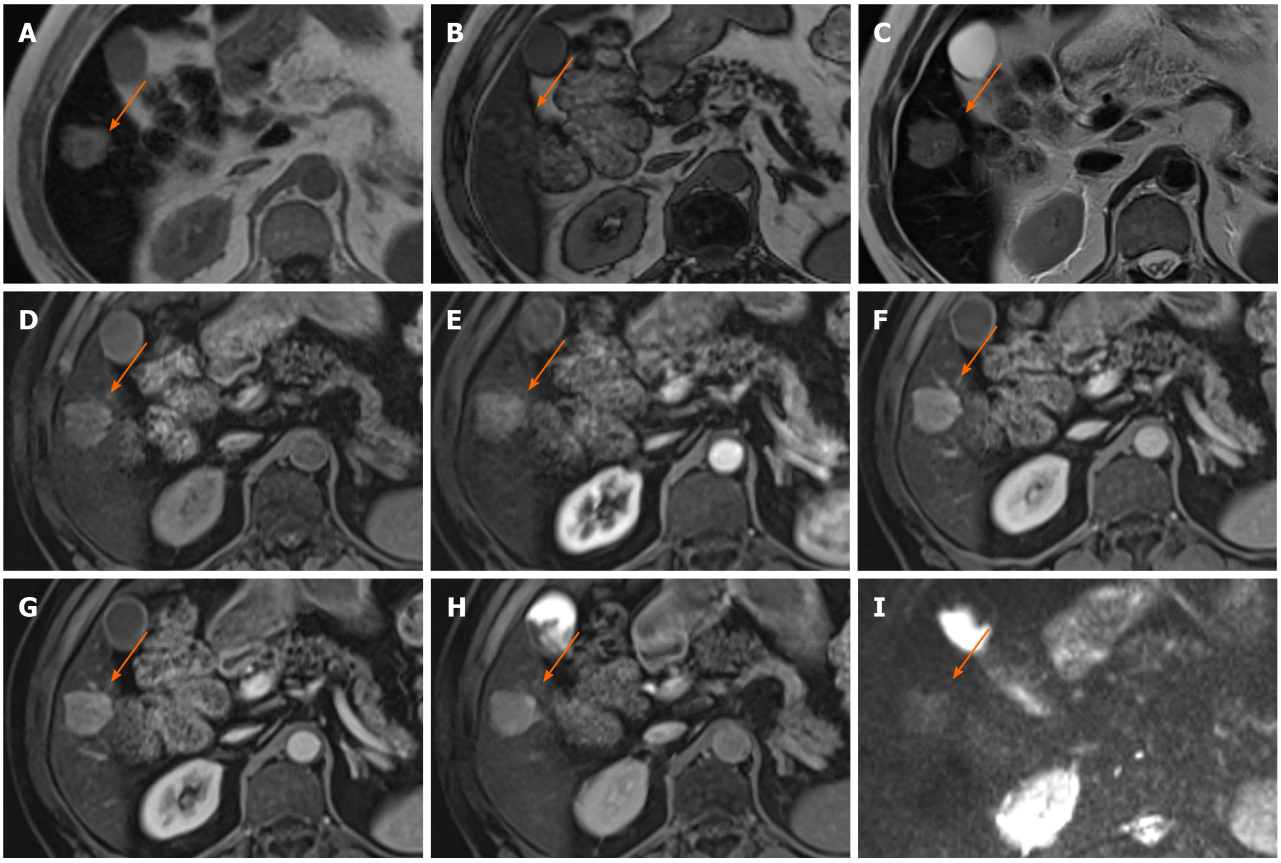

Figure 6 Hepatocellular carcinoma. A 80-year-old man presented with haematuria and was found to have an incidental liver lesion on computed tomography. His liver function tests were normal. A and B: Magnetic resonance demonstrates signal loss throughout the liver, with paradoxical increase in signal on out-of-phase (B) imaging when compared to in-phase (A), suggestive of underlying iron overload; C: Segment 5 liver lesion shows signal loss on out-of-phase sequences suggesting fat contents and is of high T1 and T2 signal; D: Pre-contrast images; E-G: Subtraction sequences were not performed, but allowing for this, there is some enhancement on arterial phase (E), which persists into portal venous (F) and delayed phases (G); H and I: There is contrast retention on hepatobiliary phase (H) and no diffusion restriction (I–b400). Further tests performed confirmed genetic hemochromatosis. Portal venous pressure measurement also showed portal hypertension. Lesional biopsy confirmed this to be a moderately differentiated hepatocellular carcinoma in a background of cirrhosis, which was subsequently ablated.

To address this, the Liver Imaging Reporting and Data System (LI-RADS) was created. It is a comprehensive system for standardising the terminology, technique, interpretation, reporting, and data collection of liver imaging. The primary blood supply of normal hepatocytes is *via* the portal venous system, in contrast to HCC which is supplied by abnormal hepatic arteries. Consequent imaging features are of a lesion which enhances during the late AP (non-rim) with subsequent progressive washout of contrast relative to background liver parenchyma and a peripheral rim of enhancement (pseudocapsule) on either PVP or delayed phase imaging[28,29]. Apparent hypointensity relative to liver in the transitional phase may potentially represent hyperenhancement of liver rather than reduced enhancement of the mass, therefore it is recommended that when gadoxetate disodium is administered as contrast media, washout is evaluated only in the PVP[30]. Additional major LI-RADS features include threshold growth (increase in size of 50% or more within 6-mo time during follow-up imaging) and size.

Hypointensity on HBP is considered an ancillary feature favouring malignancy and HBP isointensity an ancillary feature suggesting benignity[28]. However, hyperintensity on HBP phase has been demonstrated in 8.8%–13.6% of HCCs[31,32]. Such HCCs are rather difficult to differentiate from FNH on gadoxetic acid enhanced MR (Figures 5-9).

A study by Kitao *et al*[33] found that the washout pattern was observed in only 57% of HBP hyperintense HCCs at dynamic MRI *vs* 95.8% on dynamic computed tomography (CT). The reason for this is thought to be that gadoxetic acid is already taken up into tumour cells in the transitional phase by hyperintense HCCs. Therefore, the addition of CT may be helpful as AP enhancement and washout pattern at dynamic CT, as well as a decrease in ADC ratio, were shown to be independent predictors of hyperintense HCC[33]. Overall, hyperintense HCCs seem to have clinical and histologic features that might be related with more favourable outcomes[31].

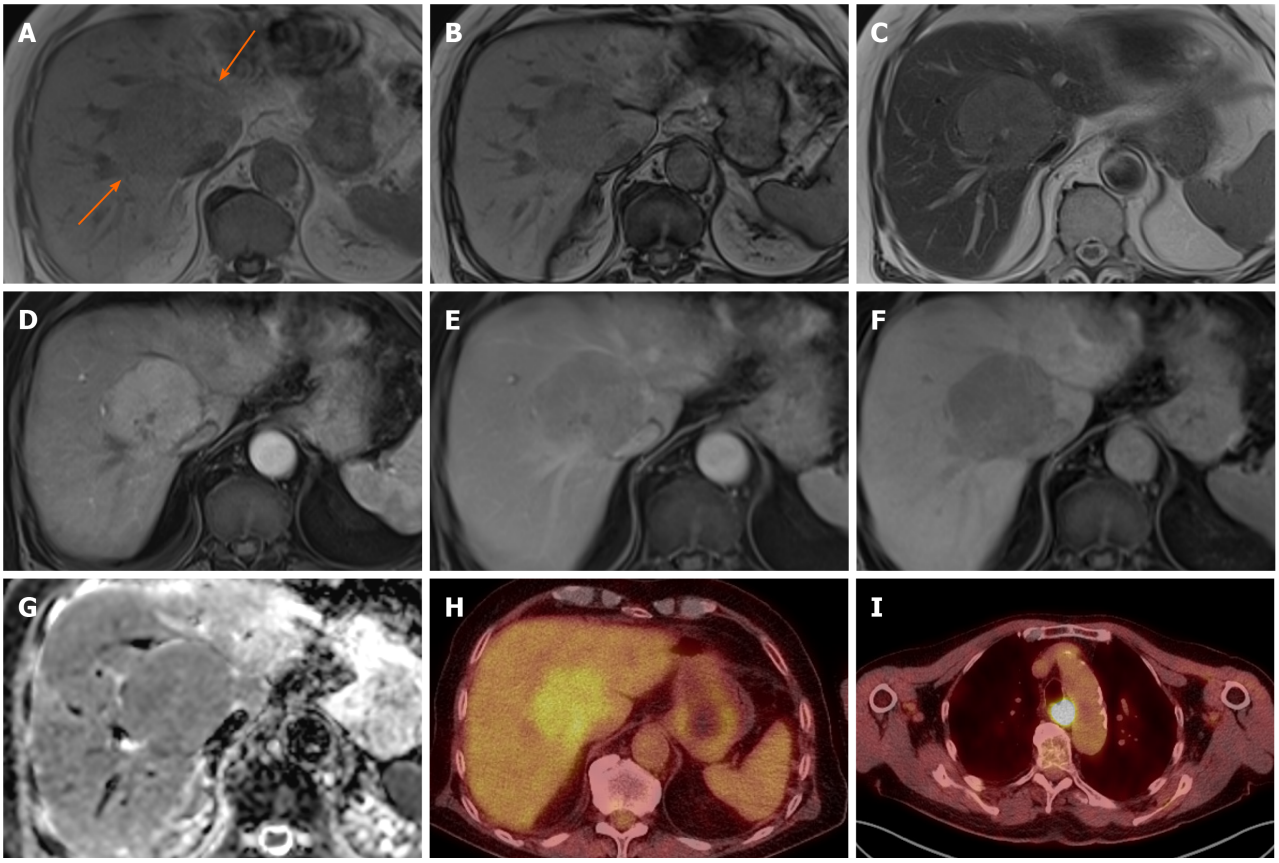

Figure 7 Hepatocellular carcinoma. A 79-year-old with previous prostate cancer has undergone a magnetic resonance (MR) pelvis and was found to have prostatic cancer recurrence and a liver mass. He has undergone staging computed tomography which showed a further area of oesophageal thickening. Endoscopy revealed oesophageal tumour and biopsy confirmed this to be a squamous cell carcinoma. MR liver and positron emission tomography (PET) scan were performed to characterise these and determine whether liver lesion is a metastasis from oesophageal or prostate primary. Alpha-fetoprotein value was 10 at time of diagnosis. A and B: In- (A) and out-of-phase (B) sequences show low T1 signal liver mass with no intratumoral fat; C: It is of mildly high signal on T2 sequences; D and E: There is homogenous arterial enhancement (D) with washout on portal venous (E) phase; F and G: No contrast retention on hepatobiliary phase (F) and isointense to low signal on apparent diffusion coefficient (G); H and I: PET scan shows tracer uptake within the liver lesion (H), however this is of lower standardized uptake value than the oesophageal cancer (I). Targeted liver lesion biopsy confirmed this to be a hepatocellular carcinoma.

An appearance of smooth hypointense rim in the HBP could also improve the detection of tumour capsule and the diagnosis of HCC[34].

Intrahepatic cholangiocarcinoma

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic tumour. Although it accounts for only 3% of gastrointestinal malignancies, the incidence of ICC has been rising worldwide[35]. Risk factors include chemical exposure, liver flukes, biliary tract disease (primary sclerosing cholangitis, hepatolithiasis, Caroli's disease), viral hepatitis, metabolic syndrome, cirrhosis, smoking and alcohol[35,36]. Of note, a large proportion of ICC patients (38.9%) have no identifiable risk factors[36] and further studies are required to explore this.

ICC can be classified into three types according to the Liver Cancer Study Group of Japan classification based on morphologic features with each type demonstrating its characteristic imaging features: Mass-forming (the most common, definite mass in the liver parenchyma), periductal-infiltrating (extends longitudinally along the bile duct, often resulting in dilatation of the peripheral bile duct), and intraductal growth (proliferating towards the lumen of the bile duct like a papilla or tumour thrombus)[37]. As part of the focal liver lesions review, we will discuss the appearances of the mass-forming ICC on gadoxetic acid enhanced MRI.

The mass-forming ICC shows an irregular, but well-defined margin with hyperintensity at T2-WI and low signal at T1-WI. Capsular retraction, encasement of vessels without the formation of a grossly perceivable tumour thrombus, and presence of satellite nodules are often seen[38]. The usual enhancement pattern demonstrated by ICC is peripheral irregular enhancement in the AP and gradual centripetal enhancement on subsequent phases. Similarly to HCC, due to the pseudo-washout effect on gadoxetic acid-enhanced MRI, it is recommended that washout is assessed on

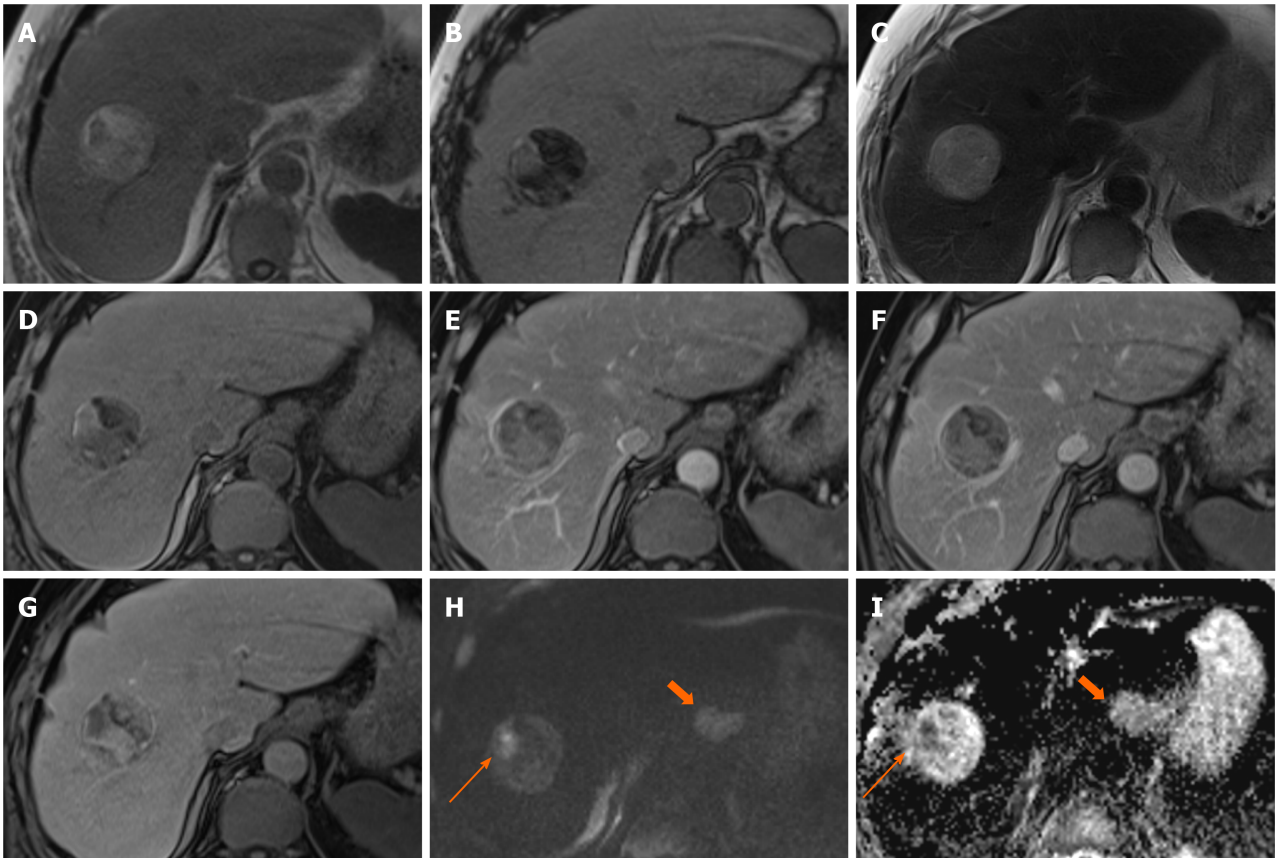

Figure 8 Hepatocellular carcinoma. A 71-year-old underwent computed tomography chest, abdomen and pelvis for anaemia which identified ascending colon thickening and a liver lesion. Colonoscopy confirmed malignant lesion in the ascending colon and histology showed this to be an adenocarcinoma. Magnetic resonance of liver was performed to characterise the liver mass. A and B: This demonstrates a well-defined lesion with the majority of it showing fat component [signal loss on out-of-phase (B) compared to in-phase (A)] except for a small part laterally; C: It is of mildly high signal on T2 sequences; D: Unenhanced sequence; E-G: There are areas of patchy enhancement on arterial (E) and portal venous (F) phases with heterogenous contrast retention on hepatobiliary phase (G); H and I: This part also shows marked diffusion restriction (long arrow, H–diffusion-weighted imaging b800, I–apparent diffusion coefficient). Diffusion sequences also identified a lymph node showing restricted diffusion (short arrow). Subsequent endoscopy was organised which demonstrated an oesophageal lesion, and biopsies of this, and the adjacent lymph node proved it to be a squamous cell carcinoma. Even with two other primaries, the liver lesion was not considered typical for a metastasis radiologically and targeted biopsy was performed. Histology showed well to moderately differentiated hepatocellular carcinoma.

PVP[39,40]. Histologically the viable tumour cells are often seen at the periphery of the tumour, while the central portion is composed of a variable degree of fibrosis. The majority of the tumours with severe fibrosis show delayed enhancement[38]. Intrahepatic mass-forming cholangiocarcinomas lack hepatocytes and in turn are often hypointense on HBP which helps to delineate the lesion itself, the satellite nodules and intrahepatic metastases due to strong enhancement of normal liver parenchyma on HBP[41]. Tumours with intermediate signal intensity on HBP tend to correlate with poor prognosis and histologically are shown to have more abundant fibrous stroma [42]. Therefore, imaging with gadoxetic acid could be used for prognostication. In a study by Choi *et al*[40] peritumoral bile duct dilatation and HBP target appearance (peripheral hypointense rim compared with the central area of the lesion) were independent factors suggestive of ICC (Figure 10).

Neuroendocrine tumours

Neuroendocrine tumours (NETs) consist of a vast heterogeneous group of malignancies which are derived from embryonic neural crest tissue found in various organs. The gastrointestinal tract accounts for 54.5%-73.7% of the tumours[43,44]. Within the gastrointestinal tract, the small intestine is the most common site, followed by the rectum, appendix, colon, and stomach. NETs comprise approximately 1%–2% of all gastrointestinal tumours. In the liver, NETs usually represent metastases from other sites, therefore other primary sites should be examined when a NET is suspected in the liver. Tumours with no identifiable primary site typically originate from unrecognised, small or "burned-out" gastroenteropancreatic NETs[45], however a primary hepatic location, while extremely rare, has been reported in the literature[46-48].

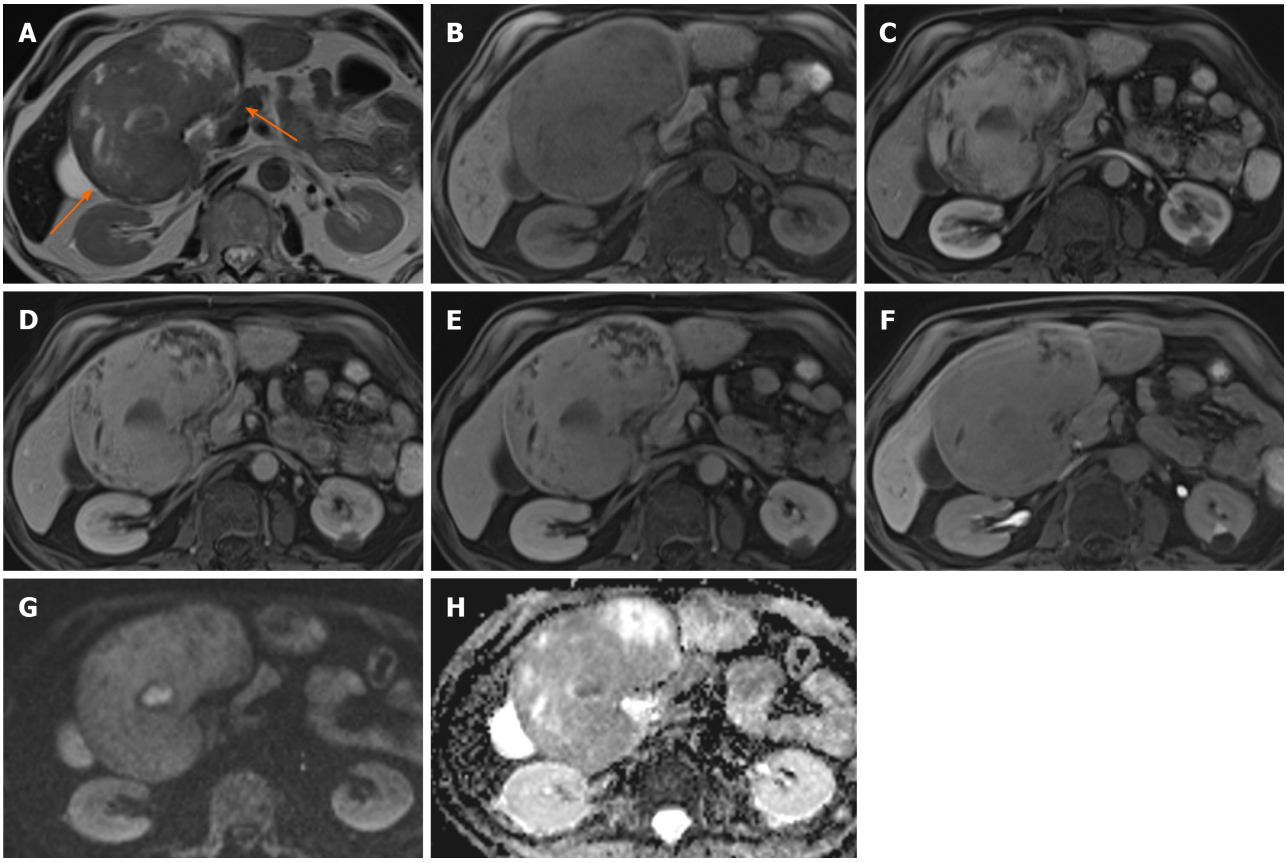

Figure 9 Hepatocellular carcinoma. A 70-year-old man with a transient episode of frank haematuria as part of the investigations into this, was incidentally found to have a large liver mass arising from the left lobe of the liver. He had previous history of tongue cancer. Liver function tests were normal and alpha-fetoprotein was 2 throughout. A: The lesion (arrowed) is mostly hypointense on T2-weighted sequence with heterogenous areas of high signal; B and C: On T1-weighted sequence (B) it shows iso- to hypointense signal and there is heterogenous arterial enhancement (C); D and E: There is some further filling in on portal venous phase (D) where the lesion is now isointense to the liver parenchyma, similarly to delayed phase (E); F: On hepatobiliary phase the mass is hypointense to background liver; G and H: Diffusion-weighted imaging sequence (G) at b value of 800 shows a focal nodule within the lesion that is markedly hyperintense and on apparent diffusion coefficient (H) hypointense in keeping with diffusion restriction. The lesion was resected and histology confirmed moderately differentiated hepatocellular carcinoma.

NET liver metastases generally are hyperintense on T2-WI. Hypervascular metastases regularly show heterogeneous intense enhancement in the AP and ring enhancement is also a frequent finding[49]. Hypovascular metastases are best appreciated on PVP, similar to CT, and appear as low-signal intensity lesions relative to the liver parenchyma (Figures 11 and 12). Perilesional enhancement is frequent in the venous phase. A peripheral low-signal intensity area may be observed on the delayed phase[49]. Because of high signal intensity on T2-WI, NET liver metastases may be difficult to distinguish from cavernous haemangioma, however, unlike NET metastases, haemangiomas do not typically washout and less commonly restrict diffusion. While variable lesion enhancement is seen with dynamic postcontrast images, NET liver metastases generally demonstrate hypoenhancement relative to liver parenchyma on HBP images[50] and HBP imaging is shown to improve detection of NET liver metastases[51,52].

Primary hepatic NETs (PHNETs) generally grow slowly and only become clinically evident at an advanced stage. They most often appear as an endocrinologically silent hepatic mass and are less frequently associated with typical carcinoid syndrome, unlike extrahepatic NETs[47]. In preoperative imaging, PHNETs are often misdiagnosed as HCC or cholangiocarcinoma. Radiological findings are similar for both primary and metastatic NETs[53]. Similarly to NET liver metastases, PHNETs tend to be hypervascular and markedly enhance, and while they are usually solid, cystic PHNETs have been described. Fluid-fluid levels have also been described in some cases[46,54] (Figure 13). Most lesions demonstrate delayed contrast wash-out due to hypervascularity and central necrosis, but progressive enhancement has also been reported[55]. ADC values typically show restricted diffusion.

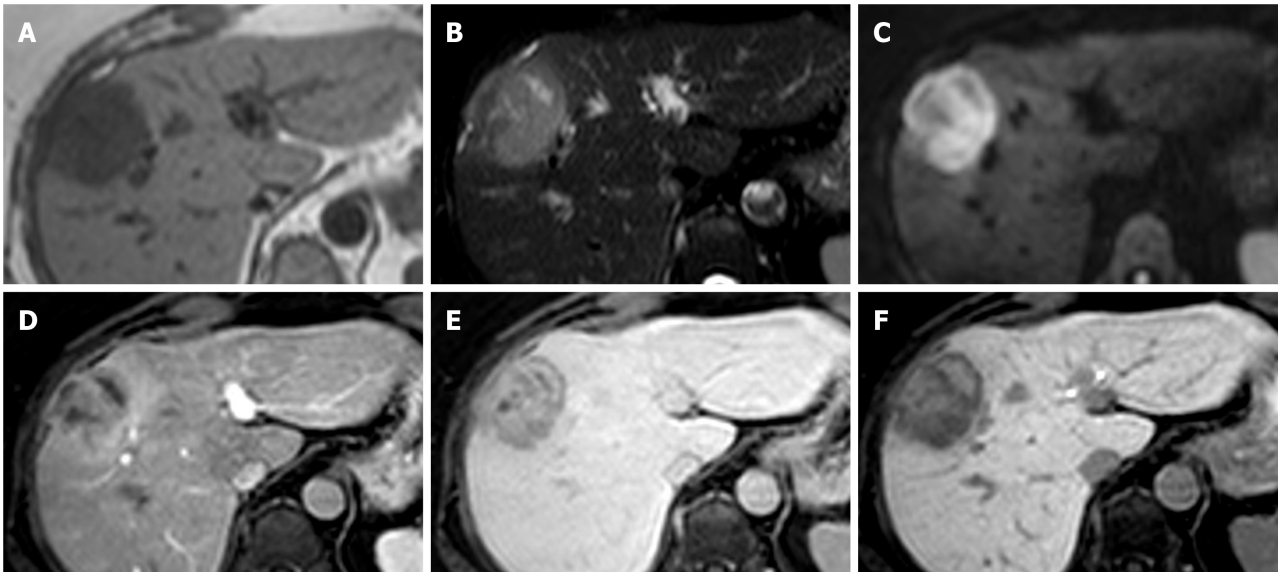

Figure 10 Intrahepatic cholangiocarcinoma. A 64-year-old female with background of hepatitis C cirrhosis was found to have a liver lesion on surveillance ultrasound. Initial magnetic resonance (MR) with extracellular contrast material was reported as likely hepatocellular carcinoma or metastasis. Biopsy confirmed cholangiocarcinoma and gadoxetic acid enhanced MR was organised to exclude satellite lesions and intrahepatic metastases. A-C: MR shows a right liver lobe lesion which is hypointense on T1-weighted imaging (A), hyperintense on T2-weighted imaging (B) and shows diffusion restriction on b800 diffusion-weighted imaging (C); D and E: On arterial phase (D) there is peripheral enhancement with progressive centripetal enhancement on delayed phases (E); F: Hepatobiliary phase shows a hypointense rim with a cloud-like inhomogeneous central enhancement. No further malignant liver lesions demonstrated.

Liposarcoma

Liposarcoma is a rare malignant mesenchymal tumour usually located in the retroperitoneal space and the deep soft tissues of the extremities, particularly those of the thigh. Hepatic location is extremely rare, few cases have been reported in the literature[56]. Early diagnosis of primary liposarcoma of liver is difficult. In liver, they are often misdiagnosed as adenomas (Figure 14).

Generally minimal enhancement is seen in liposarcomas that are well-differentiated, and more so with round cell, pleomorphic, and dedifferentiated subtypes[56]. Associated non-adipose masses, thickened or nodular septa, prominent foci of high T2 signal, and areas of enhancement are all features suspicious for liposarcoma[57]. Higher grade liposarcomas commonly contain little to no macroscopic fat and may not confound the MRI diagnosis of predominantly fatty lesions. Areas of haemorrhage and necrosis can be seen.

CONCLUSION

The various types of liver lesions demonstrate diverse imaging appearances due to common and uncommon features as well as overlapping imaging findings. Familiarising with these entities and their characteristic appearances can help in making an accurate diagnosis.

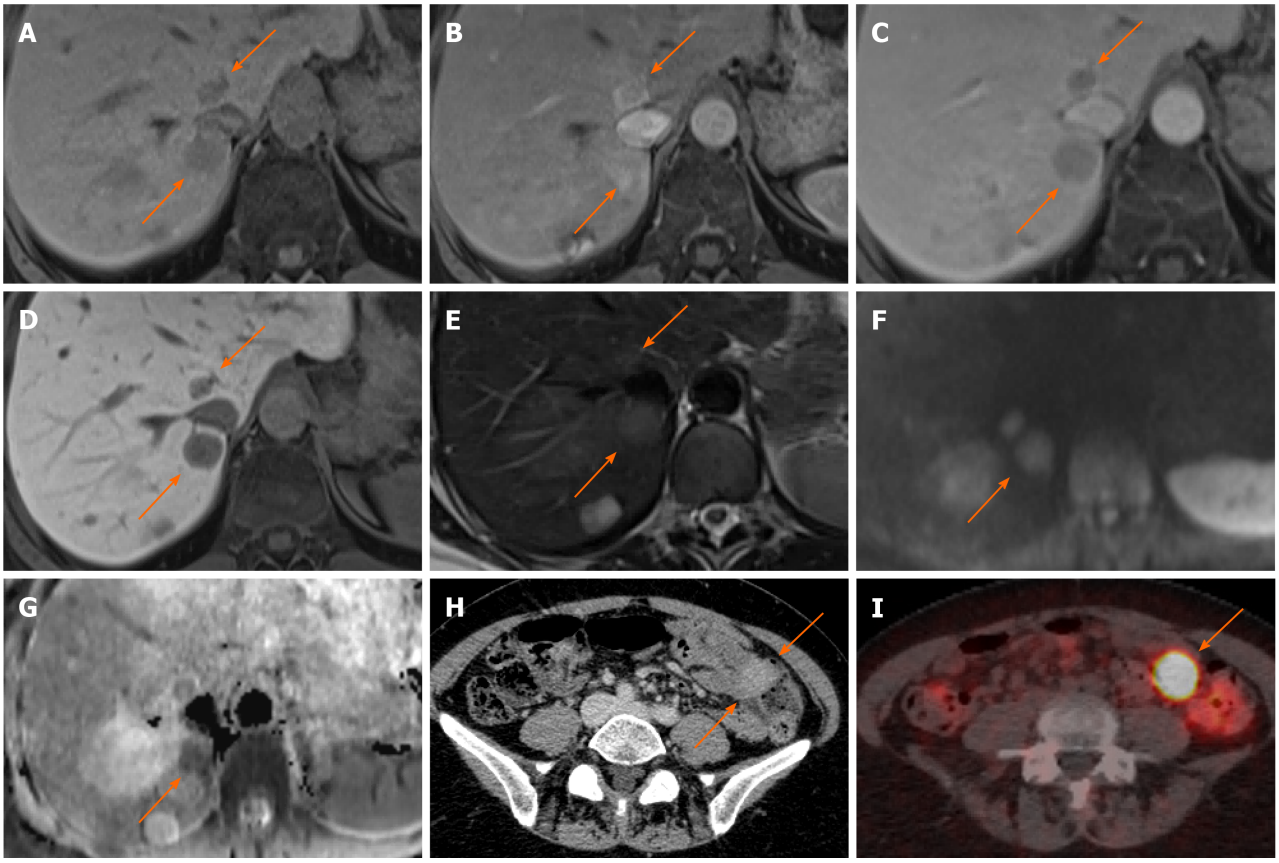

Figure 11 Neuroendocrine carcinoma metastases. A 55-year-old female with anaemia underwent computed tomography (CT) which identified multiple liver lesions. Magnetic resonance liver was performed and confirmed multiple haemangiomas and few other lesions, two of which are shown here, showing atypical appearances. A: Pre contrast phase sequence shows two lesions of low signal on either side of the inferior vena cava; B and C: On arterial phase (B) there is enhancement followed by prompt washout on portal venous (C) phase; D: There is no contrast retention on hepatobiliary phase; E: Lesions are nearly isointense to liver on T2-weighted sequence; F and G: Diffusion weighted imaging (F) at b800 shows hyperintense signal followed by low signal on apparent diffusion coefficient (G) in keeping with diffusion restriction. The nature of these was not clear, but they were suspicious for hypervascular metastases. The patient underwent a number of investigations including oesophago-gastro-duodenoscopy, colonoscopy, CT chest, abdomen and pelvis and an ultrasound scan of pelvis. None of these investigations have identified a primary source of the liver lesions. Targeted liver biopsy was performed and histology revealed well differentiated neuroendocrine carcinoma (Ki-67 = 4%); H: In retrospect, there was an enhancing lesion within the small bowel also present on previous CT; I: Subsequent Ga68-Dotatoc positron emission tomography-CT was performed which confirmed uptake within the small bowel consistent with primary tumour.

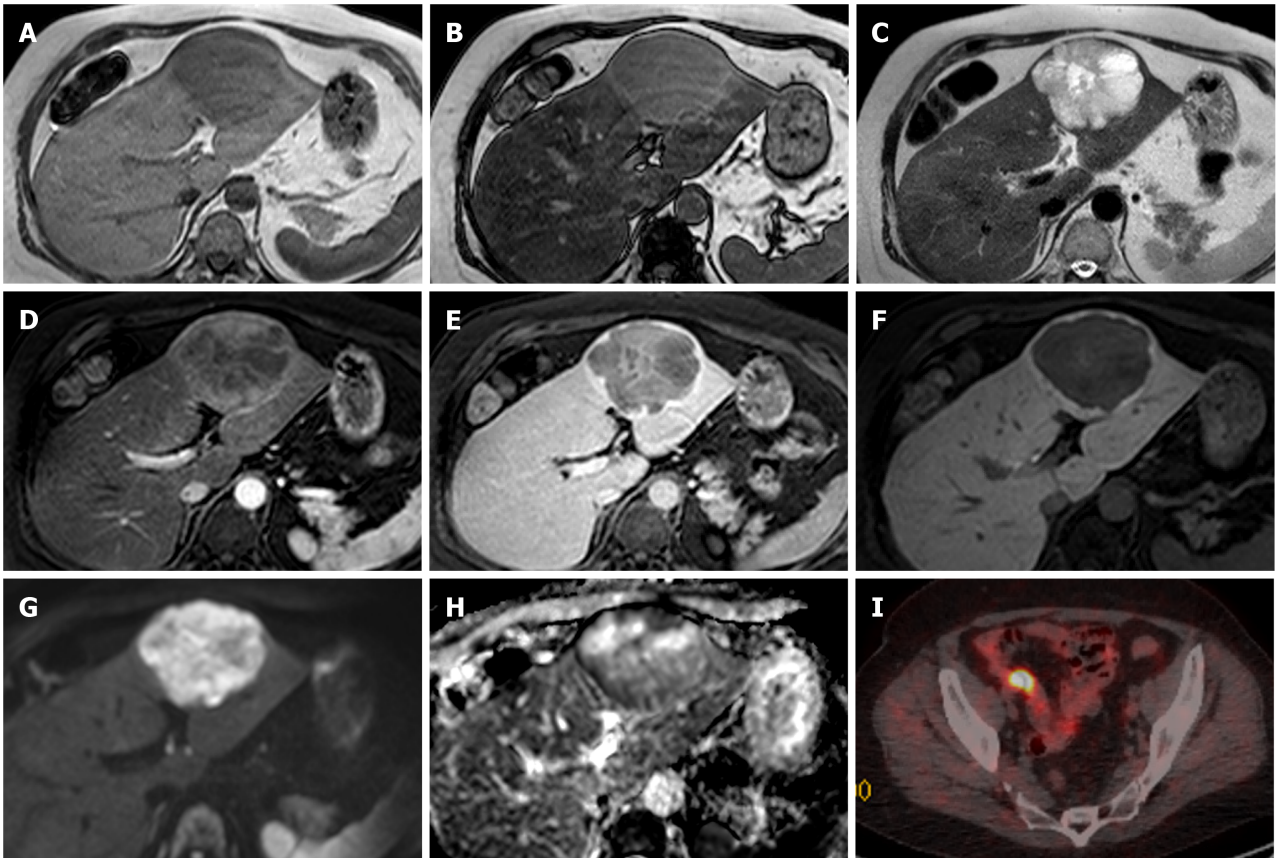

Figure 12 Neuroendocrine carcinoma metastases. A 59-year-old female was found to have a few liver lesions, the dominant lesion in the left lobe demonstrated here. A and B: In-phase (A) and out-of-phase (B) sequences show background hepatic steatosis, but no tumoral fat; C: The lesion shows heterogenous high T2 signal; D and E: There is mainly peripheral enhancement on the arterial phase (D) with washout on delayed phase (E). Delayed phase also shows an enhancing capsule; F: On hepatobiliary phase there is no contrast retention within the lesion except for the thin-rim of presumed capsule; G and H: There is high signal on diffusion weighted imaging b500 (G) with low signal seen on apparent diffusion coefficient (H), especially in the periphery. The other smaller lesions (not demonstrated here) showed similar signal characteristics. Initial staging computed tomography showed no primary tumour to suggest this is metastasis. The lesions were resected and histology confirmed low grade neuroendocrine tumour, with Ki-67 proliferation index of less than 1%; I: The patient underwent subsequent positron emission tomography scan that demonstrated the primary in the distal ileum.

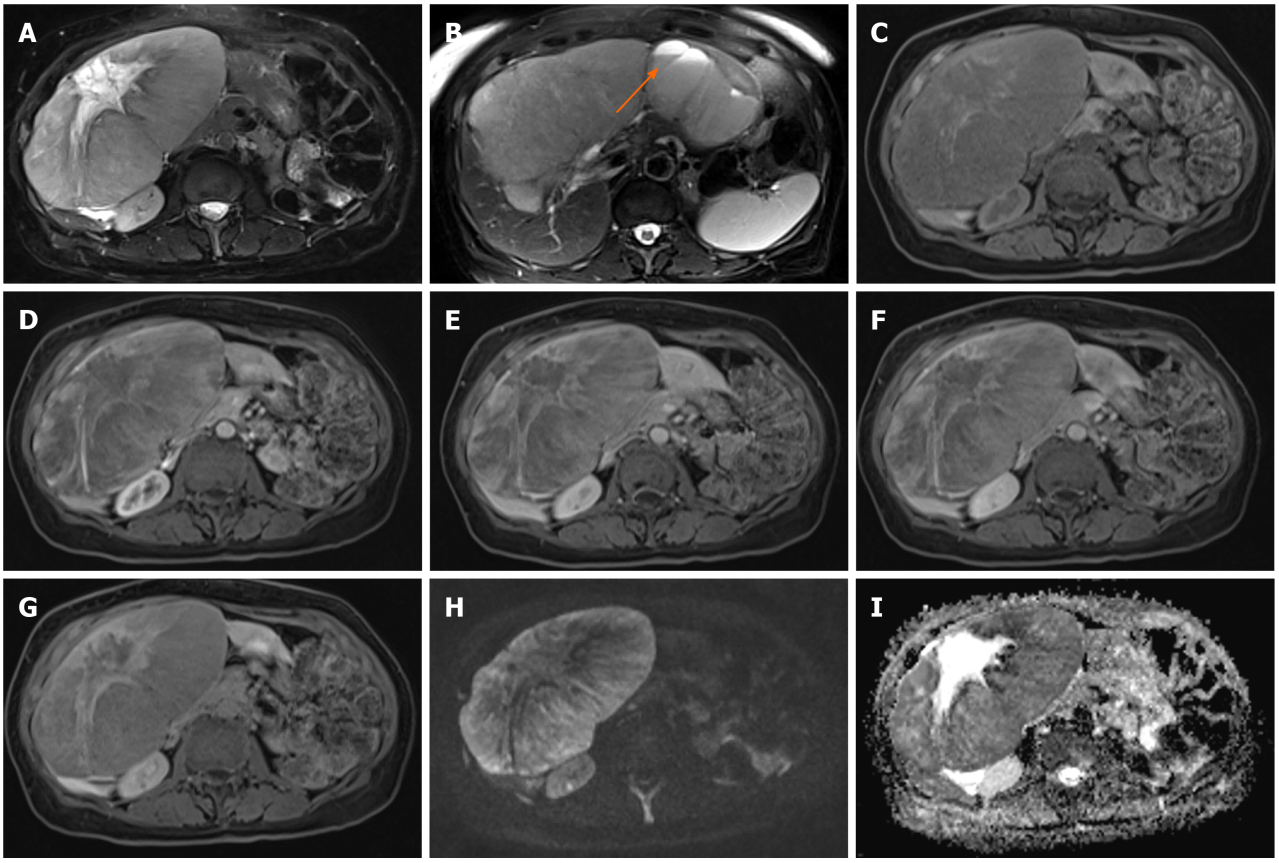

Figure 13 Neuroendocrine carcinoma. A 69-year-old female was found to have incidental large liver lesions in a non-cirrhotic liver while undergoing magnetic resonance (MR) pelvis for a uterine lesion, presumed to be fibroid. A: MR demonstrated large liver masses, the largest exophytic mass showing intermediate to high T2 signal with a high signal stellate scar; B: One of the lesions in the left liver lobe demonstrates a cystic component with fluid-fluid levels, which was presumed to represent previous haemorrhage; C: Majority of the lesions were of low T1 signal with a few hyperintense flecks surrounding the scar; D-F: There was heterogenous enhancement on arterial phase (D) with no washout demonstrated on portal venous (E) and delayed (F) phases; G: Hepatobiliary phase showed no contrast retention within the lesion except for the central scar; H and I: Diffusion weighted imaging at b800 (H) and apparent diffusion coefficient (I) show areas of diffusion restriction. These were biopsied and histology demonstrated well differentiated neuroendocrine carcinoma. The origin of this was not determinable from the immunohistochemical pattern. Overall, this was favoured to represent a primary neuroendocrine tumour of the liver as further imaging did not reveal another primary (although admittedly biopsy of the uterine lesion, radiologically presumed fibroid, was never performed). The patient represented a month later with haemorrhagic brain metastases.

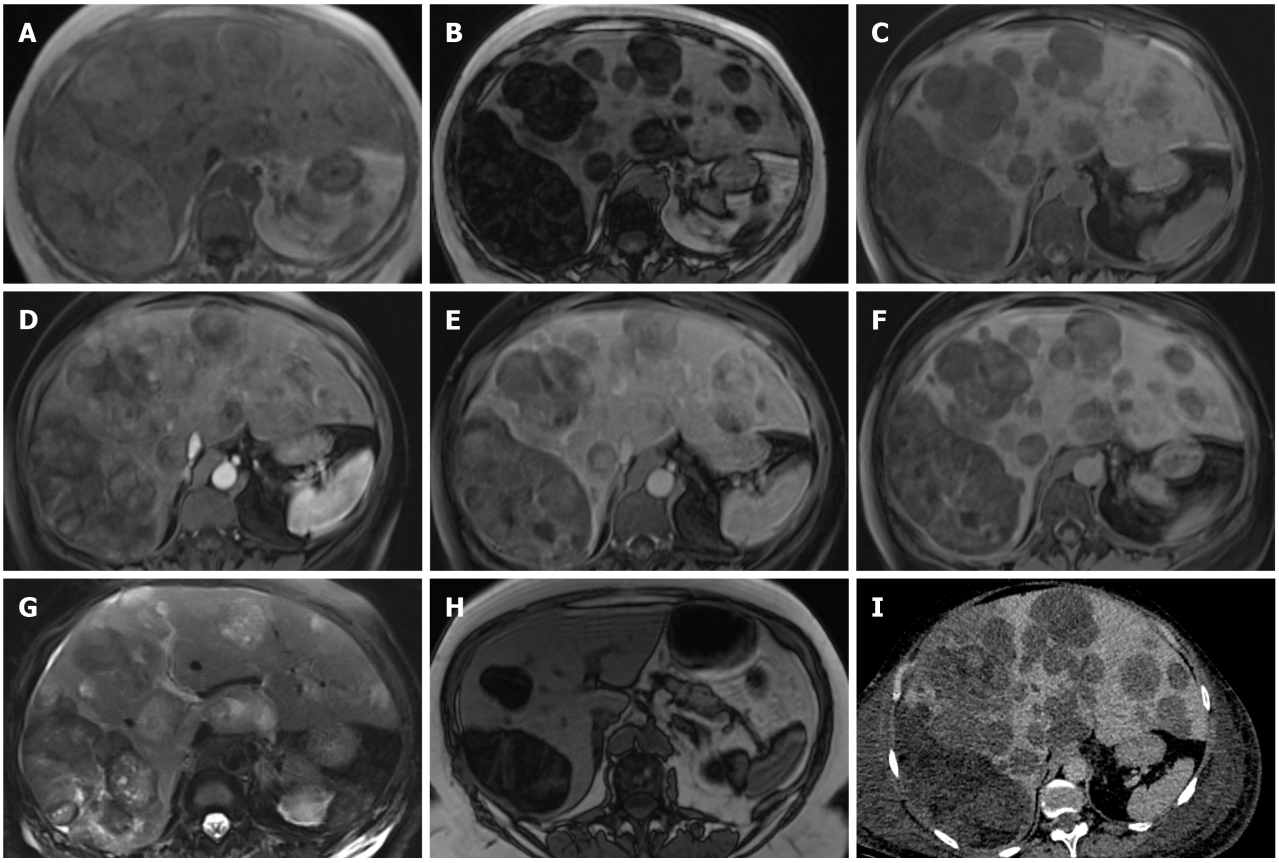

Figure 14 Pleomorphic liposarcoma. A 54-year-old underwent routine ultrasound for re-assessment of gallbladder polyps seen a year ago. Ultrasound revealed multiple liver lesions not present previously and magnetic resonance (MR) of the liver was organised. This showed multiple fat containing liver lesions favoured to represent adenomas. The patient was not on any steroid medication at the time and had no other risk factors for hepatocellular adenoma. A-G: She represented 3 mo later with right sided chest pain and computed tomography (CT) pulmonary angiogram demonstrated increase in the size and number of liver lesions, at which point a second MR liver with gadoxetic acid was performed and is shown here; A-C: MR shows multiple bilobar liver lesions of low T1 signal (C) and predominantly fat component as demonstrated by signal loss on out-of-phase sequence (B) when compared to in-phase (A); D and E: Arterial (D) and delayed phase (E) sequences show a few heterogenous areas of hyperenhancement some of which washout; F: Majority of the lesions did not retain contrast on hepatobiliary phase with only the larger lesions showing some areas of uptake, predominantly within septations; G: T2-weighted sequence (G) shows the lesions are heterogenous and of varied signal intensity; H: Image H demonstrated out-of-phase sequence on the MR performed 3 mo prior for comparison of lesion burden increase in the interim; I: demonstrates portal venous phase CT performed 1 mo since the second MR, again showing quick interval increase in size and number of the lesions. Targeted liver biopsy was performed which confirmed pleomorphic liposarcoma.

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MINIREVIEWS

Liver transplantation for benign liver tumors

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Abstract

Benign liver tumors are common lesions that are usually asymptomatic and are often found incidentally due to recent advances in imaging techniques and their widespread use. Although most of these tumors can be managed conservatively or treated by surgical resection, liver transplantation (LT) is the only treatment option in selected patients. LT is usually indicated in patients that present with life-threatening complications, when the lesions are diffuse in the hepatic parenchyma or when malignant transformation cannot be ruled out. However, due to the significant postoperative morbidity of the procedure, scarcity of available donor liver grafts, and the benign course of the disease, the indications for LT are still not standardized. Hepatic adenoma and adenomatosis, hepatic hemangioma, and hepatic epithelioid hemangioendothelioma are among the most common benign liver tumors treated by LT. This article reviews the role of LT in patients with benign liver tumors. The indications for LT and long-term outcomes of LT are presented.

Key Words: Benign liver tumor; Liver transplantation; Hepatic adenoma; Liver adenomatosis; Hepatic hemangioma; Hepatic epithelioid hemangioendothelioma

Core Tip: Liver transplantation (LT) is rarely performed for benign liver tumors. However, LT is a valid and efficient treatment option in selected patients with life-threatening complications or when surgical resection is impossible. The indications for LT for these lesions are still not well defined. This report focuses on the indications for LT and long-term LT outcomes in patients who underwent transplantation for benign liver tumors.

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INTRODUCTION

Malignant liver disease, namely hepatocellular carcinoma, currently makes up between one quarter and one-third of liver transplantation (LT) indications worldwide [1]. Patients with benign liver tumors, on the other hand, only exceptionally undergo transplantation. According to large European and United States registries, transplantations for benign liver tumors make up 1% of all LTs performed in Europe and the United States[2,3].

Benign liver tumors are relatively common, occurring in up to 20% of the general population[4]. Most are treated conservatively, and liver resection (LR) is only required in a minority of patients[5]. Despite their relative frequency, due to the generally benign behavior, there are no standardized treatment guidelines.

LT is occasionally reported in the treatment of benign liver lesions; however, due to the morbidity of the procedure, shortage of donor liver grafts, and benign course of the disease in most patients, only very selected cases may qualify for LT. Some of the indications for LT in patients with benign liver tumors include diagnostic uncertainty and/or possible malignant transformation (MT), premalignant lesions, metabolic liver disease, complications such as rupture or hemorrhage, and significant patient symptoms due to the mass-effects of the tumor[6].

Most of the literature dealing with the topic is limited to case reports or small case series. Both deceased donor and living donor (LD) options of LT are performed for benign liver lesions. However, most of the allocation systems used across the world prioritize the patients for cadaveric LT on the basis of their model for end-stage liver disease (MELD) score[7]. Patients with benign liver lesions typically have low MELD scores and normal liver function. Therefore, LDLT is often the only option for a timely transplant before life-threatening complications develop. This is particularly the case in countries with low rates of cadaveric organ donation and advanced LDLT programs [8-10]. In this report we review the recent literature and analyze the most common indications and outcomes of LT in patients with benign liver tumors.

HEPATIC ADENOMA AND LIVER ADENOMATOSIS

Hepatic adenomas (HA) are rare benign tumors of the liver, with an incidence of 3-4 *per* 100000 women[11]. They predominantly occur in women of childbearing age, often in association with prolonged oral contraceptive use[12]. Since hormonal stimulation plays a significant role in the development of HA, anabolic steroid consumption is also a risk factor[13,14]. Other environmental factors associated with HA are obesity and non-alcoholic fatty disease of the liver (NAFLD)[15,16]. In recent years, due to low estrogen contraceptive formulations and an increasing prevalence of NAFLD and metabolic syndrome, the predominant etiology of HA is shifting from hormonal use towards metabolic liver disease[17]. Other genetic or developmental conditions associated with HA include glycogen storage diseases (especially Type 1a glycogenosis), maturity-onset diabetes of the young type 3, McCune-Albright syndrome, and abnormalities of hepatic vasculature such as absence of the portal vein and portosystemic venous shunts[18-21]. Liver adenomatosis (LA) is a particular entity, initially described by Flejou, defined as the presence of more than 10 adenomas in an otherwise normal liver[22]. However, during recent years, the term adenomatosis has been extended, and it is defined as a high number of liver tumors independent of an absence of underlying liver disease[23]. There are two types of LA. The massive type is characterized by an enlarged liver, deformed liver contour, and typically large and necrotic tumors. The second type is called multifocal, with preserved liver size and contour. This type has a less aggressive course, usually presenting with one or two larger adenomas that may cause complications[24].

Although usually asymptomatic, large-sized or multiple HA can present with abnormal liver function tests, abdominal pain and distention or signs of hemorrhage [25,26]. Hemorrhage is reported to occur in 20%-40% of adenomas, usually appearing in lesions larger than 5 cm[25-28]. It is usually intratumoral; however, the tumor can

also rupture, with resulting subcapsular or intraperitoneal hemorrhage.

MT is another potential complication of HA with an overall risk of about 5%. Male gender is a particular risk, while in women, MT is noted only in tumors larger than 7-8 cm. The existence of multiple lesions reportedly does not seem to confer a specific risk [26,29,30].

HA and LA do not constitute standard indications for LT and LT is only rarely performed. Larger adenomas and adenomas complicated by hemorrhage or MT should be treated with surgical resection. However, since both HA and LA can present with life-threatening complications not amenable to surgical resection due to size, number or localization, LT may be warranted. Sometimes progressive, symptomatic growth or MT occurs after previous hepatectomy, hastening LT. Underlying liver disease can also be the primary indication for LT, such as in glycogen storage disease or vascular malformations of the liver. According to the available literature, glycogen storage disease is considered a risk factor for MT of liver adenomas[31].

According to the 2018 European Liver Transplant Registry (ELTR) report, LA represents only 0.04% of all indications for LT in Europe. The outcomes are excellent, with 1- and 5- year survival rates of 88%[32]. In 2016, Chiche *et al*[33] analyzed 49 patients from the ELTR who underwent LT for LA between 1986 and 2013. Overall, 28 (57%) patients had the massive LA form, while 21 (43%) patients had the multifocal form. Sixteen patients had glycogen storage disease, and seven patients had underlying vascular disease, supporting the notion that the first definition of LA was too restrictive. Regarding the leading indications for LT, histologically proven MT (16 patients) and suspicion of MT (15 patients) were the primary indications, while only five patients underwent LT due to hemorrhage. Out of the 15 patients with a suspicion of MT, only one patient had hepatocellular carcinoma confirmed on the surgical specimen, making this indication debatable. In the analysis of risk factors for MT, age > 30 years and history of partial hepatectomy proved to be statistically significant. Based on the results of the study, Chiche *et al*[33] suggested that LT for LA should be considered when the patient has either a major criterion (histologically proven hepatocellular carcinoma) or at least 3 out of 5 minor criteria (more than two severe hemorrhages, more than two previous resections, beta-mutated or inflammatory adenomas, underlying liver disease - major steatosis or vascular abnormalities, age > 30 years)[33].

In conclusion, HA is only exceptionally accepted as an indication for LT. Also, multiple non-resectable adenomas in the context of LA are likely to remain stable and uncomplicated, so they do not require a major operation with inherent risks such as an LT, especially in the era of organ shortage. Exceptional circumstances when LT can be considered include treatment for an underlying disease such as glycogen storage disease or vascular malformations, multiple non-resectable adenomas in men, and cases with proven or suspected MT.

HEPATIC HEMANGIOMA

Hepatic hemangiomas (HH) are the most common primary tumors of the liver, with an incidence of 0.4%-20%[34]. They are most commonly found in women 30-50 years old (female-to-male ratio, 3:1), but they can be detected in all age groups[35]. Most hemangiomas are small in size (< 4 cm), solitary and asymptomatic[35,36]. HH that measure 10 cm and larger are called giant hemangiomas, and most of them are also asymptomatic[35,36]. Rarely, HH can present as multiple lesions, as a part of a systemic hemangiomatosis syndrome[37,38]. The diagnosis of hemangiomas is usually established incidentally on imaging studies, and owing to their benign course, HH are usually managed conservatively[34]. Larger hemangiomas can cause symptoms, usually abdominal pain or discomfort[37]. Occasionally, HH can present with hemorrhage or consumptive coagulopathy, a condition known as Kasabach-Merritt syndrome (KMS)[34]. HH treatment is rarely indicated, and therapeutic modalities include arterial embolization, surgical resection, and LT. Medical therapy with steroids, vincristine, interferon-alpha, antiplatelet agents, or sirolimus with high doses of propranolol is only indicated for HH that present with KMS[39,40]. However, there is no strong evidence in favor of any pharmacological agent[40]. Apart from KMS, indications for treatment of HH are rapidly growing tumors, persistent pain, hemorrhage, risk of rupture, and symptoms resulting from compression of adjacent organs and vessels[37].

HH are a sporadic indication for LT. Based on the ELTR data, only 71 patients with HH were transplanted from 1988 to 2016, and HH represents 0.1% of all indications for

LT[32]. HH is an even less frequent indication for LT in the United States, with only 25 patients having been transplanted from October 1988 through January 2013[41]. Patients diagnosed with HH who underwent LT have 1-year and 5-year survival rates of 80%-87.8% and 74.8%-77%, respectively[32,41].

To the best of our knowledge, only 18 reports (17 case reports and 1 case series) have been published in the English literature regarding LT for HH (Table 1)[42-59]. According to a recent systematic review that included 15 of the previously mentioned studies, patients' mean age was 39.93 ± 8.7 years. Abdominal distention, respiratory distress, upper abdominal pain, excessive bleeding, and coagulopathy were the most commonly reported symptoms. Twelve patients received grafts from a cadaveric donor, while four patients received LD grafts. All patients had abnormal liver function tests before LT, and they returned to normal within a few days postoperatively. Finally, all patients were alive 90 d after LT. One patient required re-transplantation following an acute liver rejection episode, and one patient was re-operated due to abdominal bleeding[60].

In summary, despite the high incidence of HH, LT is a very rare indication for HH. However, in unresectable HH or HH with life-threatening complications, LT can be considered a safe treatment option.

HEPATIC EPITHELIOID HEMANGIOENDOTHELIOMA

Hepatic epithelioid hemangioendothelioma (HEHE) is a rare vascular tumor of the liver with an estimated incidence of less than 0.1 *per* 100000[61]. HEHE is usually diagnosed in adulthood with a mean age at diagnosis of 41.7 years (age range; 30-40 years), and a female predominance (female-to-male ratio 3:2)[62,63]. The etiology of HEHE is not well understood, although several factors have been implicated, including vinyl chloride and asbestos[63]. The hallmark of HEHE is its borderline behavior, described as the aggressiveness of the tumor graded between hemangioma and hepatic hemangiosarcoma. Tumors are often multiple or diffuse throughout the liver. Additionally, HEHE can metastasize beyond the liver. Mehrabi *et al*[63] conducted an extensive review of the literature that included 434 HEHE patients. In that study, 81% of patients had multifocal tumors while a solitary tumor was present in the remaining 19% of patients. Extrahepatic disease (EHD) was diagnosed in 36% of the patients[63]. Lungs, regional lymph nodes, peritoneum, bone, spleen, and diaphragm were the most common extrahepatic sites[63,64]. HEHEs tend to have a heterogeneous clinical presentation, ranging from asymptomatic tumors to lesions causing hepatic failure. The most frequent symptoms are right upper quadrant or epigastric pain (60%–70%), weight loss (20%), impaired general condition (20%), and jaundice (10%)[65]. Definitive diagnosis is often made through a synthesis of radiological signs and clinical features such as occurrence in young adults and longstanding clinical history[64]. Fluorodeoxyglucose-positron emission tomography imaging can be helpful in the staging of the disease before LT[66]. However, histologic examination of appropriate tissue obtained by biopsy is required for correct diagnosis. The most common misdiagnoses include angiosarcoma, cholangiocarcinoma, metastatic carcinoma, and hepatocellular carcinoma (sclerosing variant)[67].

Owing to the rarity and inconsistent behavior of these tumors, the treatment algorithm for HEHE is not standardized. The primary treatment modality is surgery, including LR and LT. It should be noted that HEHE is unresectable in most cases due to its nature, so LT is reserved for patients with multiple or diffuse tumors and/or EHD[67]. Chemo and radiotherapy regimens and transcatheter arterial chemoembolization are other therapeutic options[63,67]. In the previously mentioned study by Mehrabi *et al*[63], most patients had undergone LT (44.8%) followed by no treatment in 24.8%, chemotherapy or radiotherapy in 21%, and LR in 9.4%[63]. Surgical resection and LT had the best survival rates, with 5-year survival rates of 54.5% and 75%, respectively. 5-year survival rates were 30% after chemo or radiotherapy and 4.5% after no treatment[63]. A multicenter ELTR study which analyzed 59 patients who underwent LT for HEHE confirmed excellent results for LT[68]. Moreover, it was concluded that EHD presence is not necessarily a contraindication to LT[68]. In 2010, Grotz *et al*[69] analyzed overall survival (OS) and disease-free survival (DFS) in patients with HEHE treated with LR or LT. In both groups, there were 11 patients with comparable results. LR was associated with a 5-year OS of 86% and DFS of 62%, while LT was associated with a 5-year OS of 73% and DFS of 46%[69]. In a recent study, Noh *et al*[70] evaluated the management and prognosis of 79 HEHE patients from the Surveillance, Epidemiology and End Results program during the study period from

Table 1 List of the reported cases of liver transplantation for hepatic hemangioma

Ref.	Age (yr)/sex	Indication for LT	Type of donor	Follow-up	Condition
Klompmaker *et al*[42], 1989	27/M	KMS	LD	3 yr	Alive
Mora *et al*[43], 1995	42/F	KMS, respiratory distress	CD	16 d	Alive
Tepetes *et al*[44], 1995	4 wk/M	KMS	NA	8 d	Died, graft mal-function
Brouwers *et al*[45], 1997	4 cases	Pain (n = 2). Rupture (n = 1). KMS (n = 1)	NA	1 mo, 1 yr, 4 yr, 9 yr	Alive (n = 3). Died (n = 1)
Chui *et al*[46], 1996	33/F, 43/F	Bleeding (n = 1). Abdominal discomfort (n = 1)	CD	18 mo, 14 mo	Alive (n = 2)
Longeville *et al*[47], 1997	47/M	KMS	CD	12 mo	Alive
Russo *et al*[48], 1997	43/F	Huge mass	CD	14 d	Alive
Kumashiro *et al*[49], 2002	48/F	KMS, acute liver failure	LD	15 d	Alive
Ferraz *et al*[50], 2004	28/F	KMS, respiratory distress	CD	30 mo	Alive
Meguro *et al*[51], 2008	45/F	KMS	LD	10 mo	Alive
Aseni *et al*[52], 2010	46/M	Pulmonary embolism	CD	25 mo	
Vagefi *et al*[53], 2011	39/F	KMS	CD	NA	Alive
Unal *et al*[54], 2011	56/F	Upper abdominal pain	CD	6 mo	
Zhong *et al*[9], 2014	27/F	Diffuse mass	LD	50 mo	Alive
Yildiz *et al*[56], 2014	44/F	KMS, respiratory distress	CD	1 mo	Alive
Lange *et al*[57], 2015	46/F	Huge mass, portal vein thrombosis, ascites	CD	7 wk	Alive
Lee *et al*[8], 2018	51/F	Rapid growing tumor	LD	16 mo	Alive
Eghlimi *et al*[59], 2020	38/M	Huge mass	CD	8 mo	Alive

LT: Liver transplantation; M: Male; F: Female; LD: Living donor; CD: Cadaveric donor; KMS: Kasabach-Merritt Syndrome; NA: Non applicable.

1973 to 2014. Based on their results, patients who underwent surgical treatment (LR or LT) had significantly higher 5-year survival than those who underwent non-surgical treatment (88% *vs* 49%). In multivariate analysis, surgical therapy was the only independent prognostic factor for survival[70]. In the 2007 HEHE-ELTR report, the recurrence rate of HEHE after LT was 25%, while in the US survey that included 110 adults, the recurrence rate was 11%[68,71]. 149 patients from the ELTR registered between 1984 and 2014 were analyzed in order to identify the risk factors for post-LT recurrence of HEHE. Macrovascular invasion (HR 4.8), pre-LT waiting time of 120 d or less (HR 2.6), and hilar lymph node invasion (HR 2.2) were significant risk factors for recurrence, while EHD was confirmed not to be a risk factor[72]. A HEHE-LT score that stratified patients' risk of tumor recurrence was developed using these three risk factors. Patients with a score between 0 and 2 had a significantly better 5-year DFS than patients with a score of 6-10 (93.9% *vs* 38.5%; $P < 0.001$)[72]. This score can be used in the post-LT follow-up to decide on minimization and type of immunosuppression as well as for imaging surveillance. Furthermore, this study emphasizes the importance of routine extensive lymphadenectomy during LT. Also, mandatory waiting time should be set up in order to gain a better insight into the tumor biology and avoid futile LT[72].

CONCLUSION

In conclusion, LT is rarely indicated for the treatment of benign liver tumors, mainly due to their benign nature. Most of the complications resulting from benign liver tumors can be managed with radiological intervention or surgical resection. However, when benign liver tumors present with life-threatening complications or MT cannot be ruled out, and tumors are unresectable, LT is a reasonable and safe treatment option.

Due to their rarity, there are no standardized transplantation guidelines for benign liver tumors. Considering satisfying long-term results, studies from Europe and the United States strengthen the role of LT for benign liver tumors. Finally, a worldwide registry of patients transplanted for benign liver tumors with details about patients' history, imaging studies, and the surgical pathology would help to define precise LT criteria for this rare indication.

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MINIREVIEWS

Hepatocellular carcinoma in nonalcoholic fatty liver disease: A growing challenge

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide, and its prevalence increases continuously. As it predisposes to hepatocellular carcinoma both in the presence and in the absence of cirrhosis, it is not surprising that the incidence of NAFLD-related hepatocellular carcinoma would also rise. Some of the mechanisms involved in hepatocarcinogenesis are particular to individuals with fatty liver, and they help explain why liver cancer develops even in patients without cirrhosis. Genetic and immune-mediated mechanisms seem to play an important role in the development of hepatocellular carcinoma in this population. Currently, it is consensual that patients with NAFLD-related cirrhosis should be surveilled with ultrasonography every 6 mo

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(with or without alpha-fetoprotein), but it is known that they are less likely to follow this recommendation than individuals with other kinds of liver disease. Moreover, the performance of the methods of surveillance are lower in NAFLD than they are in other liver diseases. Furthermore, it is not clear which subgroups of patients without cirrhosis should undergo surveillance. Understanding the mechanisms of hepatocarcinogenesis in NAFLD could hopefully lead to the identification of biomarkers to be used in the surveillance for liver cancer in these individuals. By improving surveillance, tumors could be detected in earlier stages, amenable to curative treatments.

Key Words: Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Hepatocellular carcinoma; Hepatocarcinogenesis; Surveillance

Core Tip: Nonalcoholic fatty liver disease (NAFLD) is a growing cause of hepatocellular carcinoma, and liver cancer is one of the leading causes of cancer-related death worldwide. There are particular genetic and immune-mediated mechanisms for hepatocarcinogenesis in NAFLD. Moreover, hepatocellular carcinoma can develop in NAFLD in the absence of cirrhosis. Finally, the characteristics of NAFLD and its high prevalence lead to important challenges regarding surveillance for liver cancer in this population. This review will approach the most important issues concerning NAFLD-related hepatocellular carcinoma.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming one of the most common causes of liver disease worldwide[1]. According to a meta-analytic assessment of 86 studies, the global prevalence of NAFLD is 25.24%[2]. Therefore, its association with hepatocellular carcinoma (HCC) also becomes increasingly important [3]. The relevance of this association is demonstrated by the fact that NAFLD was responsible for 36300 incident cases of HCC and 34700 HCC-related deaths in 2019[4].

Although cirrhosis is considered a predisposing condition for HCC in general, diverse disease-specific mechanisms are involved in the development of NAFLD-related HCC[3,5,6]. Moreover, the observation that HCC can occur in patients with NAFLD even in the absence of cirrhosis suggests that, as in the case of hepatitis B virus infection, NAFLD itself could be etiologically linked to HCC development[7]. Over the last few years, an array of studies has shed light on the diverse genetic and immune-related mechanisms that link NAFLD to the process of hepatocarcinogenesis. Nonetheless, much work is still needed to further understand this inter-relation.

Considering the association between NAFLD and HCC, surveillance for liver cancer among patients with fatty liver has become an important topic of discussion. However, the extremely high prevalence of NAFLD and the distinct risk levels for HCC in different patients make defining the target population for surveillance quite challenging[8].

The aim of this article is to review the epidemiology of NAFLD-related HCC, the genetic and immune mechanisms involved in hepatocarcinogenesis in individuals with NAFLD, the current knowledge related to HCC in patients with NAFLD without cirrhosis, and key aspects to consider for HCC surveillance in NAFLD.

EPIDEMIOLOGY OF NAFLD-RELATED HCC

In the last few decades, HCC-related mortality has steadily increased and since the 1980s has almost tripled in the United States, where it is the fastest-rising cause of cancer-related death[9]. Notably, this increase parallels the growth in NAFLD prevalence, which increased 2 to 3-fold in a similar period of time[10], turning it into a leading etiology of cirrhosis worldwide[11]. These coinciding trends and the fact that NAFLD has been noted as an increasingly common cause of HCC in several series[12] as well as the fastest-growing cause of HCC in liver transplant candidates and recipients in the United States[13] suggest that NAFLD is a prominent contributor to HCC burden worldwide and that the prevalence of HCC will likely increase concomitantly with the global obesity epidemic[12,14]. In this context, a recent study used Bayesian models to estimate that the age-standardized incidence rate of NAFLD-related liver cancer would increase from 0.92/100000 inhabitants in 2018 to 1.18/100000 inhabitants in 2030[15].

Estimates regarding the annual incidence of HCC in patients with NAFLD-related cirrhosis in the western hemisphere range from 0.5% to 2.6%[14,16]. With regard to data from eastern hemisphere countries, a prospective study from Japan reported similar figures, with an annual incidence of 2.26% in a cohort followed for more than 15 years[17]. Another study from India reported lower figures (annual incidence of HCC of 0.5% in patients with biopsy-proven NAFLD-related cirrhosis)[18]. It is worth mentioning, though, that most of these estimates originate from cohorts followed in tertiary centers or from liver transplant registries and that population-based cohort studies are not available. Importantly, existing data suggest that older age, male sex, alcohol intake, and especially diabetes are factors that may increase HCC incidence in NAFLD-related cirrhosis[19]. The annual incidence of HCC among individuals with NAFLD who do not have cirrhosis is much lower than that reported for patients with cirrhosis, as it will be reviewed later in this article.

GENETIC ASPECTS OF NAFLD-RELATED HCC

Considering the particular characteristics of NAFLD and NAFLD-related HCC as well as the fact that liver cancer also develops in individuals with NAFLD who do not have cirrhosis, the study of the genetic aspects of hepatocarcinogenesis in NAFLD has drawn substantial attention. The main genetic mechanisms involved in the development of NAFLD-related HCC will be discussed in this section and are summarized in Figure 1.

Genetic variants associated with NAFLD-related HCC

Early NAFLD studies have identified ethnic differences and evidence of familial clustering suggestive of a hereditary/genetic component to the disease[20]. The first study to demonstrate an association between genetic variants and NAFLD was published by Romeo *et al*[21] who conducted a genome wide association analysis using quantitative proton magnetic resonance spectroscopy to measure hepatic steatosis. The genome wide association analysis showed that carriers of the rs738409 variant of the patatin-like phospholipase domain containing protein 3 (*PNPLA3*) gene, most commonly found among Hispanics, had over a 2-fold increase in intrahepatic triglycerides[21]. Subsequent studies demonstrated the same variant to be associated with NAFLD-related HCC[22,23].

Following studies described conflicting evidence of an association between the transmembrane 6 superfamily member 2 (TM6SF2) rs58542926 polymorphism and NAFLD-related HCC, potentially from its low minor allele frequency[24,25]. The membrane bound O-acetyltransferase domain containing 7 rs641738 variant was posteriorly identified in a European cohort to be associated with NAFLD-related HCC [25-30]. Another European study focusing on the identification of rare variants in NAFLD-related HCC cases found, aside from PNPLA3 and TM6SF2, pathogenic variants in apolipoprotein B gene, among others[31]. As genetic association studies have mostly included patients of European ancestry, larger and more diverse cohorts are needed given the clinical observation that Hispanics are at higher risk for NAFLD-related HCC[32].

Molecular events in NAFLD-related hepatocarcinogenesis

Association studies have provided a plethora of information regarding NAFLD-related hepatocarcinogenesis, although mechanistic studies have yet to elucidate how

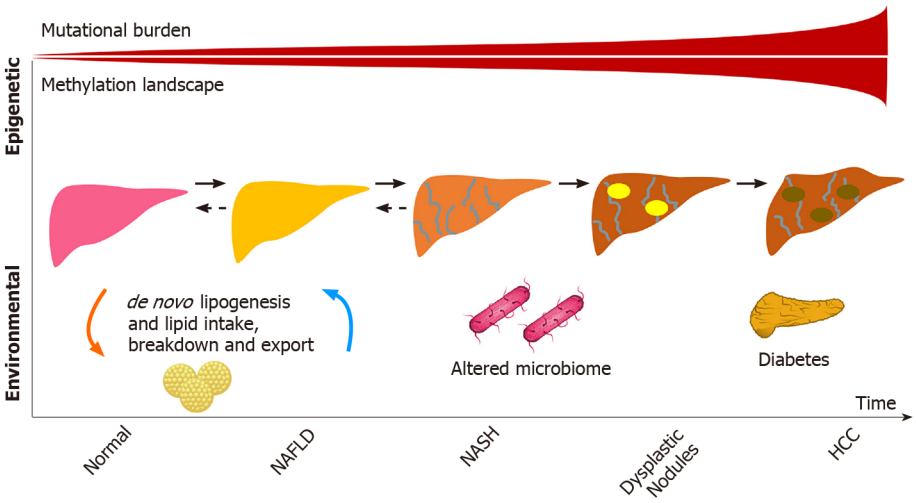

Figure 1 Main genetic factors determining nonalcoholic fatty liver disease-related hepatocarcinogenesis. HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

these variants cause disease. The observation that many of the polymorphisms involve lipid regulation raises the possibility that a lipid-rich dysregulated microenvironment may be key to HCC development. Although NAFLD-specific HCC studies are lacking, parallel mutations exist between NAFLD and other etiologies demonstrating a potential convergence in pathways that have previously been described in viral etiologies[33]. For instance, mutations in telomerase reverse transcriptase are known to play a role in the progression of dysplastic nodules and in the development of early HCC[34,35].

As hepatocyte damage increases from cirrhosis to dysplasia and eventually HCC, the mutational burden leading to cancer exponentially grows. This was well illustrated in a study by Brunner *et al*[36] who conducted whole genome sequencing of 100-500 hepatocytes from 5 healthy controls and 9 patients with cirrhosis. Structural variants and copy number variations were more commonly identified in those with cirrhosis compared to the normal controls, including in activin receptor type 2A, cyclin-dependent kinase inhibitor 2A, and AT-rich interaction domain 5A. Interestingly, similar signatures of somatic copy number variations were identified in a pilot study of 10 HCC cases in circulating tumor cells, raising the possibility of their use as biomarkers[37]. Other well described pathways include mutations in β-catenin, tumor antigen p53, and AKT/mechanistic target of rapamycin/mitogen-activated protein kinase signaling pathway, which includes tuberous sclerosis complex subunits 1 and 2, phosphatase and tensin homolog, and fibroblast growth factor 19[34].

Given the clinical and genetic heterogeneity in human HCCs, animal models have provided the pre-clinical tools to understand these pathways in NAFLD-related HCC [38]. Although NAFLD and nonalcoholic steatohepatitis (NASH) mouse models have limitations in recapitulating the human NAFLD phenotype, these animal models have proven especially relevant when comparing "obese" and "lean" NAFLD-related HCCs. Using whole exome sequencing, Shen *et al*[39] demonstrated that obese and lean NAFLD-related HCCs in mice had a different mutational burden. For instance, they identified mutations in the carboxyl ester lipase gene that caused an increase in cholesterol esters mostly in the obese mice. Similarly, Grohmann *et al*[40] studied obese and lean mouse models to show that HCC and NASH development were dependent on divergent pathways, raising the possibility of variable mechanisms in non-cirrhotic HCC development. The non-fibrotic pathway contributions were also demonstrated in European cohorts (from Germany and the United Kingdom), in which polygenic risk scores (including PNPLA3, TM6SF2, membrane bound O-acetyl-transferase domain containing 7, and glucokinase regulator) predicted the risk of HCC in patients with NAFLD. This risk was associated with hepatic steatosis (adjusted hazard ratio of 1.35, $P < 0.01$), even after correcting for hepatic fibrosis ($P < 0.05$)[41].

The advent of single cell RNA sequencing has allowed for further understanding of the cell type proportions in HCC, which was a limitation of bulk RNA sequencing given tumor heterogeneity[42], including the understanding of the inflammatory microenvironment that may have effects on treatment responses[43]. Whether similar cell type proportions and mutational signatures will be identified in NAFLD-related HCC remains to be seen in populations with and without cirrhosis.

A summary of the genetic variants and mutations described in NAFLD-related HCC is presented in Tables 1 and 2.

Epigenetic changes

Epigenetic modifiers also play a role in HCC development and account for approximately 32% of mutations found in HCC[44,45]. Many of the genes involved in structural chromosomal changes (AT-rich interaction domain 1A, AT-rich interaction domain 2, histone-lysine N-methyltransferase 2A) may not be directly involved in the pathogenesis of the disease but could be proxies to mutational changes in other genes linked by chromosomal looping captured by assay of transposase-accessible chromatin [46,47], an avenue that has not been yet explored in HCC related to NAFLD or to other etiologies of liver disease. Methylation aberrations also play a role. Recent work by Hernandez-Meza *et al*[48] demonstrated the extensive methylation landscape of different etiologies of HCC in a European cohort, with a minority represented by NAFLD. Similar to the increase in mutational burden seen from normal liver to cirrhosis, the study demonstrated that patients with HCC were more likely to have hypermethylation patterns compared to controls. Interestingly, some of these differential methylation patterns involved key lipid genes, including the transcription factor, sterol regulatory element-binding protein 1.

Other factors

Serum metabolomic and microbiome studies have also identified signatures for poor NAFLD-related outcomes[49-51], although it remains to be seen whether these are surrogates for NASH progression or if they are involved in the pathways. The role of lipopolysaccharides has been studied in this context. The increase in lipopolysaccharides in NAFLD patients, as a surrogate for oxidative stress, is likely multifactorial and linked to the gut (bacterial overgrowth, increased permeability, among other factors), nutrients (including lipids), immune response, and hepatic injury, which adds another complexity to the NAFLD-related HCC spectrum of disease and potentially partly explains disease heterogeneity[52].

The use of metabolomics to identify signatures that are pathogenic in NAFLD-related HCC is also a novelty in the field. A recent study by Buchard *et al*[53] aimed to identify differences in metabolomics in tissues of patients with NAFLD-related HCC by stratifying the cohort according to the degree of liver fibrosis. Using ^{1}H-nuclear magnetic resonance-based assays of 52 paired samples of HCC and adjacent non-tumoral tissue, the authors identified that, independently of fibrosis stage, glucose metabolism was increased in tumors as were branched chain amino acids, potentially reflecting the activation of mechanistic target of rapamycin pathways, which parallels the genetic alternations of HCC discussed previously. This study also demonstrated that HCCs had lower levels of monounsaturated fatty acids, suggesting a lipid reprogramming in HCC. Similarly, HCCs developing in the setting of advanced fibrosis also had lower monounsaturated fatty acids compared to HCCs that originated in livers with no or mild fibrosis[53]. The differences observed in tumoral *vs* non-tumoral tissues as well as in no or mild fibrosis *vs* advanced fibrosis illustrate that tumorigenesis in NAFLD may have fibrosis-independent mechanisms as suggested by Grohmann *et al*[40]. On the other hand, most patients with NAFLD who develop HCC in the absence of cirrhosis have NASH and advanced liver fibrosis instead of simple fatty liver with no or mild fibrosis, which could imply an association between fibrosis and hepatocarcinogenesis as well as common mechanisms for NASH and NAFLD-related HCC[12]. In this regard, the lipotoxicity and the metabolic reprogramming associated with steatosis are examples of pathogenic factors involved in the development of both NASH and HCC, and the inflammatory microenvironment of NASH also favors hepatocarcinogenesis[3].

Other genetic alterations that are a focus of current interest in NAFLD-related HCC are non-coding RNAs. Depending on further studies, they may provide an additional layer of complexity in epigenetic changes[45].

IMMUNE ASPECTS OF NAFLD-RELATED HCC

The mechanisms underlying the initiation and progression of HCC in the background of NAFLD are not fully understood. A number of factors including hepatic lipotoxicity, chronic inflammation, progressive fibrosis, and changes in the microbiome have all been implicated in NAFLD-related hepatocarcinogenesis. Recent studies have elegantly elucidated the role of the tumor microenvironment in this

Table 1 Summary of genetic variants described in nonalcoholic fatty liver disease-related hepatocellular carcinoma

Ref.	SNP	Associated gene	Population/cohort
Sookoian *et al*[22]; Shen *et al*[23]	rs738409 C>G	PNPLA3	American cohort; Swedish cohort; Italian cohort; British, Swiss cohort
Liu *et al*[24]; Donati *et al*[25]	rs58542926 C>T	TM6SF2	American cohort
Donati *et al*[25]; Kozlitina *et al*[26]; Falleti *et al*[27]; Vespasiani-Gentilucci *et al*[28]; Luukkonen *et al*[29]; Mancina *et al*[30]	rs641738 C>T	MBOAT7	Italian cohort

MBOAT7: Membrane bound O-acetyltransferase domain containing 7; PNPLA3: Patatin-like phospholipase domain containing protein 3; SNP: Single nucleotide polymorphism; TM6SF2: Transmembrane 6 superfamily member 2.

Table 2 Summary of genetic mutations described in nonalcoholic fatty liver disease-related hepatocellular carcinoma

Ref.	Gene	Mechanism /pathway
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*TERT*	Telomere maintenance
Brunner *et al*[36]	*ACVR2A*	Transforming growth factor-β superfamily
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*ARID5A*	Chromatin remodeling
Llovet *et al*[34]	*CDKN2A*	Cell cycle
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*CTNNB1*	β-catenin and WNT pathway activation
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*TP53*	Cellular tumor antigen, cell cycle
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*FGF19*	AKT/mTOR
Shen *et al*[39]	*Cel*	Cholesterol and lipids ester hydrolysis and absorption
Llovet *et al*[34]	*TSC*	mTOR, Hippo pathway

ACVR2A: Activin receptor type 2A; ARID5A: AT-rich interaction domain 5A; CDKN2A: Cyclin-dependent kinase inhibitor 2A; Cel: Carboxyl ester lipase; CTNNB1: β-catenin; TP53: Tumor antigen p53; FGF19: Fibroblast growth factor 19; mTOR: Mechanistic target of rapamycin; TERT: Telomerase reverse transcriptase; TSC: Tuberous sclerosis complex.

scenario[3,54-57]. Moreover, other authors have comprehensively discussed the role of cancer cell intrinsic factors that drive HCC in NAFLD[3,54,58,59]. Nevertheless, the role of the host immune system in NAFLD-related hepatocarcinogenesis must also be highlighted.

The liver is considered an immunologically privileged organ. It is constantly exposed to metabolites, toxins, and microbial products from the intestine since it derives a large part of its blood supply from the portal vein. However, there are several immune mechanisms within the liver that prevent an inflammatory hyper-response to this physiological antigenic load, including reduced expression of major histocompatibility class proteins, suppressed antigen presentation by Kupffer cells and dendritic cells, and enrichment of immunosuppressive cells like the regulatory T cells [60-62]. These mechanisms are overwhelmed in the context of NAFLD, where progressive steatosis leads to lipotoxicity, mitochondrial dysfunction, oxidative stress, and activation of cell death pathways, all of which trigger a state of chronic sterile inflammation. Unfortunately, a combination of the same factors that drive NASH progression also play mechanistic roles in the initiation of HCC in the background of this inflammatory milieu.

Progressive NASH influences both the innate and adaptive arms of the immune system, which together can enable cancer initiation and progression. The complex crosstalk among hepatocytes, adaptive immune cells, and cancer cells has been demonstrated by several studies. Wolf *et al*[54] found that infiltrating CD8+ T cells and natural killer cells contribute to NASH development and the subsequent transition to HCC. However, another study using a different mouse model of NASH showed that CD8+ T cells prevented HCC development and that a specific subset of immunosuppressive IgA+ plasma cells expressing programmed cell death ligand-1 and interleukin-10, which were abundant in NASH livers, directly suppressed liver

cytotoxic CD8+ T cells, leading to HCC development[56]. Subsequently, Ma *et al*[55] showed that the metabolic dysregulation in NAFLD causes selective loss of CD4+ T lymphocytes, thus contributing to accelerated hepatocarcinogenesis. Meanwhile, Gomes *et al*[57] have shown that T helper 17 cells are activated upon hepatocyte DNA damage in NASH and can promote HCC.

Innate immune cells like macrophages, dendritic cells and natural killer cells are also important in the pathogenesis of NAFLD-related HCC. Kupffer cells are resident macrophages that play a significant proinflammatory and profibrotic role during NASH progression. However, their role in HCC is not clear yet. Wu *et al*[63] showed that the activation of Kupffer cells positive for triggering receptor expressed on myeloid cells-1 led to secretion of proinflammatory cytokines like interleukin-6, interleukin-1β, tumor necrosis factor, C-C motif chemokine ligand 2, and C-X-C motif chemokine ligand 10, which in turn promoted HCC. In general, though, protumorigenic M2-like macrophages that drive tumor progression *via* suppressing cytotoxic T cells and inducing angiogenesis appear to be recruited from circulating bone marrow derived monocytes rather than resident macrophages[64,65]. Other immune cells like neutrophils[66-68], monocytes[69], dendritic cells[70], and natural killer cells [71,72] have also been implicated in HCC progression in NASH, highlighting the complexity of the immune mechanisms of NAFLD-related hepatocarcinogenesis (Figure 2).

HCC IN NAFLD WITHOUT CIRRHOSIS

Given some of the specificities involved in NAFLD-related hepatocarcinogenesis, HCC in the setting of NAFLD is known to occur even in the absence of liver cirrhosis, an event previously related mostly to hepatitis B virus infection[12]. The prevalence of NAFLD-related HCC in the absence of cirrhosis varies dramatically according to the geographic location of the study and even among different studies performed in a similar region of the world. Most experts estimate that between 14% and 54% of NAFLD-related HCC cases occur in patients without cirrhosis. A study from the Veterans Affairs (VA) Health System in the United States by Mittal *et al*[73] found that 42% of veterans with NAFLD-related HCC had no evidence of cirrhosis. Interestingly, a similar study by the same group the following year found the prevalence of non-cirrhotic HCC related to NAFLD to be 13%[74]. In the latter study, however, the estimation of cirrhosis was separated by different levels of confidence. Small studies from Italy and Japan have also found that 50% and 48% of NAFLD-related HCC cases, respectively, occurred in the absence of cirrhosis, suggesting that the burden of non-cirrhotic HCC in NAFLD is also significant in other parts of the world[75,76]. Finally, a meta-analysis of 19 studies found the prevalence of non-cirrhotic HCC among NAFLD-related HCC to be approximately 38%[77].

Several issues help explain the variable results from multiple studies: (1) classifying patients as to whether or not they have cirrhosis through liver biopsy is possible mainly in small studies, while this classification is much less precise in larger studies that look at International Classification of Diseases codes or large commercial clinical databases; (2) most studies in the United States have been performed in the VA System, which is inevitably biased towards a large presence of male gender among the evaluated cohorts (> 90% in most studies[32,73,74,78]); and (3) the distinction between NAFLD and NASH is not completely clear in all the studies. In this regard, a study from the Netherlands looking at almost 100 non-cirrhotic NAFLD-related HCC cases found that most individuals had a low degree of or no steatohepatitis at all, suggesting a non-inflammatory carcinogenesis path towards HCC in this setting[79].

The lack of clarity on mechanisms leading to non-cirrhotic HCC with underlying NAFLD presents a difficult dilemma for practicing providers, as it is unclear who to screen for HCC. A retrospective cohort study of 271906 patients from the VA System (mean body mass index of 31.6 kg/m^2, 28.7% with diabetes, 70.3% with hypertension, 62.3% with hyperlipidemia) suggested that diabetes and hyperlipidemia increase the risk of HCC in NAFLD[80]. However, the overall proportion of people with diabetes and NAFLD is still elevated as a total number of individuals to screen. Indeed, between 40% to 70% of individuals with diabetes have evidence of NAFLD[81]. Furthermore, it is unclear if the correlation between diabetes and HCC in patients without cirrhosis applies to other populations, as a recent study from Europe, characterizing the differences between cirrhotic and non-cirrhotic HCC in NAFLD, found an inverse association between diabetes and HCC in the non-cirrhotic group. Interestingly, non-cirrhotic HCCs in this study tended to occur in older patients and with

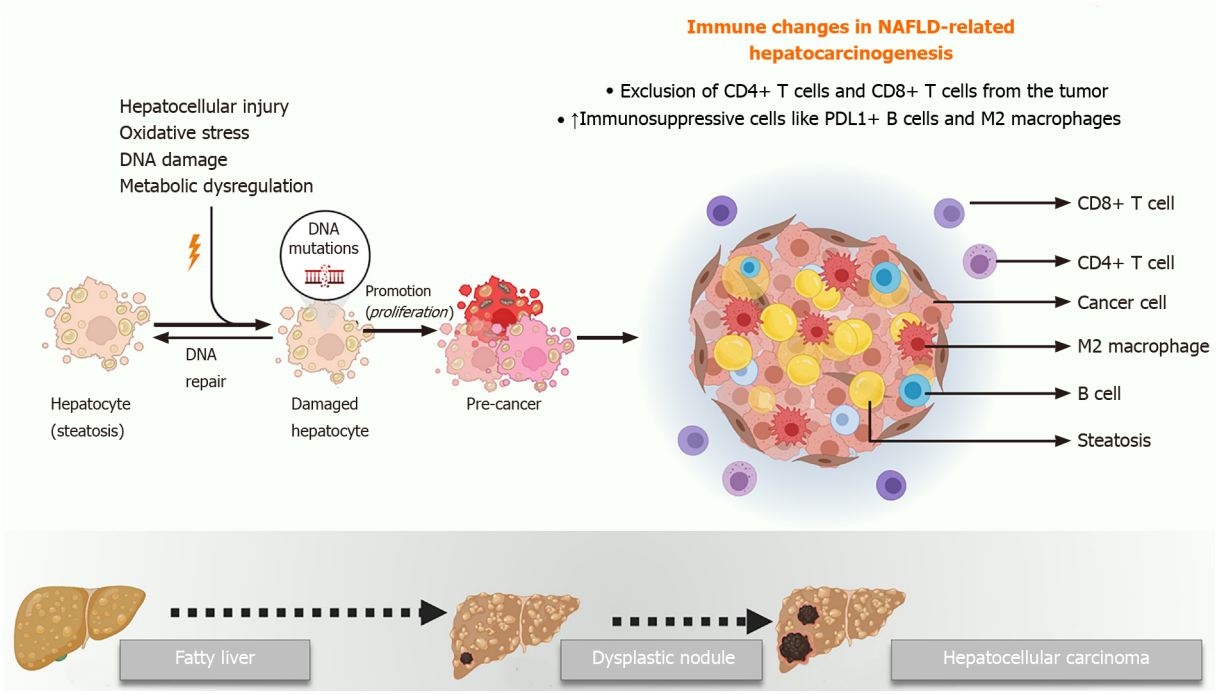

Figure 2 Main immune mechanisms of nonalcoholic fatty liver disease-related hepatocarcinogenesis. NAFLD: Nonalcoholic fatty liver disease; PD-L1: Programmed cell death ligand-1.

lower body mass index[82]. As described below, the understanding of how to surveil patients with NAFLD for HCC is in its infancy, and further studies are needed to better define those at risk.

SURVEILLANCE FOR HCC IN NAFLD

Surveillance programs aim at allowing for early detection of HCC among high-risk patients so that they have higher odds of being candidates for curative treatments. In fact, when HCC is diagnosed during surveillance, it is diagnosed in earlier stages[83-86], and patients have significantly higher survival rates[85,87]. Thus, it is of utmost importance to define which patients should be submitted to surveillance.

For individuals with an estimated annual incidence of HCC ≥ 1.5%, surveillance is considered cost-effective[8], but it is not always clear which subgroups of patients reach such a cutoff. The main risk factor for HCC in patients with NAFLD is cirrhosis, and therefore the most important international guidelines are consensual that individuals with NAFLD and cirrhosis should be surveilled for HCC with ultrasonography (US) every 6 mo[88-91]. It should be highlighted, though, that obesity and steatosis might impair the performance of US[8], and the American Gastroenterological Association recommends using either computed tomography scan or magnetic resonance imaging in cases in which US quality is deemed unacceptable[91]. Regarding the use of biomarkers, some guidelines make it optional to add alpha-fetoprotein to the surveillance program[89-91], but its performance is suboptimal, especially in NAFLD-related HCC[8], and new biomarkers should be pursued, such as those currently under study by the European-South American Consortium to Assess Liver-Originated Neoplasia.

Despite these recommendations, patients with NAFLD-related cirrhosis seem to be less likely to undergo surveillance than those with other underlying liver diseases[86,92]. In order to overcome the low adherence to surveillance, screening tools to identify individuals at higher risk for HCC could be useful. The GALAD score (gender, age, lectin-binding alpha-fetoprotein-3, alpha-fetoprotein, and des-gamma-carboxyprothrombin) has been studied in this context, and it has been recently validated in patients with NASH. In such patients, the GALAD score had sensitivity and specificity over 90% to identify individuals who would develop HCC as early as 1.5 years before the diagnosis[93].

However, some authors believe that in order to stratify patients according to their risk of developing HCC, different tools might be necessary depending on the underlying liver disease. Using data from the VA Health System database, a study evaluated 7068 patients with NAFLD and cirrhosis, with an annual incidence of HCC of 1.56%. A predictive model based on age, sex, platelet count, albumin levels, aspartate aminotransferase/alanine aminotransferase ratio, diabetes, and body mass index was developed, and it had an area under the receiver operating characteristic curve of 0.775 and 0.721 for predicting HCC in the derivation- and in the validation-cohorts, respectively. This model was able to classify patients as low-risk (< 1%/year), medium-risk (1%-3%/year), and high-risk (> 3%/year) for HCC. A classification such as this could be used, if further validated, to define subgroups that might spare surveillance[78].

As discussed above, there are subgroups of patients with NAFLD who do not have cirrhosis but are at risk of developing HCC. In a large retrospective cohort study including 296707 individuals with NAFLD and a similar number of matched controls from the VA Health System database, patients with NAFLD had 7.6-fold higher risk of developing HCC than their counterparts, and the risk was greater among men, older people, and Hispanics. However, in the NAFLD-group, the annual incidence of HCC was 10.6/1000 person-years for individuals with cirrhosis and 0.08/1000 person-years for those without it, which was considered insufficient for a general recommendation of surveillance to be made for patients without cirrhosis. The FIB-4 score was also evaluated, and, despite its association with the development of HCC, individuals with high FIB-4 scores (> 2.67) but without a diagnosis of cirrhosis were still considered to have a low risk of developing HCC[32].

Another large study evaluated four European primary care databases including over 18 million individuals and verified an incidence of HCC of 0.3/1000 person-years among patients with NAFLD, which was much higher than that of controls (hazard ratio of 3.51). When the NAFLD group was classified according to the FIB-4 score, it was possible to identify which patients were under higher risks. When compared to individuals with a FIB-4 score < 1.30, those with scores between 1.30 and 2.67 had a hazard ratio for HCC of 3.74, and the ones with scores > 2.67 had a hazard ratio of 25.2 [94]. Therefore, despite conflicting evidence, it is possible that the FIB-4 score could be used in order to select patients for surveillance.

Currently, guidelines are vague regarding surveillance for HCC in patients with NAFLD who do not have cirrhosis. The American Gastroenterological Association, in its position paper on surveillance for HCC in patients with NAFLD, recommends considering patients with NAFLD and advanced fibrosis for surveillance but recommends against routinely surveilling individuals with earlier stages of fibrosis [91]. While the position of the European Association for the Study of the Liver is similar to that[88], the American Association for the Study of Liver Diseases considers the benefit of surveillance in individuals with NAFLD who do not have cirrhosis to be uncertain and does not support it[90].

DISCUSSION

NAFLD currently affects one fourth of the global population[2]. Its increasing prevalence and the fact that it is associated with the development of liver cancer, both in the setting of cirrhosis and in its absence, make NAFLD-related HCC a growing challenge[12]. It is likely that the growth in NAFLD-related HCC will offset a decrease in viral hepatitis-related liver cancer, which is expected for the near future due to vaccination against hepatitis B virus and to the highly effective treatments for hepatitis B and C[95]. NAFLD-related HCC is already responsible for an important burden on public health, being associated with 796000 disability-adjusted life years in 2019, an increase of 33.6%in comparison to 2010[4].

This article has highlighted important genetic and immune-mediated mechanisms involved in NAFLD-related hepatocarcinogenesis. Understanding the role of certain genetic variants (especially those associated with genes such as *PNPLA3*[22,23], *TM6SF2*[24,25], and membrane bound O-acetyltransferase domain containing 7[25-30]) as well as the importance of epigenetic modifiers[44,45], the microenvironment of NAFLD, and the influences that this disease has on the innate and adaptive immune systems[54-57] will hopefully allow for a better knowledge of the clinical characteristics of NAFLD-related HCC, including the possibility of the development of liver cancer in the absence of cirrhosis. Moreover, this knowledge may help define more appropriate surveillance strategies, focusing not only in individuals with cirrhosis,

since over one third of NAFLD-related HCC cases are diagnosed in patients without this condition[77]. At present, surveillance with US every 6 mo is recommended for individuals with advanced liver fibrosis[91].

This review has limitations associated especially with the incomplete understanding of NAFLD-related HCC by the scientific community. The pathophysiology of this condition must be further studied, particularly the mechanisms leading to non-cirrhotic HCC. Moreover, there is a profound necessity for the identification of better biomarkers to detect subgroups of patients that could benefit from surveillance aside from those with cirrhosis[96].

CONCLUSION

The worldwide growing prevalence of NAFLD and its association with the development of HCC in patients either with or without cirrhosis make NAFLD-related HCC a growing challenge. Improving surveillance strategies is of the utmost importance in order for the early detection of HCC and for patients to have higher chances of being cured. Further understanding of the mechanisms leading to HCC in the setting of NAFLD will likely lead to novel molecular candidates that could be used as biomarkers to identify patients who will progress to develop a liver malignancy even in the absence of cirrhosis.

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MINIREVIEWS

Addressing hepatic metastases in ovarian cancer: Recent advances in treatment algorithms and the need for a multidisciplinary approach

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Abstract

The lifetime risk for ovarian cancer incidence is 1.39% and the lifetime risk of death is 1.04%. Most ovarian cancer patients are diagnosed at advanced stages (III, IV) because there were no specific symptoms or existing screening tests. Liver metastases have been found in up to 50% of patients dying of advanced ovarian cancer. Recent studies indicate the need for a multidisciplinary approach from initial diagnosis to oncologic surgery and chemotherapy treatment, mandating the involvement of gynecologic oncologists, surgical oncologist, medical oncologists, hepatobiliary surgeons, and interventional radiologists.

Key Words: Cancer; Metastases; Ovarian; Hepatic; Multidisciplinary

Core Tip: Each year more than 295000 women are diagnosed with and 185000 die from ovarian cancer, which remains the most lethal of all gynecologic malignancies worldwide. The management of advanced ovarian cancer has evolved over the past two decades. Surgical excision and with different minimally invasive techniques are available options for treating hepatic metastasis. A multidisciplinary approach is essential to achieve optimal treatment outcomes.

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INTRODUCTION

Each year more than 295000 women are diagnosed with and 185000 die from ovarian cancer, which remains the most lethal of all gynecologic malignancies, worldwide[1,2]. There is currently no screening test for ovarian cancer and early symptoms are usually misleading and scarce, resulting in an advanced stage at diagnosis. As a result, about two-thirds of cases are diagnosed at a late metastatic stage, and 12%-33% are International Federation of Gynecology and Obstetrics (FIGO) stage IV[3]. Ovarian cancer metastatic patterns include peritoneal and lymph node dissemination as well as hematogenous spread[4]. Peritoneal dissemination is the most common pattern of spread in FIGO stage III ovarian cancer, usually in a form of miliary tumor foci, with possible involvement of the hepatic capsule and right hemidiaphragm. According to the FIGO classification, perihepatic metastases are considered as stage III, while liver parenchymal metastases are stage IV[5]. Up to 50% of women dying of some sort of gynecologic cancer had concurrent liver metastatic disease at autopsy[6,7]. Staging, optimal cytoreductive surgery, and platinum-based chemotherapy are historically considered the standard of care for newly diagnosed advanced stage ovarian cancer. However, up to 90% of women who were optimally debulked and had adjuvant chemotherapy eventually relapse with disease progression[8]. An alternative treatment for initially inoperable disease consists of neoadjuvant chemotherapy followed by cytoreduction[9,10]. The strongest predictor of disease progression in any case is the level of cytoreduction, even in the interval setting, and it usually determines overall survival[11-13]. Complete cytoreduction is important, and exceptional surgical skill is required to achieve "no visual tumor" throughout the abdominal cavity, especially in difficult-to-treat areas, such as the upper abdomen during the operation. Complete cytoreduction may require procedures, such as peritonectomy, diaphragmatic resection, and multiple visceral resections[14-19]. Liver metastases of ovarian cancer are considered for surgical therapy, but with controversial indications and patient selection criteria. Addressing liver metastases of ovarian cancer origin still represents a barrier to complete cytoreduction. Several studies have reported the feasibility and efficacy of hepatic resection in the setting of advanced ovarian cancer [20-22]. There are several other treatment modalities of liver metastases, such as thermal radiofrequency (RFA) or microwave (MWA) ablation, cryoablation, laser induced thermotherapy (LITT), transarterial chemoembolization (TACE), computed tomography-guided high dose-rate brachytherapy (CT-HDRBT) and stereotactic body radiation therapy (SBRT). In this review, we aim to summarize recent advances in the management of ovarian cancer liver metastases. The value of the involvement of different medical and surgical specialties and subspecialties is discussed. A multidisciplinary approach to advanced ovarian cancer is essential to achieve optimal treatment outcomes.

METHODOLOGY

A review of literature on the management of liver metastases of ovarian cancer was performed. A comprehensive search of the National Library of Medicine MEDLINE/PubMed database was performed for articles published in the last two decades. The date of the last search was February 28, 2021. The search strategy included the keywords "ovarian," "cancer," "hepatic," "liver," "metastasis, -es," and "multidisciplinary." Articles relevant to the subject in the citations of each report were additionally included. Articles that were written in non-Latin alphabets were excluded for translational reasons.

SURGICAL PROCEDURES

Radical surgical resection plus postoperative treatment of liver metastases of colorectal origin have gradually evolved as a standard of care in many cancer centers, with reports of 5-year overall survival of such patients reaching 50% or more[23,24]. Results of recent studies treating patients with liver metastases of neuroendocrine origin, report a 5-year overall survival exceeding 65%[25]. Generally, recent data show a better prognosis with liver metastases originating from the genital system than with those from other non-colorectal, non-neuroendocrine primaries[26,27]. Recent trends of treatment of advanced ovarian cancer are based on the application of cytoreductive surgery; hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and radical

excision of all intraperitoneal disease, including the upper abdomen, with a curative intent and a clear survival benefit[28-30]. About 40% of women diagnosed with advanced stage ovarian cancer present with a concurrent bulky tumor load in the upper abdomen (*i.e.* the diaphragm, stomach, or liver), requiring cytoreductive surgery[31].

Liver mobilization, hepatic capsular metastases resection, liver segmentectomy, and diaphragmatectomy are surgical treatment procedures described by Wang *et al*[32]. Specifically, they recommend wedge excision or at least 1 cm of ablation depth for hepatic capsular metastases, rather than superficial excision. Diaphragmatic resection and repair rather than diaphragmatic peritoneal dissection should be applied for metastatic tumors located between the right hemidiaphragm and liver capsule. In case an anatomical resection is performed, a resection margin of more than 2 cm is required. If the metastatic disease involves porta hepatis, hepatic portal skeletonization, portal lymph node dissection should be performed.

In a study by Kamel *et al*[33] in 2011, a significant survival benefit was demonstrated for patients with ovarian cancer liver metastases treated with surgical resection *vs* patients with a similar tumor burden who had biopsy only. Median overall survival from the time of the diagnosis of liver metastatic disease was 53 mo *vs* 21 mo. Similar results were reported by a multicenter study of 2655 patients with ovarian cancer liver metastases who underwent cytoreduction in the upper abdomen[29]. The median overall survival was 54.6 mon for patients who were completely debulked. The importance of complete cytoreduction (R0) not only in the lower abdomen, but also with liver involvement was discussed by Bristow *et al*[34]. They reported an overall survival of 50.1 mo for patients who had undergone R0 Liver resection and R0 cytoreduction, *vs* a 20-mo overall survival of patients treated with an R0 cytoreduction and a non-R0 liver resection. Bolton and Fuhrman[35] conducted a study on a group of patients who had fewer than three liver metastases and another group having more than four lesions at the time of liver resection. Surprisingly, the investigators reported no difference in survival when complete excision of the hepatic tumors was achieved.

Several studies have reported on the safety and efficacy of upper abdominal cytoreductive including diaphragmatic and hepatobiliary resection[22,31,36-38], but others have reported major complications linked with that kind of surgical treatment[39]. Chi *et al*[36] reported the most common postoperative complications in a group of 141 patients treated with upper abdominal cytoreduction of liver metastases. They included pancreatic leaks, intraperitoneal ascitic fluid accumulation, and symptomatic pleural effusions. The reported overall morbidity and mortality were 22% and 1.4% respectively. A review by Gasparri *et al*[22] included studies in which liver resection was performed at either the time of primary treatment or the time of recurrence. The investigators reported no complications attributed to liver resection in the first category and only minimal complications in the second, including bilioma and transient liver function test abnormalities. The most important prognostic factors were the extent of residual disease and patient performance status. Similar perioperative outcomes and rates of complications were reported in cases of cytoreduction including either both upper and lower abdomen or solely the lower abdomen[22,40]. A major survival benefit may be safely achieved with surgical removal of liver tumor deposits during primary, secondary, tertiary and even quaternary cytoreduction[22,31]. According to Neuman *et al*[41], tumor dissemination pattern, cancer antigen (CA)-125 value, age, and initial stage of disease or level of resectability of the tumor did not seem to affect outcome. However, the presence of ascites and the location of tumor aggregates in both liver lobes ere associated with a worse prognosis.

THERMAL ABLATION TECHNIQUES

Thermal ablation techniques in liver surgery include RFA, MWA, cryoablation, and LITT. Locoregional ablation is effectively applied in patients with liver metastases considered inoperable because of surgical or anesthetic contraindications. In cases where liver lesions are parenchymal and not localized on the surface or Glisson's capsule, percutaneous local ablation is feasible and effective without the use of anesthesia. Such patients recover treatment sooner and are fit to receive adjuvant chemotherapy. Usually, hepatic metastases of ovarian cancer origin are superficial, and can only be ablated intraoperatively to protect surrounding tissues from thermal injury. Contraindications to such locoregional ablative intraoperative treatment include tumor location near the hepatic hilum, porta hepatis, or near large bile ducts. Compared with surgical removal of tumors, local ablation is usually associated with a

higher rate of recurrence, while lesions greater than 3 cm are usually not satisfactorily ablated[22]. Another obvious limitation of thermal ablation procedures compared with surgical resection is the lack of a surgical margin, as simple post ablation radiographic findings are used to determine efficacy. Only highly selected patients undergo such treatment procedures, and the local control and long-term survival benefits are still pending from large multicenter prospective studies.

RFA

RFA is a minimally invasive procedure in which high frequency alternating current is delivered through an electrode directly to the tumor, providing ablation and eventually cell death while sparing surrounding tissues from unnecessary damage. Low morbidity and mortality are attributed to this minimally invasive technique with a therapeutic intent. Many studies report a morbidity rate from 2%-5.7% and a mortality rate of less than 1% associate with RFA treatment. Patient safety is clearly greater with RFA than with liver resection, which has a reported treatment-associated morbidity of 25% and mortality of less than 5%[42-44]. RFA is indicated in selected patients with ovarian cancer liver metastases, numerous metastases, large metastases, or with foci located deep within the liver parenchyma[45-47]. Effective local tumor control has been reported in several studies of RFA in liver metastases, with a limited number of reported complications, such as bleeding, liver abscess, and rare cases of bile leakage. In 2014, Liu *et al*[47] reported no serious complications after the application of RFA in ovarian cancer liver metastases, with 1-, 3-, and 5-year overall survival rates of 100%, 61%, and 61% respectively. In 2005, Mateo *et al*[48] reported the outcomes achieved with RFA combined with excisional surgery for hepatic metastases. Prospective randomized controlled studies are eagerly awaited in order to get a better idea of the therapeutic benefit provided by the application of either RFA and/or liver resection in the treatment of hepatic metastases originating from ovarian cancer.

MWA

MWA is a minimally invasive method of thermal ablation. It uses electromagnetic energy in the microwave spectrum to increase intratumoral temperature and achieve large ablation volume[49,50]. Zhuo *et al*[51] reported that MWA (50 w × 10 min) achieved acceptable perioperative morbidity and mortality and reduced blood loss, transfusion volume, and cost compared with surgical resection of metastatic lesions. However, patients treated with MWA had a significantly higher mortality in terms of overall survival.

LITT

LITT uses neodymium-doped yttrium aluminum garnet laser light to induce therapeutic coagulation. This laser technique uses thin flexible fibers and a water-cooled applicator. A sphere of necrosis is produced from a bare fiber, while a diffuser fiber accomplishes ablation in an elliptical shape. In the multi-applicator mode, a single lesion can be ablated with the simultaneous use of up to five laser applicators [52].

Cryoablation

This ablation technique induces cell death in a target lesion by alternate freezing and thawing[53]. Gao *et al*[54] investigated the efficacy and safety of cryoablation in the treatment of ovarian cancer hepatic metastases. The post ablation local tumor progression rate was 7.14%, and the 1-year overall survival was over 90%. No serious complications (*e.g.*, liver bleeding, cryo-shock, hepatic failure, abscess, biliary fistula, renal insufficiency or others) were reported. A constellation of post ablation symptoms was observed in about half the patients, including low grade fever and malaise, and abdominal pain and was described as "postcryoablation syndrome". Elevated transaminases and right-side pleural effusion were noted in a few patients. Goering *et al*[55] found similar relapse-free rates in patients treated with cryoablation combined with hepatic resection surgery and those with surgery alone. They suggested that cryoablation could increase the number of patients eligible to surgery.

TACE

TACE has been historically used to treat primary and metastatic liver tumors. It consists of local arterial infusion of chemotherapy drugs plus embolization particles [50]. TACE is recommended for the treatment of hepatocellular cancer and liver

metastases, especially those originating from colorectal or neuroendocrine malignancies[24,56-61]. Ovarian cancer patients usually undergo cytoreductive surgery and may then receive adjuvant treatment by chemoembolization of secondary liver lesions. TACE indications for the treatment of hepatic metastases include tumors that do not respond to chemotherapy, unresectable tumors, or toxicity of chemotherapeutic agents. Generally, it is used as a last attempt to control intrahepatic metastases while preserving good liver function[62].

SBRT

SBRT, also known as stereotactic ablative radiotherapy (SABR) is a form of external beam radiotherapy that delivers a high dose of radiation in a single or a few fractions, with accuracy sufficient to hit a target and at the same time minimize the induced injury to surrounding tissues[63]. In the phase II SABR-COMET trial[64], 99 patients with hepatic oligometastases of one to five lesions from a variety of primary tumors including breast, colorectal, lung, and prostate were included. They were randomized to two groups based on whether they had received SBRT or standard palliative treatment. The authors reported a higher median overall survival in the SBRT group, 41 mo *vs* 28 mo. Toxicities greater than grade 2 were reported more often in the SBRT group (29% *vs* 9%). Three treatment related deaths (4.5%) were reported. Because of the paucity of randomized studies, the efficacy of SBRT in ovarian cancer remains elusive.

Yegya-Raman *et al*[65] conducted a systematic review of the role of SBRT in the treatment of oligometastatic gynecologic malignancies, primarily ovarian cancer. Seven of eight studies reported response rates > 75%, and 14 of 16 reported local tumor control rates of > 80%. No toxicities greaten than grade 3 were documented in 56% of the studies. In ten studies, the median progression-free survival was between 3.3 and 9.7 mo. Disease progression was usually observed outside the SBRT field. The efficacy of SBRT for management of liver metastases was similar to that of RFA, as indicated by the reported 2-year overall survival[66]. Systemic therapy is usually combined with SBRT, as it has been observed that the therapeutic combination addresses the tendency for distant progression, with less toxicity. Kunos *et al*[67] reported on the almost concurrent use of SBRT and systemic chemotherapy. The grade 3-4 toxicities that were documented were mainly hematologic and metabolic and were most likely chemotherapy related. Another combination therapy includes SBRT plus immunotherapy and has had positive results. In conclusion, the use of SBRT should be seriously considered as an alternative to surgery or chemotherapy, especially in patients with low performance status, already overtreated, or not suited for more aggressive procedures.

COMPUTED TOMOGRAPHY-GUIDED HIGH DOSE-RATE BRACHYTHERAPY

In 2004, Ricke *et al*[68,69] described the use of computed tomography-guided high dose-rate brachytherapy (CT-HDRBT) in clinical practice. CT-HDRBT is a locally applied radioablation technique administers iridium-192 through catheters into the tumor for a short time under CT guidance. The technique doe not require cooling of adjacent large vessels, and tumor size is not a burden. CT-HDRBT is recommended as an effective and feasible way to treat unresectable primary and secondary hepatic tumors. It has excellent local tumor control, time to disease progression, and overall survival outcomes[70,71]. A small study by Collettini *et al*[72] investigated the efficacy and safety of HDRBT in the treatment of ovarian cancer hepatic oligometastases. They reported that the method was safe and had an excellent local control rate. The overall 12-mo survival rate for a 12-mo period was 100%. CT-HDRBT can be effectively used to treat advanced ovarian cancer synchronous and metachronous liver metastases as a combined therapeutic approach with primary cytoreductive surgery or interval debulking.

MULTIDISCIPLINARY APPROACH

Building a multidisciplinary team (MDT) is essential for the optimal treatment of patients with advanced ovarian cancer and liver metastases. National Comprehensive Cancer Network guideline algorithms of ovarian cancer management recommend the involvement of gynecologic oncologists, pathologists if a biopsy is available, radiologists, interventional radiologists, anesthesiologists, hepatobiliary surgeons, and physicians certified to perform cytoreductive surgery[73]. All cancers should be discussed at MDT committee meetings, which time the treatment algorithms are chosen. The presence of an anesthesiologist is recommended in order to discuss the eligibility for surgery of each patient[74]. A Cochrane Review found that centralization of ovarian cancer surgical oncology services improved overall survival[75]. Management of patients by MDTs is more likely to lead to correct staging[76], evidence-based management, appropriate, and well-timed treatment[77]. As for the surgical subspecialties, intraoperative collaboration of gynecologic oncologists with colorectal and hepatobiliary surgeons is more likely to achieve a complete cytoreduction[78]. As radiographic findings, especially CT, are essential for preoperative evaluation as well as postoperative follow-up, participation of competent radiologists is valuable in patient management and decision making[79]. Interventional radiologists use a variety of techniques to perform the above mentioned minimally invasive procedures. It is clear that the involvement of different disciplines improves the quality of care and shows professionalism in gynecological cytoreductive surgery.

CONCLUSION

The management of advanced ovarian cancer has evolved over the past decade. Parenchymal hepatic metastases are no longer considered as an exclusion criterion when deciding whether a patient is eligible for optimal debulking. Various surgical and minimally invasive procedures with acceptable local control and toxicity profiles, represent valid options for treating liver metastases. Further investigation, ideally by randomized controlled trials, is needed to identify the subset of patients that will most likely benefit from each therapeutic modality. Building a MDT is of outmost importance when treating ovarian cancer liver metastases and will enhance therapeutic outcomes.

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MINIREVIEWS

Atezolizumab and bevacizumab as first line therapy in advanced hepatocellular carcinoma: Practical considerations in routine clinical practice

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. For advanced HCC, sorafenib was considered the standard of care for more than ten years. Recently the atezolizumab and bevacizumab combination has become standard of care for these patients without contraindications to either immune checkpoint inhibitors or antiangiogenic therapy. We now review the practical aspects of the atezolizumab and bevacizumab combination, including current evidence, indications, contraindications, management of adverse events, sequencing of this combination, areas of current knowledge gaps and future areas of active clinical research of this combination for busy clinicians in clinical practice.

Key Words: Hepatocellular carcinoma; Atezolizumab; Bevacizumab; Immunotherapy; Child Pugh cirrhosis; Anti-angiogenic therapy

Core Tip: There are several articles about the role of atezolizumab and bevacizumab combination in advanced unresectable hepatocellular carcinoma. However, this mini review focuses on practical issues for clinicians using this combination in hepatocellular carcinoma (HCC) patients with focus on indications, data from recent trials,

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criteria for selection of appropriate patients for this combination, sequencing strategies, overlapping toxicities, issues with Child Pugh B cirrhosis patients, future role in adjuvant settings and dealing with special subsets of HCC population.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and leading cause of cancer related death[1]. Early-stage HCC can be treated by resection, liver transplantation or ablation. Unfortunately, most patients present with an intermediate or advanced-stage disease with limited systemic options and a dismal prognosis. The multikinase inhibitor sorafenib was initially approved more than a decade ago for the management of advanced HCC[2]. Recently, four additional targeted therapies were approved for advanced HCC based on positive phase III randomised controlled trials (RCTs): Lenvatinib in the first-line setting and regorafenib, cabozantinib and ramucirumab, all in the second line after the failure of sorafenib therapy[2-5].

The recent publication of successful results of Phase III RCT IMbrave 150 has established the combination of atezolizumab and bevacizumab (Atezo and Beva) as first line therapy for advanced treatment naïve HCC with Child Pugh A cirrhosis[6]. We now review the pharmacological rationale, evolution, results, practical issues in clinical practice, current knowledge gaps and future possibilities of this combination therapy. This is an expert review based on our current clinical knowledge of this combination.

PHARMACOLOGICAL RATIONALE OF THIS COMBINATION

Atezolizumab is a monoclonal antibody against programmed cell death ligand 1 (PD-L1). PD-L1 receptors are expressed on tumour cells. The programmed cell death protein 1 (PD-1) is present on cytotoxic T lymphocytes (CTLs) and tumour cells. The interaction of PD-1 and PD-L1 is an immune inhibitory pathway. Atezolizumab reverses T cell suppression by preventing interaction between the inhibitory immune checkpoint molecules PD-1 and PD-L1. Vascular endothelial growth factor (VEGF) induces tumour angiogenesis. In addition to inducting tumour angiogenesis, VEGF also mediates immunosuppression within the tumour microenvironment by promoting immunosuppressive cells such as regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs) and tumour associated macrophages. VEGF also suppresses antigen-presenting cells and CTLs. In summary, bevacizumab not only inhibits tumour growth by inhibiting angiogenesis but also augments the immune agonistic effects of atezolizumab by reversing the immune suppressive mechanisms of VEGF pathways[7].

EVOLUTION OF ATEZOLIZUMAB AND BEVACIZUMAB COMBINATION IN THE MANA-GEMENT OF ADVANCED HCC

Phase Ib GO30140 study

In this phase I B study, there were four cohorts of various malignancies. In the HCC cohort, arm A received the combination of atezolizumab and bevacizumab in patients with unresectable HCC. The primary endpoint for this arm was overall response rates (ORR). Arm F of the same study randomised patients with unresectable HCC to atezolizumab and bevacizumab combination *vs* atezolizumab monotherapy arm. The

primary endpoint of Arm F was progression-free survival in the intention-to-treat population. The dose of atezolizumab in both the arms with or without combination was 1200 mg I.V. every three weeks. In the combination arm, the bevacizumab dose was 15 mg/kg. The critical results of the trial are summarized in Table 1[8].

Kudo[7] have comprehensively reviewed these results. As per Kudo[7], the 12% C.R. rates in arm A is very impressive as this group had patients with advanced HCC with poor prognostic factors such as α-fetoprotein (AFP) ≥ 400 ng/mL, extrahepatic spread (EHS), major vascular invasion. These results were never achieved in the tyrosine kinase inhibitors (TKI) era. The other important finding was the ORR of 62% (8/13) in intermediate stage disease with a high tumour burden.

As per Kudo[7], the Arm F is an essential proof of concept study that demonstrates the favourable results obtained in Arm A are not solely due to the efficacy of atezolizumab monotherapy but precisely due to a combination of atezolizumab and bevacizumab. The Arm F scientifically reinforces the synergistic combination of antiangiogenic therapy and immunotherapy.

In Arm A, The most common grade 3-4 treatment-related adverse events were hypertension (13%) and proteinuria (7%). Treatment-related adverse events occurred in 25 (24%) patients. There were three (3%) treatment-related deaths due to abnormal hepatic function, hepatic cirrhosis and pneumonitis.

IMBrave 150 trial

IMbrave 150 was a global, open-label, randomised phase III trial comparing atezolizumab plus bevacizumab *vs* sorafenib in systemic treatment-naive unresectable HCC [6]. Patients were randomly assigned in a 2:1 ratio either to atezolizumab plus bevacizumab or sorafenib until unacceptable toxic effects occurred or loss of clinical benefit[7]. The coprimary endpoints were overall survival and progression free survival in the intent to treat population, as assessed at an independent review facility according to Response Evaluation Criteria in Solid tumours, version 1.1 (RECIST 1.1).

The main inclusion criteria for the study were unresectable or metastatic HCC patients with ECOG-PS (Eastern Cooperative Oncology Group-Performance Status) of 0 or 1, Child-Pugh A cirrhosis. Patients with disease not amenable to curative surgical and or locoregional therapies or progressive disease after surgical or locoregional therapies were eligible. For patients with active hepatitis B virus (HBV), the trial requirement was quantitative HBV DNA < 500 IU/mL obtained within 28 d before initiation of therapy, and patients who have taken at least two weeks of anti-HBV treatment and willing to continue throughout the study duration.

The key exclusion criteria were a history of autoimmune disease and untreated or incompletely treated oesophageal or gastric varices (assessed with esophagogastroduodenoscopy) with bleeding or higher risk of bleeding. The trial required mandatory assessment of oesophageal or gastric varices within six months of initiation of trial therapy.

The most important autoimmune diseases in the exclusion criteria were myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener granulomatosis, Sjogren's syndrome, Guillain-Barre syndrome or multiple sclerosis. Patient with known fibrolamellar variant, sarcomatoid HCC or mixed cholangiocarcinoma and HCC were excluded from the study.

The patients were stratified by geographical region (Asia excluding Japan *vs* the rest of the world), macrovascular invasion or EHS of disease (presence *vs* absence), baseline alfa fetoprotein levels of (< 400 ng/mL *vs* > 400 ng/mL), ECOG of 0 or 1.

Patients assigned to the atezolizumab -bevacizumab group received 1200mg of atezolizumab plus 15mg/kg of body weight of bevacizumab intravenously every three weeks. Dose modifications were not permitted in the atezolizumab group but were allowed in the sorafenib group. Patients who transiently or permanently discontinued either atezolizumab or bevacizumab because of an adverse event were allowed to continue taking the single-agent therapy as long as the investigator determined that there was a clinical benefit. Table 2 describes the confirmed response rates, progression-free survival, overall survival and disease control rate in the IMBrave 150 trial.

Quality of life: Atezolizumab-bevacizumab delayed deterioration of patient-reported quality of life (median time to deterioration), 11.2 mo with atezolizumab- bevacizumab combination *vs* 3.6 mo with sorafenib arm. The deterioration in physical functioning and role functioning were also delayed in the experimental arm by an additional 8.2 mo and 5.5 mo, respectively.

Table 1 Results of phase Ib GO30140 study

	Arm A	Arm F	
	Atezolizumab and bevacizumab combination (*n* = 104), median follow up 12.4 mo	Atezolizumab and bevacizumab combination (*n* = 60), median follow up 6.6 mo	Atezolizumab monotherapy (*n* = 59), median follow up 6.7 mo
ORR, *n* (%)	37 (36)	12 (20)	10 (17)
CR, *n* (%)	12 (12)	1 (2)	3 (5)
DCR, *n* (%)	78 (75)	40 (67)	29 (49)
Median PFS, mo	7.4 (5.6-10.7)	5.6 (3.6-2.4)	3.4 (1.9-5.2)
HR (80%CI)	-	0.55 (0.40-0.74), *P* value (0.0108)	
12 mo PFS (%)	38		
12 mo OS (%)	63		

ORR: Over all response rates; CR: Complete response; DCR: Disease control rate; PFS: Progression free survival; HR: Hazard ratio; OS: Overall survival.

Table 2 Results of IMBrave 150 trial

Results	Atezolizumab and bevacizumab arm	Sorafenib arm	Statistically significant
Estimated OS at 6 mo (%)	84.8	72.2	
Estimated OS at 12 mo (%)	67.2	54.6	
PFS (mo)	6.8	4.3	HR for progression or death was 0.59 (0.47-0.76) *P* < 0.0001
Confirmed ORR as per independent mRECIST assessment (%)	27.3	11.9	
As per HCC specific mRECIST CR (%)	5.5	-	
Disease Control Rate (ORR + SD) (%)	73.6	55.3	

HCC: Hepatocellular carcinoma; OS: Overall survival; PFS: Progression free survival; ORR: Objective response rate; mRECIST: Modified response evaluation criteria in solid tumours.

IMBrave 150 investigators have recently published the patient reported outcomes (PROs) of this study. The PROs were prespecified exploratory endpoints of the study. The study showed clinically meaningful benefit in terms of patient reported quality of life, functioning and disease symptoms with atezolizumab and bevacizumab as compared to sorafenib. The patients completed the European Organisation for Research and Treatment of Cancer quality-of-life questionnaire for cancer (QLQ-30) and quality of life questionnaire for HCC (QLQ-HCC18). As compared to sorafenib, atezolizumab and bevacizumab combination reduced the risk of deterioration for appetite loss, diarrhoea, fatigue and pain. The benefits for fatigue and pain were maintained in QLQ-HCC18 scale too[9].

Safety: Adverse events of any grade were reported in 323 patients (98.2%) who received the atezolizumab- bevacizumab and 154 patients (98.7%) who received sorafenib. Grade 5 events occurred in 15 patients (4.6%) in the experimental group and in 9 patients (5.8%) in the sorafenib group. Table 3 tabulates the number of Grade 5 events in both arms.

The most common grade 3 or 4 adverse event with atezolizumab-bevacizumab was hypertension (15.2%). Grade III HTN is defined as Stage II HTN with blood pressure (≥ 160/≥ 100 mmHg). Serious adverse events occurred more frequently with atezolizumab and bevacizumab combination 125 patients (38%) than with sorafenib 48 patients (30.8%).

Table 3 Grade 5 events in both the arms IMBrave 150 trial

Atezolizumab and bevacizumab (*n* = 15), grade 5 adverse events	Sorafenib (*n* = 9), grade 5 adverse events
Gastrointestinal Haemorrhage (3)	Death (2)
Pneumonia (2)	Hepatic cirrhosis (2)
Empyema, gastric ulcer perforation, abnormal hepatic function, liver injury, multi-organ dysfunction syndrome, esophageal varices haemorrhage, subarachnoid haemorrhage, respiratory distress, sepsis and cardiac arrest (1 in each patient)	Cardiac arrest, cardiac failure, general physical health deterioration, hepatitis E, peritoneal haemorrhage (1 in each patient)

SELECTING APPROPRIATE PATIENTS FOR THE ATEZOLIZUMAB AND BEVACIZUMAB COMBINATION

It will be crucial for multidisciplinary teams (MDTs) to cautiously choose the most suitable patients for this combination. Patients with locally advanced unresectable tumours not suitable for locoregional therapies such as transarterial chemoembolization (TACE) and metastatic HCC with Child-Pugh A liver disease will be the most appropriate patients provided they have no other major contraindications to immunotherapy or VEGF inhibition therapy. The patients not amenable to locoregional therapies will be patients with severely impaired main portal vein flow (resulting from occlusive thrombus, tumour invasion or hepatofugal blood flow) because of dependence on the arterial inflow to adequately supply the liver[10].

TACE has not shown any survival benefit in patients with extensive bilobar involvement, so these patient will need upfront consideration of systemic therapy[11].

Stopping rules for TACE

TACE sessions are scheduled more often performed on-demand than on a predetermined time line. Decisions to continue or cease TACE are based on repeat liver imaging and the tumour response to treatment. Many algorithms have been developed to help with these decisions but are not universally validated[12]. In general, the appearance of extrahepatic metastases, vascular invasion or worsening clinical status would usually lead to ceasing further TACE procedures. Further, the concept of TACE-'refractoriness' is also to be considered. First proposed by the Japanese Society of Hepatology, the primary definition includes lack of objective response to 2 sessions of TACE (viable lesion > 50% or two or more consecutive increases in tumour number), the continuous elevation of tumour markers after TACE, vascular invasion and metastasis.

Repeated TACE procedures can lead to worsening liver function due to hepatic devascularisation[13]. This can preclude effective systemic therapies.

OPTIMIS was an international prospective observational study enrolling patients with unresectable HCC who were being considered for TACE. The authors noted that over 90% of patients continued to receive TACE despite an inadequate response. Those who transitioned to sorafenib earlier at the time of TACE-'refractoriness' had longer overall survival rates than those who were treated later. A recent Korean retrospective study also reiterated early transitioning to systemic therapy in patients without an objective response to 2 consecutive TACE procedures[14].

These patients need discussion at MDT meetings for consideration of alternative treatment options such as the atezolizumab and bevacizumab combination if there are no contraindications for this protocol.

SYSTEMATIC REVIEW AND META-ANALYSIS SUPPORTING ATEZOLIZUMAB AND BEVACIZUMAB IN FIRST LINE SETTINGS FOR MANAGEMENT OF ADVANCED HCC

In the most recent systematic review and network meta-analysis of eight first line trials with a total of 6290 patients, the combination of atezolizumab and bevacizumab was superior to lenvatinib [hazard ratio (HR) 0.63], sorafenib (HR 0.58) and nivolumab (0.68)[15].

ROLE OF ATEZOLIZUMAB AND BEVACIZUMAB IN COMBINATION WITH LOCOREGIONAL THERAPIES

Locoregional therapies such as radiofrequency ablation, TACE and cryoablation can induce multiple immunogenic effects. These procedures have multiple mechanisms to stimulate the immune system. These mechanisms are: (1) Inhibiting immunosuppressive cells like MDSC and Tregs; (2) PD-L1 upregulation; (3) Increased effector immune cells like dendritic cells, natural killer cells and T cells; and (4) Increased release of tumour antigens like glypican 1, AFP.

Several trials are examining combinations of various locoregional modalities with different immune checkpoint inhibitors (ICI). Multiple biomarkers will be evaluated in these studies including AFP, cell death biomarkers like sRAGE and circulating GPC3 cytotoxic lymphocytes[16].

TACE-induced tissue hypoxia leads to upregulation of hypoxia-inducible factor-1a, which facilitates VEGF and platelet derived growth factor expression[17]. The latter promotes neoangiogenesis and tumour revascularisation. These diverse mechanisms provide a rationale for combining atezolizumab and bevacizumab with locoregional therapies.

Currently NCT04224636 trial is recruiting patients for treatment with TACE in combination with atezolizumab and bevacizumab. There are many unanswered questions about sequencing of locoregional therapies and various ICIs.

ROLE OF ATEZOLIZUMAB AND BEVACIZUMAB IN ADJUVANT SETTINGS

Up to 70% of patients can develop recurrence in 5 years after curative intent resection for early stage HCC[18]. There is high rate of intrahepatic recurrences in patients with large tumour size, an incomplete tumour capsule, venous or microvascular invasion. There are multiple mechanisms by which surgery or radiofrequency ablation can alter the immune microenvironment of liver[19]: (1) More MDSC accumulates, leading to the immunosuppressive microenvironment; (2) The balance of proinflammatory phenotype 1 helper T cell is altered to a more immunosuppressive T-helper 2 phenotype; and (3) Tumour macrophages are polarized to an immunosuppressive M2 phenotype during postoperative wound healing. So, there is a solid rationale for considering immunotherapy in the postoperative adjuvant setting for HCC.

The major success of atezolizumab and bevacizumab in the metastatic setting has led to new trials of this combination in the adjuvant setting and in combination with other locoregional therapies. IMbrave050 (NCT0410298) is testing atezolizumab and bevacizumab *vs* active surveillance as adjuvant therapy in patients with HCC at high risk of recurrence after surgical resection or ablation. The primary outcome of the study is recurrence-free survival. The Supplementary material, Appendix 1 provides information on currently listed trials of this combination in various settings at clinical trial.gov website.

BIOMARKERS WITH THE PROGNOSTIC AND PREDICTIVE ROLE FOR THE USE OF A COMBINATION OF ATEZOLIZUMAB AND BEVACIZUMAB IN ADVANCED HCC

In the phase Ib exploratory analysis, higher expression of PD-L1 in tumour tissue, higher expression of VEGF receptor 2, and higher T-regulatory cells immunophenotype were associated with better survival[8]. Currently, this analysis is pending for the IMBrave phase III trial. In this trial, the combination showed more benefits in patients with AFP of < 400 ng/mL, viral aetiology (HBV and HCV associated HCC) had more benefits than non-viral aetiology[6]. This can be due to the immune stimulatory environment due to chronic inflammation associated with viral aetiology associated with HCC. The prevalence of microsatellite instability (MSI)-high disease and TMB is very low in HCC. In a study of 755 patients out of 542 cases assessed for MSI, only one patient (0.2%) was MSI-high and TMB-high[20].

At this stage, aetiology (viral or non-viral) should not be used in triaging the types of systemic treatments in advanced HCC. There are preclinical and clinical signals that the atezolizumab and bevacizumab combination may not be very effective in patients with HCC associated with non-alcoholic fatty liver disease (NAFLD). There is

preclinical evidence that NAFLD decreases CD4+T cells and induces tumour promoting functions in CD8+T cells, natural killer cells and Th17 cells[21,22]. More than 50% of patients with NAFLD are obese, and obesity may increase the resistance to VEGF therapy[23]. In the IMbrave 150 trial, the combination of atezolizumab and bevacizumab was less effective in patients with non-viral *vs* viral etiology with a HR of 0.91 as compared to sorafenib[6].

There is emerging evidence that WNT/B-Catenin signaling is associated with a lack of T cell infiltrates and predict resistance to immunotherapy like atezolizumab[24]. There is a proposed immunological classification in HCC, which divides HCC into three subclasses: (1) Immune (30%); (2) Immune intermediate (45%); and (3) Immune excluded class (25%). There is preclinical and clinical data of activation of WNT/B-catenin pathway leading to resistance to immunotherapy in immune excluded subtype of HCC.

In summary, there are currently no proven biomarkers that can be used to select patients for this particular combination.

COMMON OVERLAPPING TOXICITIES IN CIRRHOTIC PATIENTS TREATED WITH IMMUNOTHERAPY

Meriggi and Graffeo[25] have comprehensively reviewed the toxicities due to cirrhosis but overlap with immunotherapy agents and TKI. Due to the secretion of gastrin and vasoactive peptides, diarrhoea or loose stools can be a common symptom in patients with cirrhosis. Both immunotherapy and TKI can worsen diarrhoea. It is essential to adequately investigate the diarrhoea with stool culture, Clostridium difficile toxin assessment and standard biochemical tests. Diarrhoea associated with abdominal pain and signs of colonic inflammation is most likely related to immune-mediated colitis. It is helpful to do a baseline calprotectin when patients are admitted with diarrhoea to rule out immune-mediated colitis. Titrating the dose of lactulose used to prevent encephalopathy may be necessary to control the diarrhoea. Adequate doses of loperamide and steroids should be used to manage patients with possible immune-mediated colitis, once the common causes of diarrhoea are ruled out. Colonoscopy should be reserved for patients with severe diarrhoea with a high index of suspicion for immune-mediated colitis or those who remain steroid refractory. For those patients with steroid-resistant or refractory colitis, the use of infliximab will be challenging, given it can cause liver injury in susceptible patients.

Cancer-related fatigue is also one of the symptoms common to cirrhotic patients and can worsen with ICI therapy. Education about exercise and physical activity is crucial at the start of treatment. According to Meriggi and Graffeo[25], profound asthenia is common in HCC patients and can be multifactorial due to electrolyte imbalance, thyroid dysfunction, increased cytokine production, serotonin imbalances and vagal response activation[25]. Baseline assessment of thyroid function can dictate the need to initiate the thyroxine therapy before starting ICIs as autoimmune thyroiditis is a common side effect in the first 3-6 mo after initiation of ICIs.

Pruritis is also an overlapping symptom in HCC patients treated with ICIs. It is a common symptom of chronic liver disease and can be exacerbated by ICIs and potentially impact the quality of life.

Adrenal insufficiency caused by ICI therapy will usually pose challenges in patients with HCC. The hemodynamic changes in cirrhosis, hyponatremia due to hemodilution and use of diuretics can pose a significant challenge will mask the diagnosis of adrenal insufficiency in these patients[26].

CHILD PUGH B CIRRHOSIS AND COMBINATION OF ATEZOLIZUMAB AND BEVACIZUMAB

The IMBrave 150 trial excluded the patients with Child-Pugh B cirrhosis. Currently, the data for the use of individualized care plans, in general, is scarce in patients with HCC. The largest retrospective series of 18 patients assessed the role of nivolumab in patients with Child-Pugh B cirrhosis after progression on sorafenib. In this study cohort, > 60% of patients had ascites, and 28% of patients had a Child-Pugh B score of 9. There were higher rates of adverse events, but the frequency of irAEs (immune-related adverse events) was similar to patients with Child-Pugh A cirrhosis in the CheckMate 40 trial. Interestingly there was no significant increase in aminotrans-

ferases, which is the anticipated side effect in this subset of patients[27].

There is a single case report of the combination of lenvatinib and pembrolizumab in a patient with advanced HCC with Child-Pugh B 8 with an overall survival of 22 mo at the time of initial presentation[28]. It will be essential to see the effect of atezolizumab and bevacizumab in patients with Child-Pugh B cirrhosis. Patients with ascites will be of interest, as bevacizumab can reduced ascites in patients with various gynaecological malignancies.

ICI INDUCED HEPATITIS IN PATIENTS OF HCC

ICI induced hepatitis is a vital complication that needs particular emphasis in patients with HCC. Patients with HCC have mild hepatic dysfunction due to underlying cirrhosis, and this can make the diagnosis of ICI induced hepatitis more challenging. In the IMBrave 150 trial, 14% of patients in the atezolizumab bevacizumab arm developed a rise in ALT with 3.6% developing grade 3 or 4 increase[6]. In a large multicentre retrospective analysis of 164 patients with ICI induced hepatitis, 30.5% and 45.7% of patients developed grade 2 and grade 3 hepatitis, respectively, with a median time of onset of 61 d. The most common presentation was asymptomatic laboratory abnormalities. In patients with symptomatic presentations, flu-like symptoms like fatigue/anorexia, nausea, emesis, abdominal/back pain and arthralgia/myalgia were the most common. Steroids were used in 92.1% of patients and second-line immunosuppression was required in 22.6% of patients. On rechallenge, there was a modest risk of hepatitis recurrence. Out of 164 patients, only one had HCC and only two patients received atezolizumab as one of the ICIs[29].

ROLE OF ATEZOLIZUMAB AND BEVACIZUMAB IN MANAGEMENT OF ADVANCED HCC IN SPECIAL SUBSETS OF PATIENTS

Multifocal HCC or advanced HCC can occur in a special subgroup of patients like patients with a history of autoimmune hepatitis, pre-existing autoimmune disease, solid organ transplants, inflammatory bowel disease, significant cardiovascular disease, patients on haemodialysis, active human immunodeficiency virus (HIV) infection or patients living with HIV disease. These patients provide unique challenges during the management of advanced HCC. Pinter *et al*[24] and Rimassa *et al*[30] comprehensively review the challenges in managing these patients. Table 4 summaries the most suitable lines of therapy for these subsets of patients.

FUTURE CONSIDERATION FOR CHANGE IN THERAPEUTIC LANDSCAPE FOR SECOND LINE SETTINGS IN ADVANCED HCC FOR PATIENTS PROGRESSED ON THE ATEZOLIZUMAB AND BEVACIZUMAB COMBINATION

The choice of second-line therapies for patients developing progressive disease on atezolizumab and bevacizumab combination is uncertain. The regorafenib and cabozantinib studies included prior VEGF exposure and 3% of patient in the CELESTIAL trial received prior immunotherapy[3,4]. Sonbol *et al*[15] in their network meta-analysis speculate that cabozantinib and regorafenib may be more suitable second-line therapies as compared to sorafenib and lenvatinib as they were only used in VEGF naïve patients. The efficacy of the VEGF directed antibody ramucirumab and single-agent checkpoint inhibitors such as nivolumab and pembrolizumab is also questionable in second-line settings for patients treated with this combination. It will be important to consider trials with dual checkpoint blockade, such as the combination of the anti-CTLA-4 antibody line ipilimumab and PD-1 inhibitor nivolumab or PD-1 inhibitors with TKIs like cabozantinib and regorafenib in second-line settings for patients who have progressed on the atezolizumab and bevacizumab combination[15].

Table 4 Advanced hepatocellular carcinoma in special subset of population with absolute and relative contraindication for atezolizumab and bevacizumab combination

Special population	Absolute contraindication for atezolizumab and bevacizumab combination	Relative contraindication for atezolizumab and bevacizumab combination	Comments
Solid organ transplantation	Yes	N/A	If HCC in patients with liver transplant, transplant rejection can be potentially lethal. Sorafenib or lenvatinib are preferred first line options
HIV patients	N/A	No data	This was an exclusion criteria in IMBrave150 trial. The NCT04487067 AMETHISTA study of atezolizumab and bevacizumab in HCC is including patients with HIV disease who are stable on HAART, with CD4+T cell count ≥ 200/μL, and an undetectable viral load
Prior or active autoimmune disease (AID)	Yes, in patients when AID including autoimmune hepatitis, reactivation can be life threatening, neurological or neuromuscular disorders, poorly controlled AID on high dose immunosuppression	Can be used after discussion with patients and care givers about risk and benefit if do not fall in subgroups described in absolute contraindications	Patients with symptomatic AID are at higher risk for flare. Sorafenib or lenvatinib are preferred first line options in such patients
Inflammatory bowel disease	Bevacizumab can increase complication risk in patients with Crohn's disease with fistula	Can be used after discussion with patients and care givers about risk and benefit in patients with quiescent disease	Selective immunosuppressants like vedolizumab may be better before considering the ICP therapy
Significant cardiovascular/thromboembolic disease	N/A	Bevacizumab increases risk of HTN, thromboembolic and cardiovascular events	Can be used after discussion with patients and care givers and treating hypertension
Haemodialysis	N/A	No data available, can be considered after discussing risk and benefit and limited evidence	A recent study of 55 patients with metastatic RCC on haemodialysis showed relative safety of sorafenib, nivolumab and atezolizumab in small subgroup of patients [33]

N/A: Not applicable; HTN: Hypertension; RCC: Renal cell carcinoma; HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus; ICP: Individualized care plan.

CONCLUSION

Atezolizumab and bevacizumab is the current first-line standard of care systemic therapy option for patients with advanced or unresectable HCC unsuitable for locoregional therapy with Child-Pugh A cirrhosis with no contraindication to either atezolizumab and bevacizumab. Current ESMO and NCCN guidelines support this recommendation[31,32]. The ESMO guidelines report the substantial benefit with this combination with estimated ESMO magnitude of clinical benefit score of 5 with an absolute survival gain of additional 9.6 mo as compared to sorafenib[31].

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MINIREVIEWS

Drug-induced liver injury and COVID-19: A review for clinical practice

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Abstract

Coronavirus disease 2019 (COVID-19) consists of a systemic disease that can present many complications. The infection presents broad clinical symptoms and a high rate of transmissibility. In addition to severe acute respiratory syndrome, the patients manifest complications beyond the respiratory system. The frequency of liver damage in COVID-19 patients ranges from 14.8% to 53% of patients. One should pay attention to drug-induced liver injury (DILI) in patients with COVID-19, especially considering the off-label use of drugs in prophylactic and therapeutic regimens applied on large scales. This review aims to present relevant information on the medication used so far in COVID-19 patients and its possible hepatotoxicity. We reviewed liver damage in patients with COVID-19 on PubMed and Virtual Health Library to investigate DILI cases. Four studies were selected, involving the medicines remdesivir, tocilizumab and a pharmacovigilance analysis study. The hepatotoxicity profile of drugs presented in the literature considers use in accordance to usual posology standards for treatment. However, drugs currently used in the management of COVID-19 follow different dosages and posology than those tested by the pharmaceutical industry. The deficiency of

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uniformity and standardization in the assessment of hepatotoxicity cases hinders the publication of information and the possibility of comparing information among healthcare professionals. It is suggested that severe liver injury in COVID-19 patients should be reported in pharmacovigilance institutions, and physicians should pay attention to any considerable abnormal liver test elevation as it can demonstrate unknown drug hepatotoxicity. Liver disorders in COVID-19 patients and the use of several concomitant off-label medications — with a potential risk of further damaging the liver - should at least be a warning sign for rapid identification and early intervention, thus preventing liver damage from contributing to severe impairment in patients.

Key Words: Liver injury; Chemical and drug-induced liver injury; COVID-19; SARS-CoV-2; Pharmacovigilance

Core Tip: Coronavirus disease 2019 (COVID-19) is a multisystemic disease, and liver manifestations are an important aspect to be considered. One should pay attention to drug-induced liver injury, especially considering the off-label use of drugs in prophylactic and therapeutic regimens applied on large scales. A review of liver damage in patients with COVID-19 returned three studies involving remdesivir, tocilizumab, and a pharmacovigilance study. Liver disorders in COVID-19 patients and the use of several concomitant off-label drugs - potentially causing further liver damage - should be a warning sign for rapid identification and early intervention, thus preventing severe impairment in patients.

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INTRODUCTION

In December 2019, the world watched severe acute respiratory syndrome (SARS) spread from an epidemic in China to a pandemic with global catastrophic effects[1]. The virus causing the syndrome has been identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new pathogen in the coronavirus family, and the disease is called coronavirus disease 2019 (COVID-19)[2]. On January 31, 2021, COVID-19 was already present in 223 countries/territories, with over one hundred million confirmed cases and two million deaths. The United States presents more than 40% of confirmed cases worldwide, followed by India and Brazil[2].

The infection presents broad clinical symptoms and a high rate of transmissibility. The overall signs can vary from fever, cough, shortness of breath, body pain, and diarrhea to severe pneumonia[3]. COVID-19 is a multifactorial systemic disease with rapid progression, leading a patient to the intensive care unit (ICU) in a matter of days [4]. In mild cases of the disease, symptomatic treatment is indicated. In moderate to severe cases, support measures and the use of experimental/off-label treatments should be performed[5].

In addition to SARS, patients with COVID-19 manifest complications beyond the respiratory system[6]. The virus hosts the angiotensin-converting enzyme receptor 2 (ACE-2), which despite being expressed in 80% of lung cells, it is also located in tissues such as vascular endothelium, gastrointestinal tract, squamous epithelium of the nasal, oral mucosa, and nasopharynx[7,8]. Therefore, COVID-19 consists of a systemic disease that can present complications such as thromboembolic episodes, arrhythmias, and myocardial dysfunction, prolongation of the QT interval, acute coronary syndrome, kidney injury, hepatocellular damage, hyperglycemia, and ketoacidosis, neurological symptoms, sepsis and, in more severe cases, multiple organ failure[9].

The frequency of liver damage in COVID-19 patients ranges from 14.8% to 53% of patients[10]. In a systematic review analyzing 12882 hospitalized patients, 41.1% had elevated aspartate aminotransferase (AST), and 29.1% increased alanine aminotransferase (ALT). Elevation of AST and ALT three times above the normal upper limit is significantly associated with greater chances of unfavorable clinical outcomes [11]. Other publications demonstrate the increase in ALT/AST ratio in 16% to 62% of cases and elevated total bilirubin by 5% to 21% of the patients. Elevation of AST and ALT presented is about two times above the normal upper limit[12]. Studies suggest that aminotransferase elevations occur more frequently in severe patients[9].

The liver injury pattern consists of increased AST/ALT and less frequently decreased serum albumin, increase total bilirubin, gamma-glutamyltransferase (GT range), and alkaline phosphatase[13,14]. Liver histopathological alterations demonstrated microvesicular steatosis, portal fibrosis, inflammatory infiltration in the hepatic and ductular lobe, and multifactorial acute liver necrosis[9]. The high transmissibility of the virus and the absence of protocols for the protection of health professionals at the beginning of the pandemic made it difficult to perform autopsies and liver biopsies of patients with COVID-19 — leading to scarce histopathological data in the literature[15]. Another difficulty in establishing a liver injury pattern is the scarcity of publications reporting liver signs and symptoms in addition to laboratory findings such as jaundice, hepatomegaly, and ascites.

Liver involvement in patients with COVID-19 is currently limited to moderate to severe cases, and its damage may be transient, with liver tests returning to normal without the need for specific treatment[9,15]. The occurrence of acute or chronic liver failure is yet to be investigated. Nevertheless, the higher the serum level of AST/ALT and total bilirubin, the severer the disease, the higher the risk of a patient requiring admission to the ICU or prolonged hospital stay[16], and the greater the mortality risk [14].

Reasons for the occurrence of liver damage in COVID-19 patients are multifactorial [9]. The first hypothesis was the cytopathic injury caused directly by the virus[9]. Although the liver damage pattern found in COVID-19 patients suggests hepatocellular damage, ACE-2 is expressed in only 2.6% of hepatocytes, in contrast to the relevant expression in cholangiocytes (59%), which would suggest cholestatic damage [13]. However, the bile duct has a role in liver regeneration and immune response, and direct damage to cholangiocytes can impair this function. The presence of the virus in the vascular endothelium causes a state of hypercoagulation; thus, there is the possibility of liver damage caused by thrombosis in the porta-hepatic system[9,11].

The manifestation of hypoxemia due to pneumonia may cause liver damage due to hypoxia-reoxygenation[13]. In cardiac, circulatory or respiratory distress passive congestion and decreased blood flow to the liver may occur. Theoretically, hypoxia rescue and reperfusion of organs cause the availability of a large amount of oxygen suddenly increases the presence of reactive oxygen species, causing the release of pro-inflammatory factors and thus facilitating the occurrence of blood hyperviscosity, which aggravates microvascular lesions in the liver[13]. Septic shock is a common complication in severe COVID-19 patients and functional imbalance may be responsible for liver damage[17].

It is a consensus among experts that functional changes caused by SARS-CoV-2 in patients with moderate to severe disease may be related to systemic inflammatory response syndrome[9]. The development of an uncontrolled immune-mediated inflammatory reaction occurs by the increase in plasma cytokines and other inflammatory reagents [interleukin (IL)-1, IL-6, IL-10, and tumor necrosis factor]. This mechanism affects several organs and has supported the clinical use of anti-inflammatory corticosteroids[8].

The role of chronic liver disease (CLD) in COVID-19 patients is still controversial. Cirrhosis is a risk factor for mortality in general, with clinical complications such as sepsis and respiratory stress[18]. The prevalence of non-alcoholic fatty liver disease is increasing worldwide, and the patient's profile is similar to the SARS-CoV-2 risk group: advanced age and presence of comorbidities such as hypertension, diabetes, obesity, and cardiovascular distress[19]. CLD may interfere with the findings of liver enzyme alterations to some extent in COVID-19 — if not directly responsible, acting together with the virus to worsen liver function. Despite this scenario, liver damage might occur regardless of liver disease's previous existence[18].

One should pay attention to drug-induced liver injury (DILI) in patients with COVID-19, especially considering the off-label use of drugs in prophylactic and therapeutic regimens applied on large scales. DILI is an adverse reaction to medications, and patients using five or more drugs - for example, critically ill ICU patients with COVID-19 - are more likely to experience this type of reaction[20].

Although rare, often ranging from 1 case in 10000-100000[21], physicians and pharmacists should monitor the occurrence of this event in COVID-19 patients since the side-effect prolongs hospital stay, a critical situation in a hospital bed shortage moment[22].

Finally, the DILI adverse event can play a crucial role in COVID-19 patients. This review aims to present relevant information on the medication used so far in COVID-19 patients and its possible hepatotoxicity. We intend to condense information that supports decision-making and patient management in clinical practice in the hospital environment and make remarks on liver manifestations in light of the DILI subject.

LITERATURE REVIEW

Review of liver damage in patients with COVID-19

A review of liver damage in patients with COVID-19 on PubMed for general information on hepatic manifestations in SARS-CoV-2 was performed using the terms ("Liver Diseases" [MeSH]) AND ("sars cov 2" [MeSH]). Secondly, PubMed and VHL (Virtual Health Library) were used to explore DILI cases in COVID-19. VHL was used to expand the search for Latin American cases. The search strategy for PubMed combined the descriptors as follows ("Chemical and Drug Induced Liver Injury"[MeSH]) AND ("sars-cov-2"[MeSH]) AND ("covid-19"[MeSH]). There was no limitation by language, year of publication, or study design. The search strategy for VHL combined the descriptors as "Chemical and Drug Induced Liver Injury" AND "coronavirus infections". The first search was performed on January 6th, 2021, and was then updated on April 17, 2021.

The studies' eligibility was defined by identifying DILI cases due to medications used to treat patients with COVID-19. The studies' selection was performed by two independent reviewers, MWB and KHS, and in three sequential stages — title, abstract, and full-text readings. A third reviewer, CRB, resolved the disagreements. The following variables were analyzed: Drug, patient characteristics, assessment of liver enzymes, DILI diagnosis criteria.

The search returned 53 articles — 22 articles from the VHL and 31 articles from the PubMed database. After excluding duplicate articles and review articles, 10 available abstracts and full texts were assessed. One excluded article assessed adverse drug reactions but did not mention DILI. Another two excluded articles assessed liver injury but no mention to the medication used; a retrospective study analyzing antiviral treatment was excluded since no causality was assessed. Six studies were selected — five case reports and a pharmacovigilance analysis study of VigiBase, the World Health Organization's individual case safety reports database, as summarized in Table 1.

The results found are related to the attempt to treat critically ill patients, either by eliminating the virus or by decreasing the inflammatory manifestations developed. Tocilizumab is an IL-6 receptor antagonist and has been proposed to treat severe forms of COVID-19. IL-6 plays an important role in COVID-19-induced cytokine storm[23]. Remdesivir is a nucleotide analogue RNA polymerase inhibitor, originally developed and tested for Ebola virus disease. The drug showed antiviral activity against a broad spectrum of human coronaviruses in cell cultures and mouse models, including SARS. Recently, the Food and Drug Agency recommended Remdesivir for the treatment of patients hospitalized with severe coronavirus disease[24,26,28].

Risk of hepatotoxicity of medicines on COVID-19 patients

It is challenging to find data on hepatotoxicity. This data includes clinical trials, observational studies, series and case reports. In the case of DILI, clinical trials do not focus on assessing causality, so it is not accurate in this identification, even because it is not the objective of this study design. Retrospective observational studies have a known bias regarding data collection. However, prospective observational studies and case series are essential for the detection and understanding of DILI. In this context, the analysis of the evidence synthesis is a difficult task to perform. In terms of access, the LiverTox© database[29] website is a valuable reference for a quick consultation[30]. It classifies medicines according to the following scale: Category A (over 50 published reports), B (over 12 but less than 50), C (over four but less than 12), and D (one to three cases).

Some reservations emerged concerning the frequencies of risk of hepatotoxicity when confronted with a large series of prospective cases — mainly related to drugs presenting a risk of hepatotoxicity when it was impossible to rule out other

Table 1 Reports of drug-induced liver injury in patients with coronavirus disease 2019 (PubMed/Virtual Health Library)

Ref.	Study/site	Patient profile	Medication	DILI	Outcome
Muhović *et al*[23]	Case report; Montenegro	Man, 52-yr-old	Chloroquine, lopinavir/ritonavir, methylprednisolone, ceftriaxone and azithromycin. After 6 d: methylprednisolone, ceftriaxone, azithromycin	CIOMS/RUCAM: scored 8 points for a 'probable' cause of DILI by TCZ. Hepatocellular form of DILI diagnosed using the EASL guidelines	TCZ had a positive effect on clinical and laboratory parameters, with transaminases values normalizing in 10 d
Zampino *et al*[24]	Case series; Naples, Italy	None of the 5 treated patients had history of liver disease, visceral obesity, viral hepatitis, or prior hepatotoxic medication or alcohol intake. Liver ultrasound did not show signs of advanced liver disease. Patient 1 and 2 had history of hypertension and asthma	Before and during RDV treatment, 4 of 5 patients alsoreceived hydroxychloroquine patient 2 and 4 received ceftazidime–avibactam plus daptomycin and patient 3 meropenem and linezolid	Significant increase in AST/ALT	Adverse effect neither progressed to severe liver damage nor induced liver failure. In no cases, RDV was discontinued because of liver injury
Durante-Mangoni *et al*[25]	Case series; Naples, Italy	Four patients	All patients had been previously treated with LPV/r or darunavir/cobicistat (DRV/c) and also received hydroxychloroquine	3 patients experienced ALT and AST increase (5 times to 8 times the upper normal limit)	RDV was prematurely discontinued in patient 1 because of a *torsade de pointes* requiring cardiac resuscitation and in patient 3 because of death due to multiple organ failure. The study suggests a significant burden of adverse events
Montastruc *et al*[26]	Cross-sectional study; United States, Europe	387 reports with RDV side effects in VigiBase; 130 hepatic adverse effects, 87 from the United States; 43 from Europe; mostly men (81, 62%), mean age of 54.9 yr	In the majority of cases (122, 94%), RDV was the sole suspected drug	Increased hepatic enzymes (114, 88%), involving AST and ALT in 79 cases (61%) and bilirubin in 4 cases (3%). Other cases were reported as hepatic failure or hepatitis	Most cases were serious (94, 72%), resulting in hospitalization or prolongation of hospital stay. The use of RDV was associated with an increased risk of reporting hepatic disorders
Yamazaki *et al*[27]	Case reported; Japan	73-yr-old man. History of hypertension, hyperlipidemia, gastric ulcer, benign prostatic hyperplasia, and alcoholic hepatitis	Favipiravir was the suspected drug. Dosage was 6000 mg on day 1 and 2400 mg/d from day 2 onward, for a total of 14 d. Patient was using previously lopinavir/ritonavir combined with interferon β-1b, vancomycin and antithrombin III. After started fapinavir two more drugs were added Trimethoprim-sulfamethoxazole and micafungin	Transaminases were elevated until day 4: Aspartate aminotransferase (AST) from 70 U/L (day 0) to 112 U/L (day 4) and alanine aminotransferase (ALT) from 37 U/L to 59 U/L, respectively. Total bilirubin (T-BiL) increased until day 3 from 5.2 mg/dL to 12.6 mg/dL. On day 11, however, transaminases peaked again (AST, 268 U/L; ALT, 115 U/L) and total bilirubin was also rising	A case of cholestatic liver injury in the early stages of favipiravir treatment for COVID-19. Based on the CIMOS/RUCAM scoring system, it was classified as a cholestatic liver injury, with a score of 6 (possible)
Leegwater *et al*[28]	Case report; The Netherlands	A 64-yr-old male patient. History of hypertension and hypercholesterolemia	Remdesivir	5 d after start of remdesivir ALT was 1305 IU/L, AST 1461 U/L, alkaline phosphatase 269 U/L, total bilirubin 8 μmol/L, gammaglutamyltransferase 227 U/L and creatine kinase 103 U/L	Remdesivir toxicity was suspected based on the time-relation, the positive dechallenge, the known *in vitro* toxicity of remdesivir and the absence of alternative causes of hepatotoxicity. After stop of remdesivir the ALT/AST ratio reached normal values

CIOMS/RUCAM: Council for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method; DILI: Drug induce liver injury; EASL: European Association for the Study of the Liver; TCZ: Tocilizumab; RDV: Remdesivir.

hypotheses. Publication bias and lack of updating can also affect the assessment of the LiverTox© database[29] when considering a drug as low risk[21]. New drugs may also

go unnoticed, as the data is generally related to internal reports by regulatory agencies. Despite this bias, the LiverTox© database is still the most practical way of obtaining information on hepatotoxicity. The expansion of its use and knowledge can improve the quality of publications and more accurate detection and assessment of DILI's causality.

The evidence of hepatotoxicity available in the LiverTox© database[29] was organized considering drugs for COVID-19 treatment. Table 2 presents some of the most studied drugs for the COVID-19 treatment according to hepatotoxicity information and DILI case probability. Table 3 presents drugs that enhance the effectiveness of medical treatment.

DISCUSSION

Healthcare professionals must consider DILI in COVID-19 patients when: (1) There is an elevation of ALT five times above the upper limit of normal (ULN); and (2) Increase in ALT > 3 × ULN with an increase in bilirubin > 2 × ULN with or without alteration of alkaline phosphatase levels or with hepatic signs[31]. DILI may be present when total bilirubin is > 2.5 mg/dL in the presence of AST and ALT elevation or when international normalized ratio > 1.5 with a concomitant increase in AST and ALT[32]. DILI can be classified as hepatocellular, cholestatic or mixed, as indicated by ALT and the alkaline phosphatase test[33]. Moreover, DILI can be mild, moderate, severe, or fatal; the worst outcomes are liver transplant or death[20]. Although there are three known types of DILI, there is no consensus of what type is the most common in COVID-19 patients.

Abnormal levels for aminotransferase in DILI without other signs and symptoms should only be monitored. If the patient presents ALT 5 × > ULN with jaundice, hepatomegaly, hyperbilirubinemia, or right-upper-quadrant pain, consider further clinical investigation and interruption of suspected DILI drug[9]. Patients under off-label drugs use and investigational treatments should be longitudinally monitored for liver tests. If resources are available, monitor liver tests of patients discharged from ICU to ensure no secondary damage will occur, and liver function will be fully restored[9,13]. Most DILI cases do not need drug therapy, and patients recover after drug discontinuance. Ursodeoxycholic acid 500 mg daily use is described in the literature for hepatic protection for elevated transaminases and serum total bilirubin in non-alcoholic liver disease, however its mechanism of action remain unclear[18].

Causality algorithms should be used in the assessment of adverse drug reactions. For DILI related to COVID-19 treatment, we strongly encourage using the Roussel Uclaf Causality Assessment Method (RUCAM) due to its specificity for liver injury [34]. Briefly, the RUCAM scale assigns points to seven domains, including temporal evolution of the liver injury, risk factors (age, alcohol use, and pregnancy), concomitant use of drugs that may be hepatotoxic, and the development of repeated liver damage after the new drug is administered[35]. RUCAM may also help in the differential diagnosis of other COVID-19 related etiologies that cause AST/ALT elevation, such as myositis, ischemia, cytokine-release syndrome, and previous CLD [9].

The mortality of COVID-19 relates to SARS. Nevertheless, extrapulmonary manifestations such as liver injury may contribute to a negative clinical prognosis. There is no sufficient data to consider liver injury caused by DILI as a risk factor for mortality, but it is a safety concern since it is related to severe cases of COVID-19[9, 36], and it may increase hospital length of stay and expose patients to other comorbidities such as nosocomial infection. From a social and economic perspective, it also pressures the health system, as hospital bed shortages are a major concern in the pandemic, since resources are scarce worldwide.

The hepatotoxicity profile of drugs available in the literature considers approved therapeutic schemes applied in the medical routine. However, drugs currently used in the management of COVID-19 do not follow previously established therapies and posology when considering those tested by the pharmaceutical industry[37]. For example, in Brazil, reports of hepatotoxicity caused by ivermectin use 18 mg/d for a week as prophylaxis for COVID-19 are published in non-scientific media. Despite the small number of published cases according to Table 2, overdose — in the case of administration of non-studied dosage — may, over time, modify the risk of ivermectin hepatotoxicity. A similar situation may occur with several other drugs, leading to the need to review the frequency of adverse reactions described in the package leaflet. This scenario can be confusing in identifying DILI even when using well-established

Table 2 Hepatotoxicity of the most common drugs used to treat coronavirus disease 2019

Drug	Evidence of hepatotoxicity	Probability
Azithromycin	Liver damage is usually self-limited cholestatic hepatitis, which appears 1 wk to 3 wk after starting treatment. It may also appear after some time following medicine discontinuance. Cholestasis and elevated transaminases can persist for up to 6 mo. Despite presenting the hepatocellular and cholestatic forms of injury, cholestatic is more often related to acute liver failure, death, or liver transplantation	A
Lopinavir/ritonavir	Clinically apparent liver disease occurs in 3% to 10% of patients. The onset of symptoms or jaundice is usually 1 wk to 8 wk, and the pattern of elevations in serum enzymes varies from hepatocellular to cholestatic or mixed. The injury is usually self-limiting; however, fatal cases have been reported	D
Hydroxy-chloroquine	It has not been associated with significant elevations in serum enzymes during therapy for rheumatic diseases. When used in relatively high doses, it can trigger an acute liver injury with a sudden onset of fever and marked elevation of serum enzymes. Post COVID-19 data have not been assessed	C
Tocilizumab	It has been associated with several cases of clinically apparent liver injury with jaundice. Although the liver injury was severe, it was usually self-limiting, with complete recovery within 2 mo to 3 mo. In at least one case, however, the affected patient died of liver failure. Current recommendations are patient monitoring by routine liver tests before medication. In registration trials, serum aminotransferase elevations occurred in a high proportion (10% to 50%) of patients	C
Remdesivir	Between 10% and 50% of patients treated developed transient, mild-to-moderate serum ALT and AST elevations within 1 d to 5 d of starting therapy without changes in serum bilirubin or alkaline phosphatase levels. Elevations above 5 times ULN were reported in up to 9% of patients in several clinical trials, but the abnormalities resolved with discontinuance and were not associated with a clinically apparent injury	D
Nevirapine	Associated with significant elevations in ALT (above 5 times the ULN) in 4% to 20% of patients and symptomatic elevations in 1% to 5%	A
Ivermectin	Associated with minor, self-limiting elevations in serum aminotransferase and sporadic cases of clinically apparent liver damage. Post COVID-19 data have not been assessed	D

Adapted from LiverTox© database[27]. A: Well know hepatotoxicity; B: Highly likely hepatotoxicity; C: Probably hepatotoxicity; D: Possible hepatotoxicity; COVID-19: Coronavirus disease 2019; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ULN: Upper limit of normal.

Table 3 Hepatoxicity of adjuvant therapy medications for coronavirus disease 2019 treatment

Drug	Evidence of hepatotoxicity	Probability
Heparin	Associated with a transient elevation of 10% to 60%, but the values are generally less than 5 times the upper limit of normal and are rarely associated with symptoms or jaundice. Values above 5 times the upper limit of normal occur around 2% of those receiving high heparin doses	NR
Enoxaparin	Associated with elevations in serum aminotransferases in 4% to 13% of patients, but values greater than 5 times the upper limit of normal are not common and occur in higher doses. The typical liver injury in patients receiving low molecular weight heparins occurred with rapid onset (within 3 d to 5 d of onset), rapid recovery (from 1 wk to 4 wk), and the absence of symptoms and jaundice. Some patients have mild increases in serum bilirubin and alkaline phosphatase but generally remain within the normal range	E
Cortico-steroids	The use of glucocorticoids can result in hepatomegaly and steatosis. They can trigger or worsen non-alcoholic steatohepatitis. Long-term use can also exacerbate chronic viral hepatitis. High doses of intravenous corticosteroids, mainly methylprednisolone, have been associated with acute liver damage resulting in acute liver failure and death. Symptoms and jaundice develop 2 wk to 6 wk after discontinuance. Some cases have progressed to acute liver failure, resulting in death or the need for emergency liver transplantation	A
Voriconazole	Transient elevations in serum aminotransferase levels occur in 11% to 19% of patients on voriconazole. These elevations are generally asymptomatic and self-limited, but approximately 1% of patients require voriconazole discontinuance due to ALT elevations. Cases of acute liver failure have been described. Testing for serum bilirubin and aminotransferase levels is recommended at the time of initiation and weekly during the first month of therapy and monthly thereafter	B
Anidulafungin	Transient elevation of transaminases from 2% to 15%. There are rarely serious cases. Monitoring of liver tests during therapy is recommended, especially in patients with previous liver disease	D
Colchicine	It is rarely associated with elevations in serum aminotransferase or alkaline phosphatase. The cases of acute liver injury attributed to the overdose of colchicine were self-limiting, and the other toxicities of this agent, such as rhabdomyolysis, generally overshadowed the liver injury. No convincing cases of liver failure have been reported	C

Adapted from LiverTox© database[27]. A: Well know hepatotoxicity; B: Highly likely hepatotoxicity; C: Probably hepatotoxicity; D: Possible hepatotoxicity; E: Unlikely hepatotoxicity; NR: Not reported; ALT: Alanine aminotransferase.

causality algorithms, leading to sub notification, as drugs are used in non-previous indications.

When we analyzed Azithromycin and Hydroxychloroquine, we found that Azithromycin has a greater potential for hepatotoxicity, according to table 2.

Nevertheless, the Brazilian clinical trial 'Coalition' found a curious fact: Hydroxychloroquine alone or in addition with Azithromycin increased the levels of aminotransferases. Azithromycin was therefore not a confounder, but its interaction further increased the frequency of liver damage[38].

Besides azithromycin, many antimicrobial agents applied in the treatment of respiratory infections may cause hepatotoxicity. Fluoroquinolones, especially ciprofloxacin and levofloxacin, are responsible for frequent causes of clinically apparent liver injury and bile duct paucity[39]. Amoxicillin-clavulanate is LiverTox© A category and the most common documented cause of non-acetaminophen idiosyncratic DILI in the United States and Spain[40]. The drug causes cholestasis or mixed pattern of liver injury with significant increased alkaline phosphatase and gamma glutamyl transpeptidase markers[40-42]. Antituberculosis agents such as isoniazid are well known for their hepatotoxicity[43]; in developing countries, patients with COVID-19 and tuberculosis might be at increased risk of poor respiratory outcomes and DILI occurrence. Physicians should be aware of the available date on general antimicrobial hepatotoxicity to evaluate risk-benefit of adjuvant drug therapy.

COVID-19 is a condition yet to be duly clarified as to its extent and consequences. Despite the evidence showing the benefits of dexamethasone for the treatment, its use also made conditions such as aspergillosis pneumonia more frequent. This increase has been associated with the increased use of corticosteroids. Therefore, the treatment protocol of some antifungal drugs is associated with respiratory conditions. With the increase in the use of antifungals, known to affect the liver, it is necessary to be aware of the increased frequency of DILI associated with these drugs that were not so often used before[44].

After Ivermectin, Nevirapine, and Hydroxychloroquine, now Colchicine is under study for the treatment of COVID-19[45]. Pre-pandemic, the concept of hepatotoxicity was reported as an unlikely or even non-existent cause. Nevertheless, COVID-19 has taught us that we need to be aware of possible new adverse effects when treating new pathologies — especially those stemming from new and dosage regimens.

Most DILI reports are concentrated in a hospital environment due to the availability of diagnostic resources[46]. In a non-pandemic context when most cases are identified in a hospital environment, 50% of DILI cases are poorly diagnosed[47]. In patients with COVID-19, this situation may be even more precarious since the off-label drug use in outpatient settings — drugs such as azithromycin, hydroxychloroquine, and ivermectin — will only alert to hepatotoxicity in severe cases when a patient already requires hospitalization.

Healthcare professionals must be aware of self-medication practices with over-the-counter medicines in the treatment of COVID-19 fever and pain, such as nonsteroidal anti-inflammatory drugs[48]. Acetaminophen overdoses cause harmful acute hepatocellular injury and even in adequate doses it can slightly elevate serum aminotransferases[49]. Liver injury can occur when acetaminophen is taken for several days in supratherapeutic doses[42]. Hepatotoxicity is worsened if the patient is critically ill, presents alcoholism, malnutrition or preexisting CLD[49]. Moreover, chronic use of diclofenac can increase ALT levels; nimesulide has been described in acute liver failure and ibuprofen is associated with cholestatic DILI[41].

Studies describe the increase in AST/ALT as a synonym for liver damage and hepatotoxicity in patients with COVID-19. However, for a relevant outcome in clinical practice, it is necessary to clarify the presence of signs and symptoms in those cases. The deficiency of uniformity and standardization in the assessment of hepatotoxicity cases hinders the publication of information and the possibility of comparing information among healthcare professionals[50]. In that scenario, RUCAM may help to guide more consistent and complete data on DILI, including COVID-19 cases, undergoing clinical features, treatments used, and current diseases. The World Health Organization strengthened the report of any drug adverse event and so, DILI should also be monitored and reported to local pharmacovigilance institutions to compose the VigiBase dataset. Physicians should pay attention to any considerable abnormal liver test elevation as it can demonstrate unknown drug hepatotoxicity. The only certainty that we have is that after COVID-19, knowledge about drug use and abuse will be updated. For that, we should pay attention to increasing DILI reports.

CONCLUSION

COVID-19 is a multisystemic disease, and liver manifestations are a crucial aspect to be considered. The pandemic moment experienced presents new clinical situations

that need different perspectives and approaches. It is important to verify the occurrence of hepatic manifestation in different populations, as there may be a relationship with the different therapeutic schemes used to treat the disease.

Pharmacovigilance actions using validated tools such as the RUCAM algorithm can establish a causal relationship between drugs and DILI and disseminate relevant information for clinical decision-making. The set of liver disorders in COVID-19 patients and the use of several concomitant off-label drugs should be at least a warning sign of potential further liver damage. Rapid identification and early intervention can prevent liver damage contributing to severe impairment in patients.

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MINIREVIEWS

Probiotics in hepatology: An update

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Abstract

The gut–liver axis plays an important role in the pathogenesis of various liver diseases. Probiotics are living bacteria that may be used to correct disorders of this axis. Notable progress has been made in the study of probiotic drugs for the treatment of various liver diseases in the last decade. It has been proven that probiotics are useful for hepatic encephalopathy, but their effects on other symptoms and syndromes of cirrhosis are poorly studied. Their effectiveness in the treatment of metabolic associated fatty liver disease has been shown both in experimental models and in clinical trials, but their effect on the prognosis of this disease has not been described. The beneficial effects of probiotics in alcoholic liver disease have been shown in many experimental studies, but there are very few clinical trials to support these findings. The effects of probiotics on the course of other liver diseases are either poorly studied (such as primary sclerosing cholangitis, chronic hepatitis B and C, and autoimmune hepatitis) or not studied at all (such as primary biliary cholangitis, hepatitis A and E, Wilson's disease, hemochromatosis, storage diseases, and vascular liver diseases). Thus, despite the progress in the study of probiotics in hepatology over the past decade, there are many unexplored and unclear questions surrounding this topic.

Key Words: Gut-liver axis; Pathogenesis; Gut dysbiosis; Gut microbiota; Gut microbiome; Liver disease; Probiotics; Hepatic encephalopathy; Cirrhosis; Metabolic associated fatty liver disease; Alcoholic liver disease; Primary sclerosing cholangitis

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Core Tip: Probiotics are useful for hepatic encephalopathy, but their effects on other symptoms and syndromes of cirrhosis are poorly studied. Their effectiveness in the treatment of metabolic associated fatty liver disease has been shown both in experimental models and in clinical trials, but their effect on the prognosis of this disease has not been described. The beneficial effects of probiotics in alcoholic liver disease have been shown in many experimental studies, but there are very few clinical trials to support these findings. The effects of probiotics on the course of other liver diseases are either poorly studied or not studied at all.

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INTRODUCTION

It has been 10 years since the *World Journal of Gastroenterology* published an article titled "Probiotics in Hepatology"[1]. The following decade was marked by tremendous progress in the study of the gut-liver axis[2,3]. It was shown that the gut microbiota plays an important role in the development of various liver diseases. Probiotics are drugs that target it[4]. The aim of this review is to describe the current data on the use of probiotics for the treatment of liver diseases.

SCIENTIFIC BASIS FOR THE USE OF PROBIOTICS IN LIVER DISEASES

Gut dysbiosis[5-7], small intestinal bacterial overgrowth[8,9] and an increase in the permeability of the intestinal wall[10] leads to bacterial translocation in cirrhosis[11,12]. The latter leads to systemic and liver inflammatory reaction, as well as hemodynamic changes[13], and contributes to the development of complications of cirrhosis, such as ascites, esophageal varices, and hepatorenal syndrome[2,11,12]. In addition, the gut microbiota produces a variety of neuroactive products of protein metabolism, which are normally removed by the liver and abundantly enter the bloodstream, leading to the development of hepatic encephalopathy, in cirrhosis[14].

The gut microbiota plays an important role in the regulation of metabolism in our body. It modifies bile acids (deconjugation, conversion of primary into secondary), which through their receptors [farnesoid X receptor (FXR) and Takeda G-protein receptor 5], have a variety of effects on the metabolism[15,16]. In addition, the gut microbiota forms short-chain fatty acids (SCFA), which through their receptors, also have a complex effect on metabolism and maintain intestinal barrier integrity[17]. Gut dysbiosis leads to disorders of these regulatory functions, which can result in metabolic changes.

Alterations in gut microbiota and increased intestinal permeability were also described in alcoholic liver disease[18,19], metabolic associated fatty liver disease (MAFLD)[20], primary sclerosing cholangitis[21,22], and autoimmune hepatitis[23]. Gut dysbiosis was also reported in primary biliary cholangitis[24], Wilson's disease [25], hepatitis B[26] and hepatitis C[27] recently.

At the same time, probiotics have shown their ability to correct gut dysbiosis[28], increase production of SCFA[29], and reduce the increased permeability of the intestinal barrier[30]. All this constitutes the scientific basis for their use in the treatment of liver diseases.

A simplified diagram of the gut-liver axis is shown in Figure 1.

PROBIOTICS FOR CIRRHOSIS

According to the latest meta-analysis of randomized controlled trials (RCT), the use of probiotics is effective in the treatment of minimal hepatic encephalopathy and prevents the development of overt hepatic encephalopathy. Probiotics are as effective

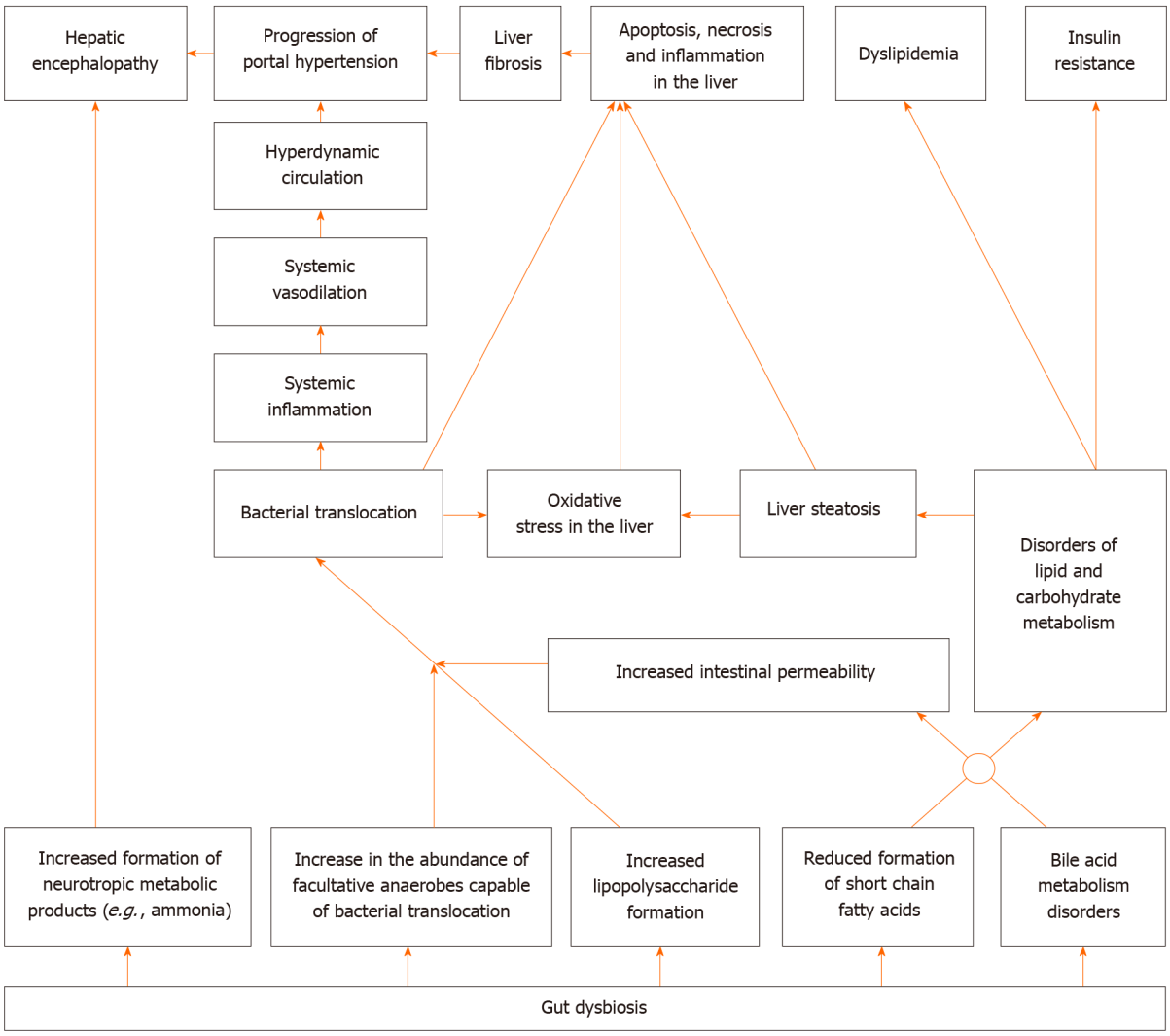

Figure 1 Simplified diagram of the gut-liver axis.

in treating minimal hepatic encephalopathy as rifaximin, lactulose, and L-orinithin-L-aspartate. There was no effect of probiotics on mortality. The addition of lactulose to probiotics did not significantly affect the effectiveness of the treatment. Probiotics lower blood ammonium levels more than lactulose. The addition of lactulose to probiotics paradoxically increases blood ammonium levels. The use of probiotics was not accompanied by the development of significant side effects[31]. Other recent meta-analyses have reached similar conclusions[32,33].

Several RCTs that studied the effect of probiotics on other indicators in cirrhosis been published.

The use of probiotics (*Clostridium butyricum* combined with *Bifidobacterium infantis*) in minimal hepatic encephalopathy led to a decrease in the abundance of harmful Enterococcus and Enterobacteriaceae in the gut microbiome. The blood levels of markers of bacterial translocation [lipopolysaccharide (LPS)], intestinal permeability (D-lactate) and damage to the intestinal epithelium (diamine oxidase) also decreased in these patients[34]. The use of probiotic beverage Yakult 400 also led to a decrease in the abundance of Enterobacteriaceae in the gut microbiome[35]. In another RCT, administration of Lactobacillus GG for 8 wk led to an increase in the proportion of beneficial bacteria (*Lachnospiracea* and *Clostridia XIV*) and a decrease in the proportion of harmful ones (*Enterobacteriaceae*). Moreover, this was accompanied by a decrease in endotoxemia and systemic inflammation[36].

Administration of a probiotic for cirrhosis leads to an improvement in cognitive functions and an increase in gait speed, but does not significantly affect the risk of falling and the hand grip muscular strength[37].

A recent meta-analysis showed that administration of probiotics for cirrhosis does not significantly affect C-reactive protein (CRP) and interleukin (IL)-6 Levels, but leads to a decrease in tumor necrosis factor alpha (TNF-α) level[38].

Probiotic *Lactobacillus casei* (*L. casei*) Shirota application for 6 mo did not have a significant effect on neutrophil function, the blood level of LPS and most cytokines, frequency of bacterial DNA detection in blood, intestinal permeability (but it was baseline normal), quality of life, indicators of the complete blood count, or liver and kidney function in non-severe cirrhosis (Child-Pugh scores < 11)[39].

The use of a multi-strain probiotic containing several species of *Bifidobacterium* and *Lactobacillus* for non-severe cirrhosis (Child-Pugh scores < 12) showed similar results [40]. However, the intake of this probiotic led to an increase in the abundance of *Faecalibacterium prausnitzii*, *Syntrophococcus sucromutans*, *Bacteroidetes vulgatus*, *Prevotella*, and *Alistipes shahii* in the fecal microbiome. At the same time, the abundance of *Bifidobacterium bifidum*, *Lactobacillus acidophilus* (*L. acidophilus*), and *L. casei* remained unchanged[41].

One of the most studied probiotics for cirrhosis is VSL#3, a mixture containing eight bacterial strains. Its use for 6 mo led to a decrease in the Child-Pugh and MELD scale values, the blood level of IL-1b and IL-6, TNF-α, aldosterone, renin, brain natriuretic peptide, ammonia, and indole, as well as the risk of hospitalization, but did not significantly affect mortality[42]. Its use for 2 mo in patients with large esophageal varices without a history of bleeding improves their response to propanolol[43]. Administration of this probiotic for 28 d did not lead to any significant change in the blood content of the plasminogen activator inhibitor and vascular endothelial growth factor, but led to an increase in the blood levels of large endothelin and nitric oxide and a decrease in the blood levels of thromboxane B2[44]. In addition, the use of this probiotic for 6 wk led to a decrease in the hepatic venous pressure gradient, cardiac output, and heart rate and an increase in systemic vascular resistance and sodium levels in the blood, but did not significantly affect the mean pulmonary artery pressure [45]. However, the last two studies were not controlled. VSL#3 also prevents the development of endothelial dysfunction in experimental models of cirrhosis[46].

Probiotics reduce the risk of development of re-bleeding from esophageal varices after endoscopic treatment in cirrhosis according to a retrospective study. Moreover, the larger the dose of the probiotic, the more pronounced the effect[47].

The probiotic tolerance was excellent and there were no significant side effects in any of the cited studies. However, cases of the development of spontaneous bacterial periotinitis[48] and fatal endocarditis[49] caused by probiotic strains, which was consumed by a patient with cirrhosis for a long time, are described.

Summarizing these data, we can deduce the aforementioned facts. The effectiveness of probiotics in the treatment of minimal hepatic encephalopathy and in the prevention of development of overt hepatic encephalopathy has been confirmed by a series of meta-analyses and is beyond doubt. In addition, most studies have shown an improvement in the profile of the gut microbiota after following administration. At the same time, the influence of probiotics on other characteristics of patients with cirrhosis (intestinal permeability, bacterial translocation, systemic inflammation and others) differs from study to study. Perhaps this is due to the fact that different probiotic strains were used, which had different effects on these indicators. It would be helpful to conduct studies that directly compare probiotics that have shown and not shown an effect on these biomarkers.

The suggested mechanism of action of probiotics in cirrhosis is shown in Figure 2.

PROBIOTICS FOR ALCOHOLIC LIVER DISEASE

The use of probiotics led to a decrease in the level of steatosis, inflammation, oxidative stress, and cell death in the liver, a decrease in the level of biomarkers of systemic inflammation, bacterial translocation, gut dysbiosis, dyslipidemia, damage to the intestinal epithelium, and intestinal permeability in experimental alcoholic liver disease (Table 1)[50-54]. Probiotics restore the alcohol-damaged epithelial barrier in the intestines by epidermal growth factor receptor activation[55]. Functioning of this receptor is also required for the protective effect of probiotics in alcoholic liver disease [55]. Probiotics suppress alcohol-induced apoptosis of hepatocytes[56].

These effects are not just due to the living bacteria themselves, which are part of the probiotics, but also the supernatant of their culture[57].

However, unlike many published experimental results, there are very few clinical trials on the effectiveness of probiotics in alcoholic liver disease. There was no effect of the probiotics (*Lactobacillus subtilis* and *Streptococcus faecium*) on total protein, cholesterol, or IL-1b levels in the blood according to RCT. The probiotics blocked the growth of blood LPS level in alcoholic hepatitis, but only in the cirrhosis subgroup.

Table 1 Effects of probiotics on different disorders in experimental alcoholic liver disease

Disorder	Biomarker changes	Ref.
Liver steatosis	↓ Liver mass, ↓ content of triglycerides, free fatty acids, and cholesterol in the liver tissues	[50-52, 54]
Liver inflammation	↓ Myeloperoxidase activity, expression of tumor necrosis factor alpha gene and neutrophil infiltration in the liver	[54]
Oxidative stress in liver	↓ Level of nitric oxide and malondialdehyde and ↑ level of glutathione and catalase in the liver tissue	[50,51, 54]
Death of hepatocytes	↓ Serum aminotransferases	[50-54]
Systemic inflammation	↓ Serum IL-6 and tumor necrosis factor alpha	[51-53]
Bacterial translocation	↓ Serum lipopolysaccharide	[51-54]
Gut dysbiosis	↑ Firmicutes, Clostridiales and Lactobacillales; ↓ Proteobacteria and Campylobacterales	[51,53]
Damage to the intestinal epithelium	↓ Serum diamine oxidase	[53]
Increased intestinal permeability	↓ Serum D-lactate, ↑ the amount of occludin and other protein of tight junction in the gut epithelium, ↓ intestinal permeability for dyes	[50,52-54]
Dyslipidemia	↓ Serum cholesterol and triglycerides	[50,52-54]

The number of *Escherichia coli* decreased in the feces in the probiotics groups. Changes in the levels of other biomarkers were not compared between the probiotic and placebo groups in this RCT[58].

It was shown that probiotics led to a more pronounced decrease in the activity of transaminases in the blood than standard therapy while significantly having no effects on the level of total bilirubin and GGT in alcoholic steatohepatitis in an earlier RCT [59].

Thus, the encouraging results of the use of probiotics in the treatment of alcoholic liver disease, obtained in experimental models, need to be confirmed by a large number of clinical trials.

PROBIOTICS FOR METABOLIC ASSOCIATED FATTY LIVER DISEASE

The use of probiotics led to a decrease in the level of steatosis, lipogenesis, oxidative stress, and inflammation in the liver, a decrease in the level of biomarkers of insulin resistance, bacterial translocation, gut permeability, and systemic inflammation and a decrease in blood level of lipids and glucose and in expression of the inflammation activator receptor genes (toll-like receptors 4 and 9, and NLRP3) in the liver in experimental MAFLD (Table 2)[60-66]. It also leads to a decrease in the LPS content and an increase in the bile acid content in feces[62,67], increases the content of cholesterol 7α-hydroxylase, which converts cholesterol to bile acids, and transporters of bile acids into bile in the liver[62], enhances the transfer of Nrf2 (transcription factor of antioxidant defense genes) to the nucleus[66], transfers metabolism from carbohydrate utilization to fat utilization[63], increases the acetate and butyrate level in feces[68], improves gut microbiome structure by increasing the abundance of gram-positive bacteria such as Firmicutes and decreasing gram-negative bacteria such as Bacteroidetes, Proteobacteria, and Fusobacteria[69], but does not affect the degree of cholesterol reabsorption[63].

Some of these effects can be achieved using the supernatants of the cultures of live probiotics[70].

Butarate, formed by probiotic strains, enhances the formation of tight junction proteins, as well as activates 5' adenosine monophosphate-activated protein kinase (inhibits lipogenesis) and increases the lifetime of Nrf2 in cell culture[71].

Consuming yogurt four times or more per week reduces the risk of developing MAFLD[72].

A number of systematic reviews with meta-analyses describing the effect of probiotics on the course of MAFLD were published recently. The meta-analysis, including 105 studies of patients with MAFLD and/or its underlying disorders (obesity and/or diabetes), showed that administration of probiotics leads to a decrease

Table 2 Effects of probiotics on different disorders in experimental metabolic associated fatty liver disease

Disorder	Biomarker changes	Ref.
Liver steatosis	↓ Liver mass, the size and number of lipid droplets, the content of triglycerides, free fatty acids, and cholesterol in the liver tissues	[60-66]
Obesity	↓ Body mass, subcutaneous fat	[61-63, 65,66]
Intensified lipogenesis	↓ Expression of the genes of sterol regulatory element-binding protein 1c (SREBP-1c), 3-hydroxy-3-methylglutaryl-CoA reductase, acetyl-CoA carboxylase 1, acetyl-CoA acetyltransferase 2, and fatty acid synthase, ↑ activated 5' adenosine monophosphate-activated protein kinase (SREBP-1c inhibitor)	[60,62, 65,66]
Reduced lipolysis	↑ Expression of the gene of peroxisome proliferator-activated receptor alpha (fatty acid catabolism enhancer) and acyl-CoA oxidase	[60,62]
Bile acid metabolism disorders	↑ Expression of the gene of bile salt export pump, farnesoid X recetor, cholesterol 7a-hydroxylase, sodium taurocholate cotransporting polypeptide, ↓ the content of bile acids in the liver tissues	[62]
Oxidative stress	↓ Total reactive oxygen species, lipid peroxidates, and malondialdehyde and ↑ glutathione, superoxide dismutase, and catalase in the liver tissue	[60,61, 65,66]
Liver inflammation	↓ Expression of the genes of tumor necrosis factor alpha, interleukin 1-beta and 6 and the content of NF-κB in the liver	[60]
Death of hepatocytes	↓ Serum aminotransferases	[60,61, 65]
Insulin resistance	↓ HOMA-IR, insulin, resistin	[63,64]
Systemic inflammation	↓ Serum tumor necrosis factor alpha, interleukin 1-beta and 6	[60,64]
Bacterial translocation	↓ Serum lipopolysaccharide	[64]
Increased gut permeability	↑ Amount of proteins of tight junction in the gut	[64]
Disorders of the metabolism of carbohydrates and lipids	↓ Serum total cholesterol, low density lipoprotein cholesterol, glucose, triglycerides, and free fatty acids, ↑ expression of the gene of low-density lipoprotein receptor	[60-62, 65,66]

in body weight, body mass index, waist circumference, body fat mass, visceral and subcutaneous adipose tissue mass, fasting glucose, glycated hemoglobin, insulin, HOMA-IR, ALT, AST, triglycerides and CRP[73]. A meta-analysis that included 22 studies of patients with MAFLD showed that probiotics lower weight, body mass index, ALT, AST, GGT, ALP, total cholesterol, LDL-C, triglycerides, glucose, insulin, TNF-α, leptin, and liver steatosis and do not significantly affect waist circumference, waist-to-hip ratio, fat mass, serum albumin, HDL-C, HOMA-IR, CRP, or IL-6[74]. Other meta-analysis came to broadly similar conclusions[75]. The fourth meta-analysis showed that administration of probiotics for MAFLD resulted in a decrease in liver fibroscan stiffness[76].

Several new RCTs have been published following these meta-analyses.

The use of a multi-strain probiotic for 1 year in patients with metabolic associated steatohepatitis (MASH) resulted in a decrease in the severity of ballooning necrosis and fibrosis, without significantly affecting steatosis and inflammatory infiltration of liver compared to the placebo. Moreover, the level of bilirubin, ALT, ALP, leptin, TNF-α, IL-1b, IL-6, and LPS decreased in the blood, without significant difference in HOMA-IR and body weight[77].

The use of another multi-strain probiotic for 12 wk led to, among other things, a decrease in liver fat content according to MRI data and an increase in the *Bacteroidetes/Firmicutes* ratio[78].

A combined probiotic (Bifidobacterium, Lactobacillus and Enterococcus, 1 g two times per day, 3 mo) in histologically verified MAFLD lowered the serum levels of ALT, AST, GGT, total cholesterol, triglycerides, and HOMA-IR and the value of the histological scale of steatohepatitis activity NAS, and proportion of patients with dysbiosis, but did not significantly affect the serum levels of total bilirubin and high density lipoprotein cholesterol[79].

The use of a probiotic (*L. casei, L. rhamnosus, L. acidophilus, Bifidobacterium longum*, and *B. breve*) for MASH led to a decrease in the serum levels of triglycerides, ALT, AST, GGT, and ALP, but did not significantly affect fasting blood sugar, the serum levels of cholesterol and its fractions, CRP, weight, body mass index, percent body fat, waist circumference, and waist-to-hip ratio[80].

In general, it can be argued that RCTs and their meta-analyses have confirmed most of the results obtained in experimental models of MAFLD. However, we did not find

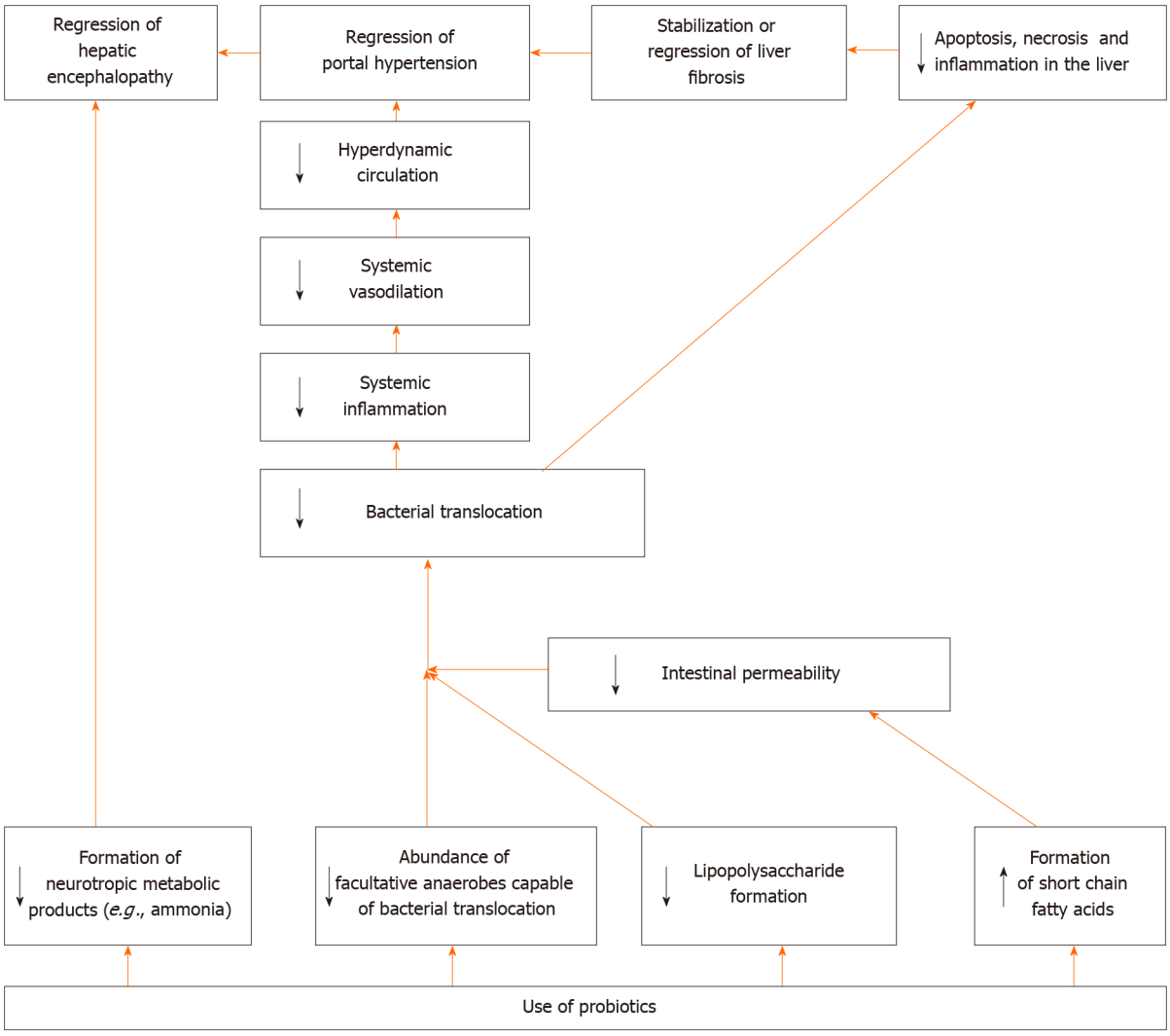

Figure 2 Suggested mechanism of action of probiotics in cirrhosis.

any studies that described the effect of probiotics on prognosis in this disease, which is a challenge for future researchers. Based on the data obtained, the following mechanism of the development of positive effect of probiotics in MAFLD can be assumed (Figure 3).

PROBIOTICS FOR VIRAL HEPATITIS

Unlike for cirrhosis, alcoholic and MAFLD, probiotics have hardly been researched as drugs for viral hepatitis. Perhaps this is due to the fact that, unlike these diseases, effective therapy for viral hepatitis already exists.

Long-term use of a probiotic *Enterococcus fecalis* strain FK-23 in chronic viral hepatitis C led to a decrease in ALT and AST levels, with no significant effect on viral load, blood total protein, urea and hemoglobin levels, and platelet count in an uncontrolled clinical study[81].

Bifidobacterium adolescentis SPM0212 showed antiviral effects against hepatitis B virus in cell culture[82].

PROBIOTICS FOR CHOLESTATIC DISEASES

The use of *L. rhamnosus* GG reduced the biochemical and histological signs of hepatitis, cholestasis, and fibrosis after ligation of the common biliary duct in mice. Perhaps the reason for this is that probiotics increases the activity of FXR in the intestine. This receptor enhances the formation of fibroblast growth factor 15 (FGF15) in response to

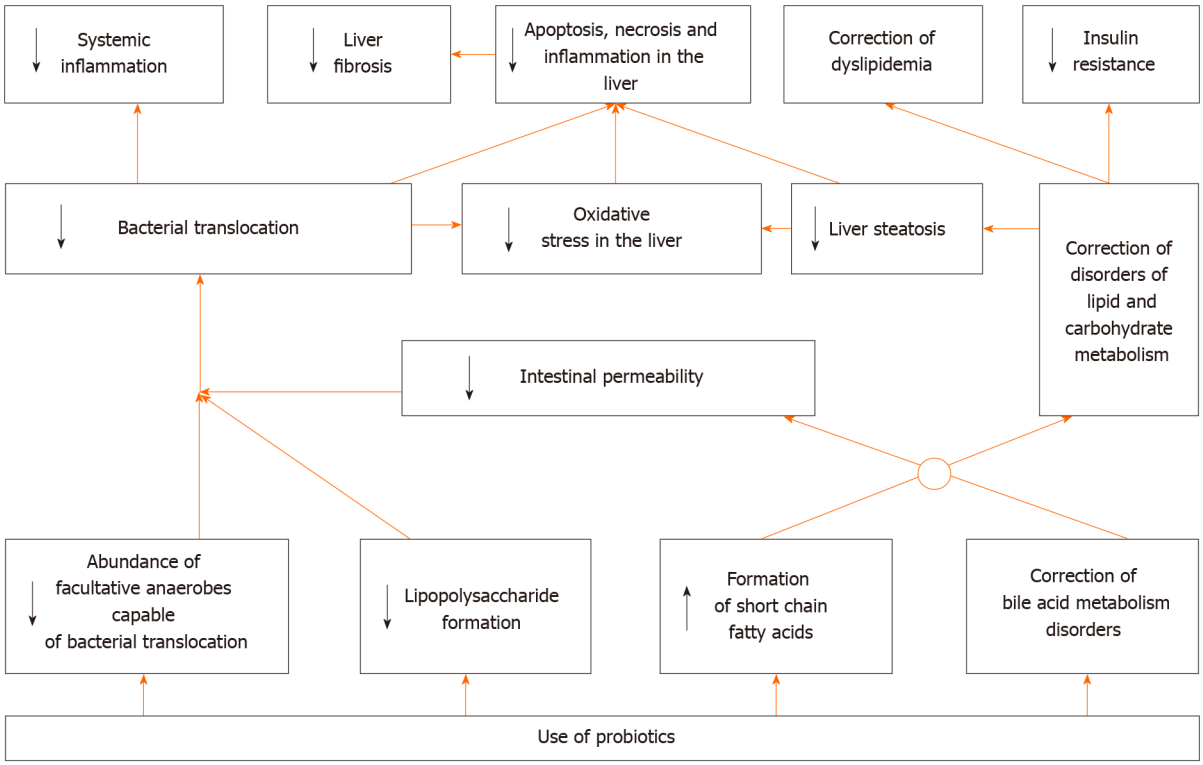

Figure 3 Suggested mechanism of action of probiotics in metabolic associated fatty liver disease.

stimulation with bile acids. FGF15 reduces the production of bile acids in the liver due to negative feedback. With cholestasis, few bile acids enter the intestine, this receptor is not activated enough, the FGF15 content in the blood decreases, and the formation of bile acids in the liver increases. The latter, with cholestasis, have a toxic effect on the liver, which is manifested by its inflammation and fibrosis. The intake of this probiotic led to an increase in the activity of FXR in the intestine and FGF15 in the blood, which close this feedback, protecting the liver from autointoxication with bile acids. This hypothesis is supported by the fact that the use of powerful antagonists of FXR blocks the positive effect of the probiotic in this case and the culture supernatant of this probiotic increases the activity of this receptor in tissue cultures[83].

In addition, *L. rhamnosus* GG increases the content of Firmicutes and Actinobacteria in the gut microbiota, which convert primary bile acids into secondary ones, which are poorly absorbed, and therefore, removed with feces. There is a significant increase in the content of bile acids due to secondary ones, with an absolute and relative decrease in the content of primary bile acids in the feces of such animals. That is, administration of this probiotic for cholestasis leads not only to a decrease in the formation of new bile acids, but also to an increase in the removal of already formed ones with the feces[83].

L. rhamnosus GG has a similar protective effect in another model of cholestatic liver damage, in which the excretion of bile acids is blocked due to the knockout of the gene of their transporter multidrug resistance protein 2[83]. The use of *L. casei rhamnosus* was as effective as neomycin in preventing cholangitis in patients with biliary atresia who underwent Kasai operation[84].

The use of probiotics for primary sclerosing cholangitis combined with inflammatory bowel diseases did not have a significant effect on pruritus, fatigue, serum level of bilirubin, ALP, GGT, AST, ALT, prothrombin, albumin, and bile salts in a very small RCT that included 14 patients[85].

We did not find any other clinical trial of probiotics in chronic cholestatic diseases. Given the very encouraging results of the experimentary study, a large RCT on this topic seems to be very interesting.

PROBIOTICS FOR AUTOIMMUNE HEPATITIS

We found only one study describing the effect of probiotics on liver condition in experimental autoimmune hepatitis. It was shown that they lead to a decrease in the

formation of TNF-α, IL-6, and IL-1b in the liver, as well as in the proportion of Th-17 cells among CD4+ lymphocytes in the liver and spleen[86]. Further experimental studies and clinical trials are needed to clarify the usefulness of probiotics in the treatment of this disease.

CONCLUSION

In conclusion, the study of probiotics in hepatology is uneven. It has been proven that they are useful in hepatic encephalopathy, but their effect on other symptoms and syndromes of cirrhosis is poorly studied. Their effectiveness in the treatment of MAFLD has been proven both in experimental models and in clinical trials, but their effect on the prognosis of this disease has not been described. The beneficial effects of probiotics in alcoholic liver disease have been well shown in many experimental studies, but there are very few clinical trials to support them. The effect of probiotics on the course of other liver diseases is either poorly studied (primary sclerosing cholangitis, chronic hepatitis B and C, autoimmune hepatitis), or not studied at all (primary biliary cholangitis, hepatitis A and E, Wilson's disease, hemochromatosis, storage diseases, vascular liver diseases, *etc*.).

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ORIGINAL ARTICLE

Clinical and Translational Research

Development of a risk score to guide targeted hepatitis C testing among human immunodeficiency virus patients in Cambodia

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Abstract

BACKGROUND

The World Health Organization recommends testing all human immunodeficiency virus (HIV) patients for hepatitis C virus (HCV). In resource-constrained contexts with low-to-intermediate HCV prevalence among HIV patients, as in Cambodia, targeted testing is, in the short-term, potentially more feasible and cost-effective.

AIM

To develop a clinical prediction score (CPS) to risk-stratify HIV patients for HCV coinfection (HCV RNA detected), and derive a decision rule to guide prioritization of HCV testing in settings where 'testing all' is not feasible or unaffordable in the short term.

METHODS

We used data of a cross-sectional HCV diagnostic study in the HIV cohort of Sihanouk Hospital Center of Hope in Phnom Penh. Key populations were very rare in this cohort. Score development relied on the Spiegelhalter and Knill-Jones method. Predictors with an adjusted likelihood ratio ≥ 1.5 or ≤ 0.67 were retained, transformed to natural logarithms, and rounded to integers as score items. CPS performance was evaluated by the area-under-the-ROC curve (AUROC) with 95%

0309).

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confidence intervals (CI), and diagnostic accuracy at the different cut-offs. For the decision rule, HCV coinfection probability ≥1% was agreed as test-threshold.

RESULTS

Among the 3045 enrolled HIV patients, 106 had an HCV coinfection. Of the 11 candidate predictors (from history-taking, laboratory testing), seven had an adjusted likelihood ratio ≥ 1.5 or ≤ 0.67: ≥ 50 years (+1 point), diabetes mellitus (+1), partner/household member with liver disease (+1), generalized pruritus (+1), platelets < 200 × 10^9/L (+1), aspartate transaminase (AST) < 30 IU/L (-1), AST-to-platelet ratio index (APRI) ≥ 0.45 (+1), and APRI < 0.45 (-1). The AUROC was 0.84 (95%CI: 0.80-0.89), indicating good discrimination of HCV/HIV coinfection and HIV mono-infection. The CPS result ≥0 best fits the test-threshold (negative predictive value: 99.2%, 95%CI: 98.8-99.6). Applying this threshold, 30% (n = 926) would be tested. Sixteen coinfections (15%) would have been missed, none with advanced fibrosis.

CONCLUSION

The CPS performed well in the derivation cohort, and bears potential for other contexts of low-to-intermediate prevalence and little onward risk of transmission (*i.e.* cohorts without major risk factors as injecting drug use, men having sex with men), and where available resources do not allow to test all HIV patients as recommended by WHO. However, the score requires external validation in other patient cohorts before any wider use can be considered.

Key Words: Hepatitis C virus; Hepatitis C/human immunodeficiency virus coinfection; Clinical prediction rule; Targeted screening; Cambodia; Development prediction model

Core Tip: We developed and internally validated a clinical prediction score to stratify human immunodeficiency virus (HIV) patients for risk of hepatitis C (HCV) coinfection, and derived a decision rule to guide prioritization of HCV testing. The score incorporates readily available clinical and laboratory predictors, and had, in the Cambodian derivation cohort, a good ability to discriminate between HCV/HIV coinfection and HIV mono-infection. Key populations were rare in the Cambodian HIV cohort.

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INTRODUCTION

Interferon-free antiviral treatment has replaced the combination of pegylated interferon and ribavirin as standard-of-care for chronic hepatitis C[1]. These new treatments are highly efficacious, short in duration, well-tolerated and hold, as becoming increasingly affordable, real promise of worldwide scalability[2]. On the other hand, less than 5% of people living with hepatitis C virus (HCV) in low and middle income countries (LMIC) were aware of their status end of 2016[3]. To boost identification of HCV infected individuals, particularly in LMIC, the World Health Organization (WHO) launched a first set of HCV testing guidelines in 2017[4]. Routine testing throughout the whole population is recommended where HCV seroprevalence is of intermediate (≥ 2%) or high (≥ 5%) level, and targeted testing in all other settings. Clinical suspects, people who inject drugs (PWID), men having sex with men (MSM), people in prisons, birth cohorts, and people living with human immunodeficiency virus (HIV) (PLWH) are the main targets for this latter.

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Though feasibility in resource-limited settings was considered when formulating the WHO recommendations, it is unlikely that many LMIC will be able to implement them at full-scale in the short-term, due to operational (human resources, diagnostic capacity, stigma), but also financial constraints[5]. There are no large global financing initiatives in the pipeline for viral hepatitis at the short-to-medium term, and countries are in the meantime left to find their own financial solutions[6]. This seriously impacts the scale of what can be implemented.

In this regard, and based on the prevalence we registered in Cambodia, and even lower rates of HCV/HIV coinfection found in several HIV cohorts in Sub-Saharan Africa[7-10], we anticipate that some LMIC with large, primarily heterosexually-infected, HIV cohorts (and little forward transmission risk) may opt not to offer HCV testing to all HIV patients, at least in the short-to-medium term. Applying 'screen all' strategies in such cohorts is resource demanding and yields low positivity. To preserve resources, countries may rather choose to prioritize testing, in first instance, only for those at higher risk.

With the possibility of very successful treatment and growing availability of cheap WHO prequalified screening tests[11], the threshold to offer testing should, however, be low enough, to avoid maximally that HCV/HIV coinfected are denied treatment because of restrictive testing strategies. The critical question is thus whether it is possible to identify accurately, and in a simple manner, a subgroup of HIV patients in which the 'probability of being HCV infected and having to be treated in the short-term' is so low that it would be reasonable not to offer them HCV testing or postpone it until more resources become available. Or phrased differently, to preserve the limited budget for testing and treating those with a higher risk of being HCV co-infected.

Easy-to-use tools to guide such targeted HCV testing in HIV populations, other than prioritization of key populations or older birth cohorts, do not exist. Though many LMIC have some birth cohort effect in their epidemics, it is generally less neat than in North-America and Europe, as drivers of generalized HCV exposure were removed at much later date or only partially[12-14]. Birth-cohort testing might thus be too restrictive. In our previous study in Cambodia, 55% of HCV/HIV coinfections would have been missed if only PLWH older than 50 years would have been tested[7].

As for other pathologies and conditions[15-18], diagnostic prediction models combining several readily available elements from patient history, physical examination, and lab tests may more accurately risk- stratify HIV patients and support clinical decisions regarding the need to prioritize HCV testing.

Using data from our HCV diagnostic study in Cambodia, we developed and internally validated a clinical prediction score (CPS) to risk-stratify HIV patients for HCV coinfection, and derived a decision rule to guide prioritization of HCV testing. In addition to the full CPS, we also explored alternative risk scores, one with only socio-demographic/clinical predictors and another primarily lab-based.

MATERIALS AND METHODS

Source of data, study site and participants

For developing the score, we used data of a cross-sectional HCV diagnostic study conducted in the HIV cohort of Sihanouk Hospital Center of Hope (SHCH) in Phnom Penh, Cambodia (clinical trials.gov NCT02361541). It is one of the largest primary care HIV cohorts in Cambodia with, as most other Cambodian HIV cohorts, primarily heterosexually-infected HIV patients. Key populations (history/current injecting drug use: 0.2%, history/currently engaged in sex work: 0.2%, self-identified MSM: 0.6%) were rare. Data were prospectively collected following a pre-specified protocol for HCV diagnostic work-up and predictors. The information on predictors (by history-taking, physical examination and laboratory tests) was collected without knowledge of the results of HCV diagnostic testing. Details of the study and diagnostic results have been published previously[7].

In brief, all consecutive adult HIV patients without history of HCV treatment and visiting the HIV clinic of SHCH between November 2014 and May 2016 underwent, if consenting, a structured health and HCV risk factor screening immediately followed by lab testing (hepatitis C, hepatitis B, CD4, platelets and liver tests (transaminases). HCV testing was done according to the classic two-test algorithm; initial testing for HCV antibodies followed by confirmatory HCV-RNA testing in case of HCV antibody positive or borderline results. In total, 3045 (out of 3562 in the cohort) adult HIV patients were enrolled, of whom 106 had a current HCV infection (*i.e.* HCV-RNA

detected).

Approval for this study was provided by the Institutional Review Board of the Institute of Tropical Medicine Antwerp, the Ethics Committee of the Antwerp University Hospital (Belgium), and the Cambodian National Ethics Committee for Health Research. All enrolled participants provided written informed consent. The statistical methods and analysis of this study were reviewed by Jozefien Buyze from the Institute of Tropical Medicine, Antwerp, Belgium.

Development of the clinical prediction score

Outcome of interest: The outcome event was having a current HCV infection, which was defined as having a detectable HCV-RNA viral load as measured by the quantitative COBAS® AmpliPrep/COBAS® TaqMan® HCV PCR Test, v2.0, on the COBAS® TaqMan® 48 Analyzer (Roche Diagnostics Ltd, Mannheim, Germany). The lower limit of detection was 15 IU/mL. Further in this paper, we refer to 'current HCV infection' as 'HCV infection or coinfection'.

Candidate predictor variables: The clinical variables we explored as predictors were selected based on the distribution of the variables in our study data[7], reported associations in the literature and clinical plausibility, with preference for readily available and objective parameters. Potential predictors considered were: age (years), gender (female/male), platelet count ($\times 10^9$ cells/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), AST-to-platelet ratio index (APRI), having diabetes mellitus (yes/no), any of the following symptoms: fatigue, myalgia/arthralgia, anorexia/weight loss (yes/no), presenting generalized pruritus without obvious skin lesions (yes/no), having a household member and/or partner with liver disease (yes/no), and poor CD4 recovery on antiretroviral treatment (ART), *i.e.* CD4 below 200 after 3 years or more on ART (yes/no). Known major risk factors for HCV infection (history/current injecting drug use, sex work, being homosexual) were not considered as they were very uncommon in this cohort[7]. As we were mainly interested in the joint effects of the different variables to predict the probability of HCV infection and less to get an idea of the individual contribution of each variable, we did not exclude potentially correlated variables as long as they validly contributed to improving the predictive ability of the model[19,20].

Derivation cohort and sample size: We did not calculate a formal sample size for this CPS development study. We included the data of all 3,045 adult HIV patients enrolled in the cross-sectional study in the data set for derivation of the score to allow an adequate assessment of the potential predictors following the rule of thumb to have 10 outcome events per explored predictor variable[21].

Score development: We used the Spiegelhalter and Knill-Jones method adapted by Berkley *et al*[22] and Stéphan *et al*[23] to develop the score. The continuous candidate predictors (age, platelets, AST, ALT, APRI) were dichotomized guided by Receiver Operating Characteristic (ROC) curves at the point with the highest sum of sensitivity and specificity, and rounded to values that are easy to use in clinical practice. Crude likelihood ratios (LHR) were calculated for all candidate predictors. Candidate predictors with a crude LHR ≥ 2 or ≤ 0.5 were, in a next step, used in a multivariable logistic regression model to calculate adjusted LHRs. The predictors with an adjusted LHR ≥ 1.5 or ≤ 0.67 were selected for the CPS. The adjusted LHRs were transformed to their natural logarithm, and rounded to the nearest integer to calculate the score (relative weight) of each predictor. By summing the scores of all risk factors presented by a patient the total predictor score for each patient was obtained. A value of 0 was assigned to missing data.

Score performance: The CPS's performance to differentiate patients with HCV coinfection *vs* those without HCV coinfection (discrimination) was evaluated by the area-under-the-ROC curve (AUROC) with 95% confidence intervals (CI). AUROCs of 0.7-0.79, 0.8–0.89, ≥ 0.9 were respectively considered acceptable, good, and outstanding in terms of discrimination[24]. In addition, diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value) was calculated at the different cut-offs of the score. Statistical analysis was done using Stata 14 and R 3.4.2 software.

Derivation and performance of the decision rule to guide prioritization of HCV testing

As clinically useful decision threshold (test-threshold in our case), we opted for the CPS cut-off which dichotomizes the HIV patients in a subgroup with probability of

HCV coinfection < 1% and a subgroup with probability ≥ 1% (Figure 1). This latter group could be prioritized for HCV testing, while for those with probability below 1% testing could be postponed if 'testing all' is not feasible or not affordable in the short-term.

We considered the harm/benefit of 'testing and not testing' at patient (access to treatment) and public health level (onward transmission, cost) (Table 1). Generally, due to the introduction of nearly 100% curative, well-tolerated generic DAA treatment options the potential harm of not testing has become much more important in recent years. In addition, HCV coinfected HIV populations in resource-constrained settings might be at higher risk of advanced HCV disease as they have often started ART late or with less optimal regimens. Pondering this, but also the possibility to repeat the risk scoring regularly (as HIV patients are in chronic care follow-up), we opted for a 1% probability threshold for the decision rule (*i.e.*, giving false negatives much more weight than false positives). Logically, this threshold is lower than the WHO recommended threshold range (2%-5%) for HCV testing in the general population[4].

The proportion of missed HCV coinfections, and the number of patients needed to test (NNT) to identify one HCV/HIV coinfection were calculated as measures of performance (clinical usefulness) of the decision rule in the derivation cohort.

Internal validation of the CPS

Finally, in order to correct for over-optimism (over-fitting) caused by the use of the same data set for both the derivation of the score and the evaluation of its predictive ability, we assessed internal validity of the CPS performance with a bootstrapping procedure (0.632+ estimator)[25]. We determined the performance (proportion of missed coinfections) of the CPS and the decision rule derived from each bootstrap sample in the original derivation set. This bootstrap-derived performance provides a more realistic estimate of the CPS performance in similar new patient cohorts.

Development of alternative scores

We explored two reduced models: (1) using only the six clinical and socio-demographic candidate predictors (clinical CPS); and (2) starting from lab-based (ALT, AST, platelets, APRI) and socio-demographic (gender, age) candidate predictors (lab CPS). Both were developed and assessed in the same way as the full CPS. The clinical model was explored with the intention to provide a feasible alternative for HIV programs where ALT, AST and platelet count results are not routinely available. The lab model might be easier to use in large programs equipped with electronic databases which can flag patients to be prioritized for HCV testing.

RESULTS

Description of the HIV derivation cohort

A total of 3,045 ambulatory HIV patients of Sihanouk Hospital Center of Hope were included. Their median age was 43 years (interquartile range - IQR: 36-48), 43% were male patients, and 98% were on antiretroviral therapy (ART) for a duration ranging from 2 mo to 13 years. Most were on nevirapine- (n = 1189) or efavirenz-based (n = 1539) ART. HIV virological failure was rare (3.4%). The cohort counted only few people (n = 31) who reported a history or current engagement in sex work, being homosexual, or past or current injecting drug use.

In this cohort, 230 patients tested positive for HCV antibodies, two had a borderline result. Of these 232, 106 had a detectable HCV-RNA, our outcome of interest. None of the coinfected reported past/current sex work, being MSM, or injecting drug use. Distribution of the candidate predictors in the cohort and the missing values are further specified in Table 2.

Prediction score for HCV/HIV coinfection

In Table 3, we list the 11 candidate predictors, all in dichotomous format, as taken forward in the score building. We report the unadjusted associations (crude positive and negative likelihood ratios) between the candidate predictors and having a HCV coinfection. After univariable analysis, two potential predictors (poor CD4 recovery on ART, gender) were dropped as the crude LHRs were not ≥ 2 or ≤ 0.5. From the remaining candidate predictors, seven with adjusted LHR ≥ 1.5 or ≤ 0.67 were retained in the final multivariable score model. The adjusted LHRs are shown in the last two columns. Among the retained predictors, three rely on laboratory testing results

Table 1 Harm and benefit of hepatitis C virus testing and not testing

Harm of testing (false positives)	Benefit of testing	Harm of not testing (false negatives)	Benefit of not testing
Low, but existing:	High (for some):	High (for some):	Important in some contexts:
Cost of tests, human resources (lab & counseling)	If diagnosed positive: good treatment available (high cure rate, few side effects, short /life-saving for cirrhotic patients/ but treatment often not urgent)	Denial of live-saving, highly efficacious and affordable treatment	Cost-saving in resource-constrained environment with many competing interests
Stress related to waiting for results	Impact on further transmission (but less weight in HCV populations with low risk profile)		
Budget allocated to HCV testing not available for other health priorities			
Divert resources /timely access from those most in need (in case of testing all)			

HCV: Hepatitis C virus.

(platelet count, AST, APRI).

The relative weight (further called score) of the retained predictors is detailed in Table 4. Only APRI (whether ≥ 0.45 or < 0.45) contributed in both directions, and none of the predictors weighed more than + 1 or -1. The total score for each individual patient can range from -2 to + 6.

The distribution of the total individual scores in the HIV cohort, by coinfection status, and probability of HCV coinfection by each final score is presented in Figure 2. None of the patients in the derivation cohort had a score above 5. The majority (n = 2,219, 70%) had -2 or -1 as score. The probability of HCV coinfection ranged from 0.6% when the score was -2, to 75% for those with the highest score. A score ≥ 0 seems to fit best as test-threshold by dichotomizing in a large sub-group with predictive probability of HCV coinfection < 1% *vs* a smaller group with probability ≥ 1%.

Performance of the full CPS and derived decision rule for targeted HCV testing

The CPS yielded an AUROC of 0.84 (95%CI: 0.80-0.89), indicating good discrimination between HCV/HIV coinfection and HIV mono-infection. Diagnostic accuracy for different cut-offs of the risk score is detailed in Table 5.

The score ≥ 0, identified above as meeting our pre-defined criteria of clinically useful threshold to guide prioritization of HCV testing, had a negative predictive value (NPV) of 99.2% (95%CI: 98.8%-99.6%) or differently put, the probability of HCV coinfection among those with score < 0 was 0.8%.

Applying this test-threshold, only 30% (n = 926) of the HIV patients would have been prioritized for HCV testing. In this subgroup, 90 HCV coinfections (85%) would have been diagnosed decreasing the number needed to test (NNT) from 29 to 10. Sixteen HCV coinfections would have been missed, but none of these missed HCV diagnoses had advanced fibrosis (*i.e.*, ≥ 9.5 kPa as measured by transient elastography). In line with international guidelines, triple HBV/HCV/HIV coinfections should also be prioritized for testing and treatment. In this derivation cohort, they were rare (n = 2), but not missed by the prioritization rule.

Adjusting for over-optimism (over-fitting), the bootstrap 0.632+ estimate of proportion of missed HCV coinfections was 18%, compared to 15% in the original derivation set.

Development of alternative scores (clinical CPS, lab CPS)

In the alternative 'clinical' model, five predictors (age ≥ 50 years, diabetes mellitus, partner/household member with liver disease, generalized pruritus, fatigue/myalgia-arthralgia/anorexia-weight loss) were retained in the final model, each with a relative weight of +1 point. Gender was dropped after univariable analysis. The AUROC was 0.69 (95%CI: 0.64-0.74), indicative of poor discrimination of HCV/HIV coinfection and HIV mono-infection. Figure 3 further illustrates the poor discrimination of the clinical score, which moreover did not allow to identify a sub-group with predicted HCV infection probability below 1%.

Table 2 Characteristics of the derivation cohort, including the candidate predictors

Characteristics	Missing values	*n* = 3045	Candidate predictor
HIV patients with HCV coinfection, *n* (%)	0	106 (3.5)	
Male, *n* (%)	0	1,307 (42.9)	√
Age, yr, median (IQR)	0	42.5 (36.3-48.1)	√
Key populations[1], *n* (%)	0	31 (0.1)	
Receiving ART, *n* (%)	0	2,972 (97.6)	
On NNRTI-based ART, *n* (%)		2,728 (91.8)	
On PI-based ART, *n* (%)		232 (7.8)	
Other, *n* (%)		12 (0.4)	
Duration on ART, years, median (IQR)	0	6.9 (4.4-9.1)	
HIV viral load < 50 copies/mL, *n* (%)	368	2,517 (96.6)	
CD4, cells/μL, median (IQR)	11	464 (339-609)	
Poor CD4 recovery on ART[2], *n* (%)	13	117 (4.0)	√
ALT, IU/L, median (IQR)	0	28 (20-43)	√
AST, IU/L, median (IQR)	0	26 (21-36)	√
Platelets, × 10^9 cells/L, median (IQR)	0	266 (221-312)	√
APRI, median (IQR)	0	0.29 (0.21-0.41)	√
Fatigue, myalgia/arthralgia, or anorexia/weight loss, *n* (%)	0	301 (9.9)	√
Diffuse pruritus, *n* (%)	0	120 (3.9)	√
Diabetes mellitus, *n* (%)	6	113 (3.7)	√
Hepatitis B surface antigen positive, *n* (%)	0	311 (10.2)	
Partner or household member with liver disease, *n* (%)	10	185 (6.1)	√

[1]homosexual, history or current injecting drug user, or history or currently engaged in sex work.
[2]CD4 below200 after 3 years or more on ART.
ART: Antiretroviral therapy; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: AST-to-platelet ratio index; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

For the primarily laboratory test based model, four predictors were retained in the final model (age ≥ 50 years: + 1 point, APRI ≥ 0.45: + 1, APRI < 0.45: - 1, platelets < 200 10^9/L: + 1, AST < 40 IU/L: -1). Gender and ALT were dropped. The AUROC of the lab CPS showed good discrimination of HCV/HIV coinfection and HIV mono-infection, and was 0.83 (95%CI: 0.79-0.87). The best-fit cut-off for the test-threshold of ≥ 1% predicted probability was a lab CPS score ≥ 0. Applying this cut-off, 22 HCV coinfections would have been missed, including two with advanced fibrosis. The NNT was 9.5, as 800 persons would have been prioritized for testing, to identify 84 coinfections.

DISCUSSION

We developed (and internally validated) a clinical prediction score to risk-stratify, primarily heterosexually-infected HIV patients for HCV coinfection, for use as first step in the identification of HIV patients to be prioritized for HCV testing when resources are insufficient to test all.

The risk score uses elements from history taking, physical examination and laboratory test results which are readily available or easily obtainable in most HIV programs, and are a combination of age, an exposure-related factor (partner/household member with liver disease) and variables related to severity of liver disease. Its overall performance in the derivation cohort in terms of discriminating HCV/HIV coinfected and HIV mono-infected was good (AUROC 0.84, 95%CI: 0.80-0.89), and we

Table 3 Crude and adjusted likelihood ratios of the candidate predictors for hepatitis C virus coinfection

Predictor variables after dichotomization	Number of HIV patients	Outcome events, *n* (%)	Crude likelihood ratios (LHR)		Adjusted likelihood ratios (aLHR)	
			Positive LHR	Negative LHR	Positive aLHR	Negative aLHR
Male gender	1307	45 (3.4)	0.99	1.01	-	-
Age ≥ 50 years	601	45 (7.5)	2.55	0.71	**2.18**	0.72
Platelets < 200 × 10^9 cells/L	442	49 (11.1)	3.46	0.62	**1.69**	0.82
AST ≥ 30 IU/L	1190	88 (7.4)	2.21	0.28	1.48	**0.53**
ALT ≥ 40 IU/L	887	69 (7.8)	2.33	0.49	-	-
APRI ≥ 0.45	633	78 (12.3)	3.88	0.33	**2.42**	**0.48**
Having diabetes	113	13 (11.5)	3.76	0.90	**2.14**	0.94
Presenting fatigue OR myalgia/arthralgia OR anorexia/weight loss	301	21 (7.0)	2.11	0.88	-	-
Generalized pruritus	120	10 (8.3)	2.61	0.94	**2.04**	0.95
Having a partner OR household member with liver disease	185	10 (10.3)	3.21	0.87	**3.62**	0.85
Poor CD4 recovery on ART	117	5 (4.3)	1.34	0.99	-	-

In **bold** the adjusted likelihood ratios ≥ 1.5 or ≤ 0.67. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; APRI: AST-to-platelet ratio index; ART: Antiretroviral therapy; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

Table 4 Predictors and their weight in the clinical prediction score

Predictor	Score
Age ≥ 50 yr	+1
Having diabetes mellitus	+1
Having a partner and/or household member with liver disease	+1
Presenting generalized pruritus	+1
Platelets < 200 × 10^9 cells/L	+1
APRI ≥ 0.45	+1
APRI < 0.45	-1
AST < 30 IU/L	-1
Possible range of the score	- 2 to + 6

APRI: AST-to-platelet ratio index; AST: Aspartate aminotransferase.

were able to derive a clinically useful decision rule for HCV testing prioritization along our pre-set requirements (test-threshold at ≥ 1% predicted probability of HCV coinfection, and substantially decrease the number needed to test (NNT)). In our study population, not testing those with predicted probability < 1% would have decreased the NNT from 29 to 10, while missing 15% of the HCV/HIV coinfections, and thus outperforming birth cohort testing[7]. If externally validated, our score and decision rule may thus be a practical way forward for countries not able or not opting to fully implement the WHO recommendation to test all HIV patients for hepatitis C[4]. Resource-constrained countries carry the largest burden of HCV/HIV coinfection.

With this paper, we do not intend to advocate in a general manner for targeted HCV testing in all HIV populations. We agree with the WHO guidelines that HIV populations are a convenient population sub-group to be targeted as a whole, as they often have a higher HCV prevalence than the general population, and are easy to reach [4,26]. 'Testing all repeatedly for HCV, accompanied by appropriate preventive

Table 5 Diagnostic accuracy at different cut-offs of the clinical prediction score

Cut-off	HIV patients, *n* (%)	Sensitivity, % (95%CI)	Specificity, % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)
Score ≥ -1	1871 (61.4)	93.4 (86.9-97.3)	39.7 (37.9-41.5)	5.3 (4.3-6.4)	99.4 (98.8-99.8)
Score ≥ 0	**926 (30.0)**	**84.9 (76.6-91.1)**	**71.6 (69.9-73.2)**	**9.7 (7.9-11.8)**	**99.2 (98.8-99.6)**
Score ≥ 1	670 (22.0)	74.5 (65.1-82.5)	79.9 (78.4-81.3)	11.8 (9.5-14.5)	98.9 (98.4-99.2)
Score ≥ 2	325 (10.7)	59.4 (49.5-68.9)	91.1 (90.0-92.1)	19.4 (15.2-24.1)	98.4 (97.9-98.9)
Score ≥ 3	103 (3.4)	33.0 (24.2-42.8)	97.7 (97.1-98.2)	34 (24.9-44.0)	97.6 (97.0-98.1)
Score ≥ 4	18 (0.6)	10.4 (5.3-17.8)	99.8 (99.5-99.9)	61.1 (35.7-82.7)	96.9 (96.2-97.5)
Score ≥ 5	4 (0.1)	2.8 (0.6-8.1)	99.97 (99.8-100)	75 (19.4-99.4)	96.6 (95.9-97.2)

In **bold** the diagnostic accuracy results (number of HIV patients, sensitivity, specificity, PPV, and NPV) for the cut-off at score ≥ 0. This is the cut-off best fitting as threshold-to-test. PPV: Positive predictive value; NPV: Negative predictive value; HIV: Human immunodeficiency virus.

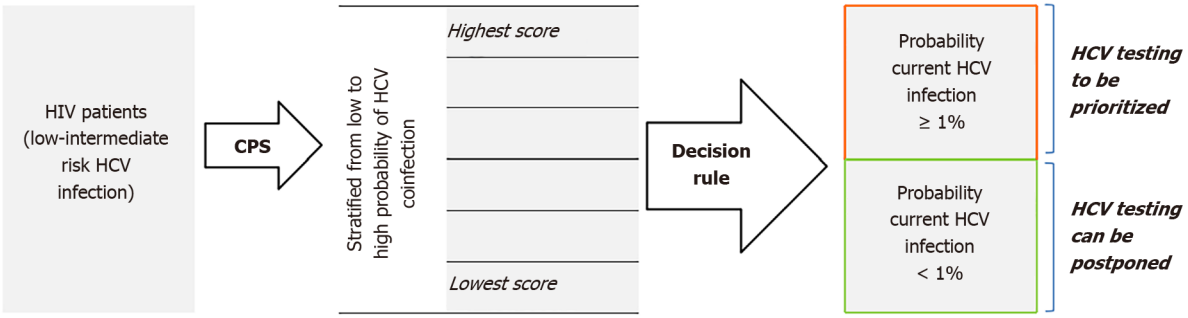
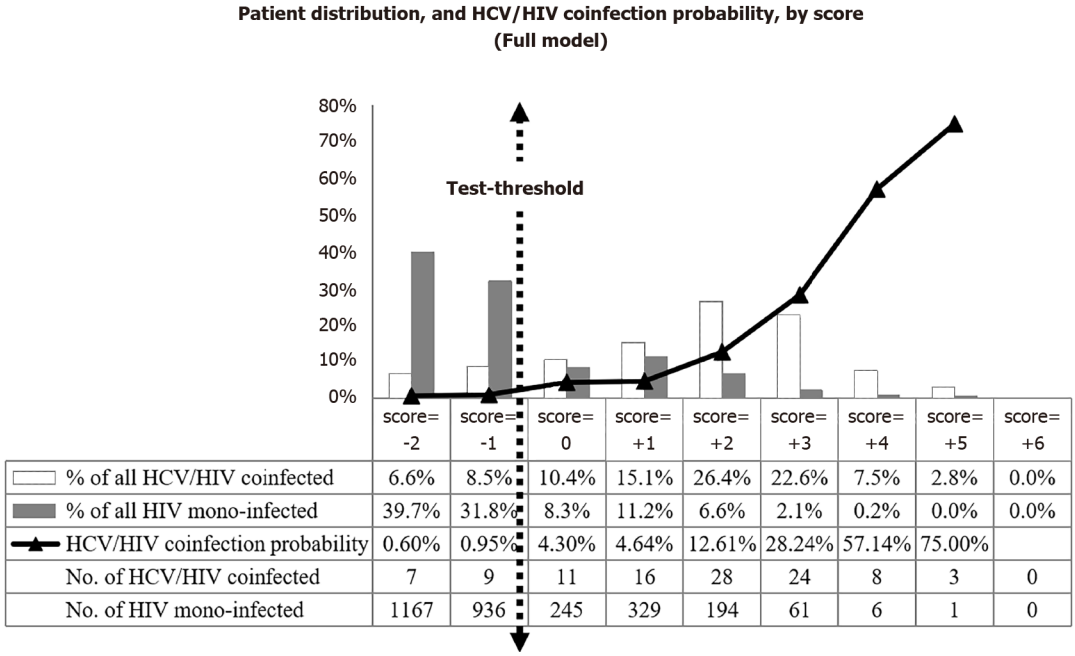


Figure 1 Threshold for the decision rule for targeted hepatitis C virus testing. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; CPS: Clinical prediction score.

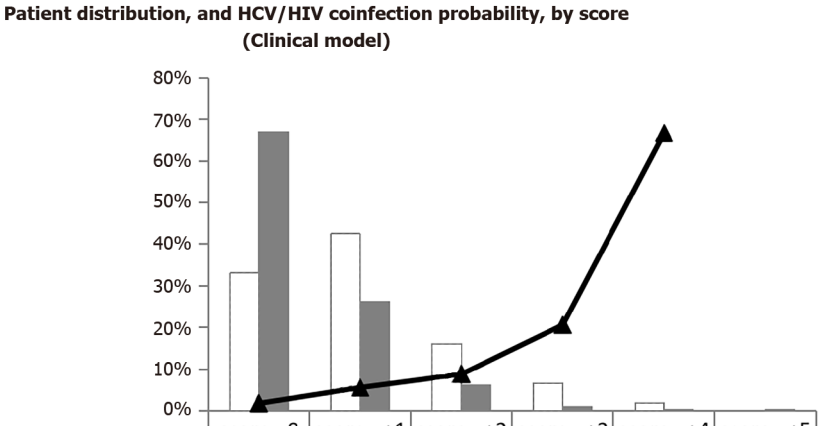
counselling' should be aimed for whenever feasible as part of a comprehensive package of care for people living with HIV (including timely initiation of ART and treatment of comorbidities as HCV), especially as nearly 100% curative HCV treatment options are now available. However, lack of resources, and low in-country HCV coinfection prevalence in large HIV cohorts with little ongoing transmission risk, are valid contextual arguments that countries may use to opt differently[8-10,27]. As also the argument that HIV coinfection leads to faster HCV disease progression (and therefore priority) has become debatable in the early ART era[8-10,27,28], some countries may indeed opt for a more restricted HCV testing approach combined with early initiation of ART. Anticipating this, it seemed to us timely to develop this score for targeted HCV testing.

The study and the resulting risk score have a number of strengths. The study was conducted and reported in accordance with the methodological standards for development of clinical prediction rules, as outlined in the TRIPOD statement and detailed in the S1 TRIPOD checklist[29]. Data collection was done prospectively, and blinded from the HCV diagnostic results. Missing data were rare. The model was built following the Spiegelhalter Knill-Jones (SKJ) approach, a statistical method that combines elements of the Bayes theorem and logistic regression. While combining, it also sidesteps disadvantages of both conventional methods (*i.e.*, the Bayes' assumption of independence of predictors; and the mathematical, user-unfriendly output of logistic regression). SKJ allows and adjusts for dependency between predictors, and provides output in adjusted LHRs which are more easily understood and interpreted by clinicians[22,23,30]. The model we developed is clinically sensible as all predictors retained in the final score are plausibly related to infection risk (older age and having a household member/partner) or severity of liver disease (increased APRI, low platelets, diabetes, generalized pruritus without skin abnormalities)[7,31,32]. This, as well as the fact that the score can be repeated at regular intervals and that initially missed cases can be picked up later, may favor acceptability by clinicians. The score has a good discriminative ability and performed particularly well to identify a large subgroup of HIV patients that can be considered as a very low-risk group for HCV coinfection



	score= -2	score= -1	score= 0	score= +1	score= +2	score= +3	score= +4	score= +5	score= +6
% of all HCV/HIV coinfected	6.6%	8.5%	10.4%	15.1%	26.4%	22.6%	7.5%	2.8%	0.0%
% of all HIV mono-infected	39.7%	31.8%	8.3%	11.2%	6.6%	2.1%	0.2%	0.0%	0.0%
HCV/HIV coinfection probability	0.60%	0.95%	4.30%	4.64%	12.61%	28.24%	57.14%	75.00%	
No. of HCV/HIV coinfected	7	9	11	16	28	24	8	3	0
No. of HIV mono-infected	1167	936	245	329	194	61	6	1	0

Figure 2 Patient distribution by coinfection status, and probability of hepatitis C virus coinfection by score of the full clinical prediction score. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.



	score= 0	score= +1	score= +2	score= +3	score= +4	score= +5
% of all HCV/HIV coinfected	33.0%	42.5%	16.0%	6.6%	1.9%	0.0%
% of all HIV mono-infected	66.9%	26.1%	6.0%	0.9%	0.0%	0.0%
HCV/HIV coinfection probability	1.75%	5.54%	8.76%	20.59%	66.67%	
No. of HCV/HIV coinfected	35	45	17	7	2	0
No. of HIV mono-infected	1965	768	177	27	1	1

Figure 3 Patient distribution by coinfection status, and probability of hepatitis C virus coinfection by score of the clinical prediction score. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

(probability < 1%). From a program perspective, this opens perspectives of substantial optimization of resource utilization for HCV testing.

There are also several limitations. It is a model development study, with internal validation to correct for over-optimism by bootstrapping, but no external validation was done yet. Further validation in different settings will thus be crucial before decisions on generalizability can be taken[33]. Inherent to the score building method used (Spiegelhalter Knill-Jones), continuous variables had to be categorized. This may have led to information loss[34,35]. The SKJ method adjusts for dependency between predictors (confounding), but in a more restricted manner than the conventional logistic regression. Each result (present or absent) of a particular predictor/test is being shrunk to the same degree[30]. Taking into consideration these potential weaknesses, we used our dataset to compare the performance of logistic regression,

CART and SKJ to predict HCV/HIV coinfection. Logistic regression missed less HCV coinfections, but would refer 98% of HIV patients for HCV testing. The SKJ method had the highest area under the ROC curve and missed less coinfections than CART. CART delivered a better positive predictive value[36]. Another potential weakness of the score is its dependence on some lab tests (mainly transaminases). Though we aimed to use information which is readily available or easily obtainable in HIV programs, these lab tests might not be done regularly anymore in some programs. The clinical score (without lab tests) did unfortunately not perform well. On the other hand, the alternative score without clinical variables did perform reasonably well, and can, if validated, be a handy alternative in certain HIV programs. Routine electronic HIV databases containing these variables could flag patients to be prioritized for HCV testing without any need for further data collection by the clinician.

To further improve cost-effectiveness of HCV testing, the potential of the risk score to identify subgroups best to be tested with the classical two-step algorithm (HCV antibody test followed by HCV-RNA testing), or one-step test procedure (HCV-RNA) could also be further explored.

CONCLUSION

We successfully developed and internally validated a practical score, based on readily available clinical data, to risk-stratify HIV patients for HCV coinfection. In our setting, a large cohort of primarily heterosexually-infected Cambodian HIV patients, the score has shown promising potential to substantially reduce the number needed to test (to 30% of the cohort) without compromising access to testing and treatment for HIV patients with advanced HCV disease, especially as this score can be repeated regularly. Confirmation of these promising findings through external validation is required before its use in other low-risk HIV cohorts (*i.e.*, with few MSM or injecting drug users) in settings with limited resources can be considered.

ARTICLE HIGHLIGHTS

Research background

The advent of direct-acting antivirals has revolutionized hepatitis C (HCV) treatment and has generated interest in the global elimination of hepatitis C as a public health problem. To allow timely scale up of treatment, efficient HCV testing strategies are crucial. By the end of 2017, only about 20% of those living with hepatitis C knew their status, with significantly lower proportions in low and middle income countries (LMIC).

Research motivation

In the absence of funding initiatives dedicated to viral hepatitis, it is expected to remain difficult for LMIC to offer broad access to HCV testing. Depending on local resources and epidemiology, offering targeted HCV screening might be a more feasible option. However, easy-to-use tools to guide such targeted HCV testing, other than prioritization of key populations or older birth cohorts, do not exist.

Research objectives

To develop and internally validate a clinical prediction score for targeted HCV screening combining age and factors linked to liver disease severity, aiming to identify most of the chronic hepatitis C patients in low-risk human immunodeficiency virus (HIV) populations, but especially those in more urgent need of treatment.

Research methods

Score development relied on the Spiegelhalter and Knill-Jones method which was applied on a cross-sectional dataset from a large HIV cohort in Phnom Penh, Cambodia. Predictors independently associated with current HCV infection (HCV RNA detected) with likelihood ratio ≥ 1.5 or ≤ 0.67 were retained in the score. Performance of the score was estimated by the area-under-the-ROC curve and diagnostic accuracy at the different cut-offs. For the decision rule, HCV coinfection probability ≥ 1% was agreed as test-threshold.

Research results

We developed (and internally validated) a clinical prediction score to risk-stratify, primarily heterosexually-infected HIV patients for HCV coinfection, for use as first step in the identification of HIV patients to be prioritized for HCV testing when resources are insufficient to test all. The risk score uses elements from history taking, physical examination and laboratory test results which are readily available or easily obtainable in most HIV programs. In the Cambodian derivation cohort, the score would have enabled identifying 85% of the coinfected while reducing the need for testing by 70%. At the best-fitting threshold-to-screen (score ≥ 0), a negative predictive value of 99.2% was obtained, and no cases with advanced fibrosis were missed.

Research conclusions

The score for targeted HCV screening performed well in the derivation cohort and bears potential to substantially reduce the number needed to test without compromising access to testing and treatment for HIV patients with advanced HCV disease. Confirmation of these promising findings through external validation is required before recommendations on wider use can be made.

Research perspectives

The validity of the score should be tested in other HIV cohorts with low onward risk of transmission, starting from similar HIV cohorts in Cambodia but also in HIV populations in other settings.

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ORIGINAL ARTICLE

Retrospective Study

Elevated liver enzymes portends a higher rate of complication and death in SARS-CoV-2

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Author contributions: Currier EE and Jafri SM designed the research study and performed the research; Currier EE, Jafri SM, and Dabaja M analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

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Abstract

BACKGROUND

Severe acute respiratory syndrome coronavirus 2, or coronavirus disease-2019 (COVID-19), has infected millions worldwide since its discovery in Wuhan, China in December 2019, but little is still known about the disease process. Preliminary research in China notes liver function tests (LFTs) abnormalities are common in COVID-19 patients, suggesting decreased hepatic function, and that abnormalities in LFTs are related to complicated disease course and negative outcomes. However, there has been limited large-scale data assessing COVID-19's association with liver dysfunction and negative outcomes.

AIM

To investigate how COVID-19 affects the liver function and disease course in patients infected with the virus treated at Henry Ford Hospital from March to September 2020.

METHODS

A total of 8028 patients infected with COVID-19 were identified and included in the study at a single academic center. Data from medical charts on laboratory testing including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and bilirubin levels, past history of liver disease, and disease course indicators including hospital admission, intensive care unit (ICU) admission, intubation, and death were recorded and analyzed. Elevated liver enzymes were defined as ALT/AST greater than 60, AP greater than 150, or bilirubin greater than 1.5, super-elevated liver enzymes were defined as ALT/AST greater than 120, AP greater than 300, or bilirubin greater than 3.0.

RESULTS

A total of 8028 COVID-19 patients were identified and included in the study. Data from medical charts on LFTs (namely, AST, ALT, AP, and bilirubin levels), past

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history of liver disease, and disease course indicators (hospital/ICU admission, intubation, death) were recorded and analyzed. LFTs from 3937 patients were available for interpretation. 45% were found to have elevated or super-elevated LFT. When compared to COVID-19 patients without elevated LFTs, this cohort was found to have significantly higher odds of hospital admittance, ICU admission, intubation, and death (all $P < 0.001$). 248 (3.1%) had a history of liver disease. Those with elevated and super elevated LFTS had significantly higher odds of having a past history of liver disease ($P < 0.001$).

CONCLUSION

The findings from this study suggest that in patients who have tested positive for COVID-19, those with elevated and super elevated liver enzyme levels have significantly higher odds of hospital admittance, ICU admittance, intubation and death in comparison to those COVID-19 patients without elevated liver enzyme levels.

Key Words: COVID-19; Hepatology; Liver damage; Complications; Elevated liver function test

Core Tip: This study suggests that in coronavirus disease-2019 (COVID-19) positive patients, those with elevated and super elevated liver function tests (LFTs) have significantly higher odds of hospital admittance, intensive care unit admittance, intubation, and death in comparison to those COVID-19 patients without elevated LFTs (all $P < 0.001$). LFT elevations may serve as an indicator for medical professionals in the treatment of COVID-19 patients and may allow for proactive treatment of those patients at increased risk of complications.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), or coronavirus disease-2019 (COVID-19), was first reported in Wuhan, China in December 2019, and as of March 2020, the World Health Organization declared COVID-19 a global pandemic[1]. While millions of people have been infected and have died from COVID-19 worldwide, still much is unknown about COVID-19's disease process and the systemic effects of the disease[1]. However, preliminary research on COVID-19 shows that the disease may have a significant impact on the gastrointestinal and hepatic systems.

Early studies have shown that gastrointestinal (GI) symptoms are common in COVID-19 patients and symptoms such as nausea, diarrhea, *etc*, are present in approximately 10% of cases[2,3]. It has been noted that liver function test (LFT) abnormalities are common, however, the incidence has ranged widely from preliminary data, from 14.8% to 78%[2-5]. Abnormal LFTs, namely increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, and alkaline phosphatase (AP), have been reported, which indicates decreased hepatic functions[2-11]. Thus, these noted LFT abnormalities in COVID-19 patients suggest that COVID-19 may negatively impact liver function[4-6,8]. Furthermore, three meta-analyses have both shown that patients presenting with abnormal LFTs had a significant association with an increased risk of complication risk course [*i.e.* intensive care unit (ICU) admission, intubation, death][2,8,10]. Little is still known about the impact of pre-existing hepatic conditions on COVID-19 outcomes (*i.e.* cirrhosis, post-liver transplant, *etc*)[4].

The current hypothesis behind COVID-19's involvement of the hepatic system is multifactorial liver damage, secondary to systemic inflammatory response syndrome, hypoxia-reperfusion injury, cytokine-storm induced damage, drug-induced liver damage, sepsis-mediated damage, and/or multiorgan failure[2,4,5,11,12]. However, little is known about the mechanism behind hepatic damage.

The current available research is limited in that almost all of the data was obtained from China, as few studies, especially large-scale studies, outside China have been published[2,3,10]. Furthermore, most of the current research published is limited in the study sample sizes, leading to current meta-analyses receiving data from a large number of hospitals. In these studies patients were held to different clinical cutoffs when advancing medical interventions, which could have negatively impacted the accuracy of the data and determination of the significance of abnormal LFTs and its impact on disease complications. To date, there has been no published large-scale research investigating the relationship between COVID-19 patient's LFTs and their relationship to a complicated disease course in the United States. Additionally, epidemiologic studies of COVID-19's impact have shown that Black and minority populations are disproportionally represented in the number of cases, complications, and deaths due to the virus[13,14]. While this is postulated to be due to increased incidence of comorbidities, increase odds of living in high-density areas, and lack of access to healthcare, more studies with populations that reflect demographics of COVID infection are needed to assess COVID-19's association with liver dysfunction across a diverse population[15].

The significance of this research is to investigate how SARS-CoV-2/COVID-19 affects liver function and disease course in patients infected with the virus treated at Henry Ford Hospital from March to September 2020. As studies have linked liver dysfunction with severe disease and negative disease outcomes, it is important to confirm the preliminary research currently available. If COVID-19 is continued to be linked to liver dysfunction, this information can help clinicians determine the level of care patients need and proactively treat potentially complicated disease processes.

We hypothesize that COVID-19 patients with elevations in LFTs will have higher chances of a complicated and severe disease process.

MATERIALS AND METHODS

With approval from the institutional review board at Henry Ford Health System (HFHS), the study used the medical records of patients treated at HFHS to identify patients who tested positive for COVID-19. Medical records from individuals who had tested positive from the beginning of the pandemic until September 2020 were isolated and included in the study. No individuals were excluded from the study. For this type of study formal consent is not required.

After isolating the patient population, all records of liver enzyme levels (AST, ALT, AP, bilirubin), medical history of liver disease (defined as medical documentation of alcoholic liver disease, toxic liver disease, hepatic failure, hepatitis, inflammatory liver disease, hepatic fibrosis, liver transplant, and other liver diseases- not elsewhere classified), and complicated disease course (designated by a hospital admission, ICU admission, intubation, and death) were recorded. Individuals with a past medical history of liver disease were screened through retrospective chart review and identified by a prior diagnosis of one of the above conditions; details on disease severity, length, *etc* were not recorded. However, those with history of liver disease were separated into another cohort due to the possibility of liver enzyme elevation secondary to liver disease and not the COVID-19 disease process.

Descriptive statistics of demographic variables and hospital-related outcomes are provided. Continuous data are reported as mean ± SD, while categorical data are reported as counts and column percentages [*n* (%)]. Prevalence rates for elevated and super elevated liver enzymes are computed using binary indicator variables. Logistic regression is used to calculate odds ratios and their 95%CIs for the outcomes of interest. Statistical significance is set at $P < 0.05$. All analyses are performed using SAS 9.4 (SAS Institute Inc., Cary, NC, United States).

RESULTS

There is a total of 8028 unique patient medical record numbers used in this descriptive analysis. Table 1 displays the descriptive statistics of these patients. Of the 8028

Table 1 Patient demographics

Variable	Response	All patients (*n* = 8028)
Sex	Female	4638 (58%)
	Male	3389 (42%)
	Unknown	1 (0%)
Race	Black	4268 (53%)
	Other	1219 (15%)
	White	2541 (32%)
Hispanic	No	6921 (86%)
	Unknown	768 (10%)
	Yes	339 (4%)

patients included, 4638 (57%) are female, 3389 (42%) are male, and 1 (0%) is unknown. Additionally, 4268 (53%) are Black, 2541 (32%) are White, and 1219 (15%) are another race; 6921 (86%) are not Hispanic, 339 (4%) are Hispanic, and 768 (10%) are unknown. Patients were classified by Hispanic *vs* non-Hispanic to identify those who are Central or South American/Latino who are considered "White" on this hospital's demographic information but are of Hispanic descent.

Binary indicator variables for history of liver disease, death, hospital admission, ICU admission, and intubation were created. Table 2 displays the counts, percentages, and 95%CIs for these hospital-related outcomes. ICU admission and intubation are recorded for only those patients who were admitted to the hospital, noted by the change of *n*. Of the 8028 patients, 245 (3.1%) had a history of liver disease, 673 (8.4%) died, and 5199 (64.8%) were admitted to the hospital. Of the 5199 admitted to the hospital, 807 (15.5%) were admitted to the ICU, and 637 (12.3%) were intubated.

Table 2 displays the descriptive statistics for elevated liver enzymes. There was a total of 115846 lab values from 3937 patients. When we assessed elevated liver enzymes, we looked at this at the patient level – if they have ever had elevated liver enzymes. Binary indicator variables were created for ever having any elevated liver enzyme, and this was further broken down by specific enzymes (AST, ALT, AP, and bilirubin). Elevated liver enzymes are defined as an AST greater than 60, ALT greater than 60, AP greater than 150, or a bilirubin greater than 1.5.

There are 1722 patients who had elevated liver enzymes, 2114 who never had an elevated liver enzyme, and 101 patients who were indeterminable. Approximately 45% of patients had an elevated liver enzyme level, 34% of patients had an elevated AST, 27% of patients had an elevated ALT, 10% of patients had an elevated AP, and 12% had an elevated bilirubin.

In Table 2, we looked at super elevated liver enzyme levels, which is double the elevated threshold (AST greater than 120, ALT greater than 120, AP greater than 300, or a bilirubin greater than 3). There were 714 patients who had super elevated liver enzymes, 3116 who never had super elevated liver enzymes, and 107 patients who were indeterminable. Approximately 19% of patients had a super elevated enzyme level, 12% with AST, 12% with ALT, 2% with AP, and 3% with bilirubin.

Lastly, Figure 1 displays the logistic regression models examining the effect of elevated and super elevated liver enzymes on each outcome. Presence of elevated liver enzymes and super elevated liver enzymes are associated with increased odds of liver disease, hospital admittance, death, intubation and ICU admittance (all $P < 0.001$).

DISCUSSION

The findings from this study suggest that in patients with a positive COVID-19 test, those who have elevated and super elevated liver enzyme levels have significantly higher odds of hospital admittance, ICU admittance, intubation and death in comparison to those COVID-19 patients without elevated liver enzyme levels. While little is known about COVID-19's effect on organ systems during infection and recovery, the link between elevated LFTs and poor outcomes is important and suggests that COVID-19 negatively impacts liver function; this is also consistent with

Table 2 Hospital outcomes and elevated liver enzyme prevalence rates

Outcome	Count (%) (95%CI)
	***n* = 8028**
History of liver disease	245 (3.1) (2.7, 3.5)
Death	673 (8.4) (7.8, 9.0)
Hospital admission	5199 (64.8) (63.7, 65.8)
Outcome	Count (%) (95%CI)
	n = 5199
ICU admit	807 (15.5) (14.6, 16.5)
Intubation	637 (12.3) (11.4, 13.2)
Outcome	Count (%) (95%CI)
Any elevated liver enzyme	1722 (44.9) (43.3, 46.5)
Elevated AST	1297 (33.5) (32.0, 35.0)
Elevated ALT	1052 (26.7) (25.4, 28.2)
Elevated AP	392 (10.1) (9.2, 11.1)
Elevated bilirubin	468 (12.0) (11.0, 13.1)
Any super elevated liver enzyme	714 (18.6) (17.4, 19.9)
Super elevated AST	480 (12.4) (11.4, 13.5)
Super elevated ALT	468 (11.9) (10.9, 13.0)
Super elevated AP	94 (2.4) (1.9, 3.0)

ICU: Intensive care unit; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AP: Alkaline phosphatase.

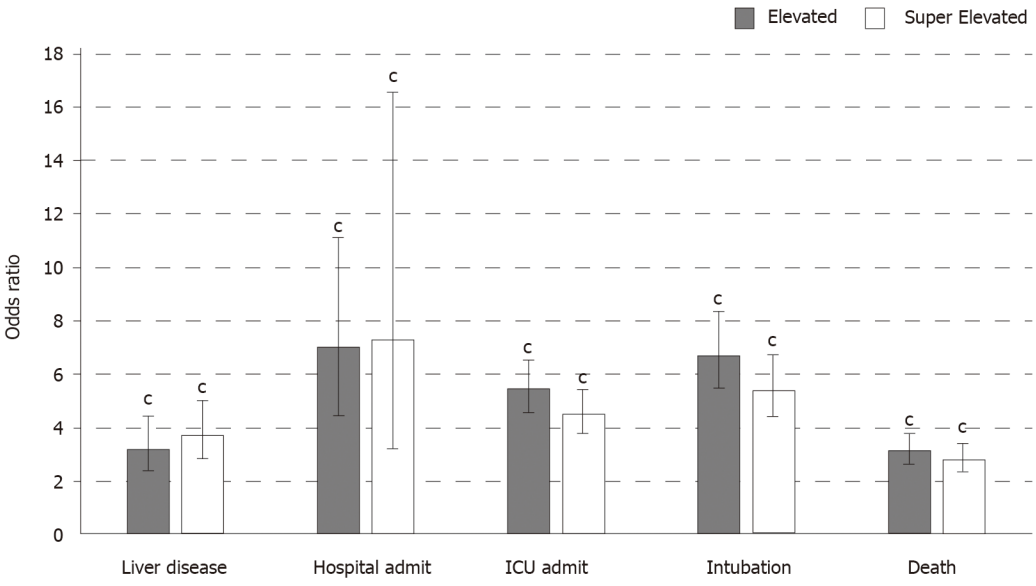

Figure 1 Logistic regression for elevated and super elevated liver enzyme levels with 95%CI. $^{c}P < 0.001$.

early data from other studies[2-11].

Of the 8028 patients identified in this study, LFTs from 3937 patients were available for statistical interpretation. Of this cohort, 45% were found to have elevated or super-elevated LFTs and when compared to COVID-19 patients without elevated LFTs, this cohort was found to have significantly higher odds of hospital admittance, ICU admission, intubation, and death (all $P < 0.001$). The data suggest that the risk of hospital admission and the necessity for more aggressive medical interventions (*i.e.*

intubation, ICU admission) are more common in those with elevated LFTs. Thus, elevations in LFTs may serve as an indicator for medical professionals in the preventative treatment of complicated COVID-19 patients. By identifying those patients who have poor liver function and are thus linked to a more complicated disease course, providers may be able to monitor, proactively treat patients at increased risk, and mitigate disease complications.

Interestingly, however, this data does not show that elevation in LFTs is linearly correlated with outcomes, as seen by the odds ratio of ICU admission, intubation, and death in patients with super elevated enzyme levels being less than those with elevated enzyme levels (Figure 1). The cause of this relationship is unknown; however, we hypothesize that those with super elevated liver enzymes may have been clinically identified as severe COVID-19 cases earlier and been treated more aggressively. Retrospective research has shown that those with LFT elevations at time of admission were more likely to receive aggressive mediation interventions than those with normal LFTs (58% compared to 31%)[15]. Therefore, this lack of linear relationship may be related to early clinical treatment of patients who present with LFT abnormalities, compared to those who develop elevations throughout their hospital stay or who have moderate elevations.

Little is still known about COVID-19's effect on liver function, however, the findings from this study indicating COVID's negative impact on liver function is consistent with the limited preliminary COVID studies in China on outcomes and predictive markers of disease[16]. As noted in the previous studies, abnormal LFTs are seen as predictive markers of a complicated disease process, thus indicating hepatic dysfunction. A weakness in previously available research is the homogeneity of the population studied, with most research being derived from almost solely Asian and South Asian populations. This study, however, consisted of 53% Black, 32% white, 15% other, and 4% Hispanic persons. Therefore, this cohort is more closely representative of the current demographics affected by COVID-19 in the United States, where Black people are more likely to be infected and die from COVID-19[17,18]. Thus, these findings suggest that the relationship between LFT elevations and disease complications is not limited to race and can be applied to populations outside of the Asian community and countries.

Of the 8028 patients identified in the study, 248 (3.1%) had a history of liver disease. Those with elevated and super elevated LFTs had significantly higher odds of having a past history of liver disease ($P < 0.001$). This is important as previous research on underlying liver disease and COVID-19 infection has been limited due to sparse data on persons with underlying liver disease[19]. This data indicates that LFT abnormalities are consistent with complicated disease process in those who have a history of liver dysfunction, as seen in those without liver disease. While it is unclear if LFT elevations were due to the effects of the COVID-19 disease process or is secondary to their underlying liver disease, we do hypothesize the COVID's negative impact on liver function exacerbates already lowered liver function in these patients, thus increasing their odds for complications.

This study does have several weaknesses. While over 8000 patients were treated for COVID-19 at the hospital, liver enzymes were only available for about half of those included in the study. This discrepancy may be due to a high number of ambulatory patients who were tested for COVID-19, but whose disease process was self-limited and did not require medical intervention beyond diagnoses and supportive care. Furthermore, this research did not investigate the medications patients in the study received and as some medications used to treat COVID-19 have been linked to elevation in LFTs, this may confound some of the elevations seen in this study[20]. Additionally, as the study was retrospective, there were a variable number of lab tests available to analyze for each patient (*i.e.* some had multiple LFTs available while others had a single test). Thus, some patients may have had high LFTs during the disease course, but this was not captured on the available lab results. In research going forward, an area for improvement would be to find consistent lab values to compare and limit the possibility of missed LFT fluctuations. In addition, capturing and assessing LFTs from ambulatory patients not requiring hospitalization.

CONCLUSION

In conclusion, abnormal liver biochemistry, namely AST, ALT, AP, and bilirubin, is very common in COVID-19 patients, noted in 45% of our patient population. Abnormal LFTs are closely linked to disease complications and the prognosis for

COVID-19 patients. These findings are consistent with other early research and support that COVID-19 is related to hepatic dysfunction. Importantly, as LFT elevation has been linked to severe disease outcomes, patients with elevations should be monitored closely and treated prophylactically to mitigate disease complications. Going forward, chronic effects of COVID-19 infection of hepatic function will be important to monitor as indicators of acute liver dysfunction is common in COVID-19 patients.

ARTICLE HIGHLIGHTS

Research background

Preliminary research on coronavirus disease-2019 (COVID-19) shows that the disease may have a significant impact on the gastrointestinal and hepatic systems. Namely, early research shows that liver function test (LFT) abnormalities are common, however, the incidence has ranged widely from preliminary data, from 14.8% to 78%. Furthermore, three meta-analyses have both shown that patients presenting with abnormal LFTs had a significant association with an increased risk of complication risk course [*i.e.* intensive care unit (ICU) admission, intubation, death], but there is currently limited single-site, large scale research on the link between LFT abnormalities and COVID outcomes.

Research motivation

The motivation of this research is to identify a link between LFT abnormalities and COVID-19 outcomes.

Research objectives

The objective of this research was to identify if there was a link between LFT elevation and outcomes in COVID-19 patients. This study did support the hypothesis that those with LFT abnormalities are at increased risk of complicated disease processes and death. Clinically, this is very important as LFT abnormalities may identify patients at risk for disease complications and may lead to early medical intervention.

Research methods

Of 8028 patients infected with COVID-19 were identified and included in the study at a single academic center. Data from medical charts on laboratory testing including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and bilirubin levels, past history of liver disease, and disease course indicators including hospital admission, ICU admission, intubation, and death were recorded and analyzed. Elevated liver enzymes were defined as ALT/AST greater than 60, AP greater than 150, or bilirubin greater than 1.5, super-elevated liver enzymes were defined as ALT/AST greater than 120, AP greater than 300, or bilirubin greater than 3.0.

Research results

Of 8028 COVID-19 patients were identified and included in the study. Data from medical charts on LFTs (namely, AST, ALT, AP, and bilirubin levels), past history of liver disease, and disease course indicators (hospital/ICU admission, intubation, death) were recorded and analyzed. LFTs from 3937 patients were available for interpretation. 45% were found to have elevated or super-elevated LFT. When compared to COVID-19 patients without elevated LFTs, this cohort was found to have significantly higher odds of hospital admittance, ICU admission, intubation, and death (all $P < 0.001$). 248 (3.1%) had a history of liver disease. Those with elevated and super elevated LFTS had significantly higher odds of having a past history of liver disease ($P < 0.001$).

Research conclusions

The findings from this study suggest that in patients who have tested positive for COVID-19, those with elevated and super elevated liver enzyme levels have significantly higher odds of hospital admittance, ICU admittance, intubation and death in comparison to those COVID-19 patients without elevated liver enzyme levels. While this research is unsure of the cause of this relationship, this research supports that LFT changes could serve as an indicator of COVID-19 outcomes and serve as a metric for evaluating those at risk for severe complications.

Research perspectives

In research going forward, an area for improvement would be to find consistent lab values to compare and limit the possibility of missed LFT fluctuations. In addition, capturing and assessing LFTs from ambulatory patients not requiring hospitalization would increase the validity of the link between LFTs and outcomes.

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META-ANALYSIS

Global prevalence of hepatitis B virus serological markers among healthcare workers: A systematic review and meta-analysis

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Abstract

BACKGROUND

The hepatitis B virus (HBV) infection is a global public health concern that affects about 2 billion people and causes 1 million people deaths yearly. HBV is a blood-borne disease and healthcare workers (HCWs) are a high-risk group because of occupational hazard to patients' blood. Different regions of the world show a highly variable proportion of HCWs infected and/or immunized against HBV. Global data on serologic markers of HBV infection and immunization in HCWs are very important to improve strategies for HBV control.

AIM

To determine the worldwide prevalence of HBV serological markers among HCWs.

METHODS

In this systematic review and meta–analyses, we searched PubMed and Excerpta Medica Database (Embase) to identify studies published between 1970 and 2019 on the prevalence of HBV serological markers in HCWs worldwide. We also manually searched for references of relevant articles. Four independent investigators selected studies and included those on the prevalence of each of the HBV serological markers including hepatitis B surface antigen (HBsAg), hepatitis e antigen (HBeAg), immunoglobulin M anti-HBc, and anti-HBs. Methodological quality of eligible studies was assessed and random-effect model meta-analysis resulted in the pooled prevalence of HBV serological markers HBV infection in HCWs. Heterogeneity (I^2) was assessed using the χ^2 test on Cochran's Q statistic and H parameters. Heterogeneity' sources were explored through subgroup and metaregression analyses. This study is registered with PROSPERO, number CRD42019137144.

RESULTS

We reviewed 14059 references, out of which 227 studies corresponding to 448 prevalence data among HCWs (224936 HCWs recruited from 1964 to 2019 in 71 countries) were included in this meta-analysis. The pooled seroprevalences of current HBsAg, current HBeAg, and acute HBV infection among HCWs were 2.3% [95% confidence interval (CI): 1.9-2.7], 0.2% (95%CI: 0.0-1.7), and 5.3% (95%CI: 1.4-11.2), respectively. The pooled seroprevalences of total immunity against HBV and immunity acquired by natural HBV infection in HCWs were 56.6% (95%CI: 48.7-63.4) and 9.2% (95%CI: 6.8-11.8), respectively. HBV infection was more prevalent in HCWs in low-income countries, particularly in Africa. The highest immunization rates against HBV in HCWs were recorded in urban areas and in high-income countries including Europe, the Eastern Mediterranean and the Western Pacific.

CONCLUSION

New strategies are needed to improve awareness, training, screening, vaccination, post-exposure management and treatment of HBV infection in HCWs, and particularly in low-income regions.

Key Words: Healthcare workers; Hepatitis B virus; Seroprevalence; Hepatitis B surface antigen; Hepatitis e antigen

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Core Tip: This study showed that healthcare workers (HCWs) are at an intermediate level (2%-8%) of hepatitis B virus (HBV) infection worldwide. The study also shows that globally, about half of HCWs are immune to HBV. Resource-limited areas with the lowest HBV immunization levels also have the highest HBV infection levels. To achieve the goal of HBV eradication by 2030, new strategies are needed to improve awareness, training, screening, vaccination, post-exposure management and treatment of HBV-infected HCWs, and especially in low-income regions.

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INTRODUCTION

Hepatitis B virus (HBV) is one of the main causes of liver disease. HBV infection remains asymptomatic in most infected people but also causes acute or chronic infections which can progress to liver failure, fulminant hepatitis, cirrhosis, hepatocellular carcinoma, and death[1-3]. Globally, hepatitis B is a major public health concern, with approximately a third of the world's population infected, including about 360 million chronic infections and 1 million deaths per year[4]. The HBV infection prevalence varies widely across World Health Organization (WHO) regions, with the African and Western Pacific regions bearing the highest burden (6.1% and 6.2% in the general population, respectively)[5,6].

HBV is transmitted parenterally through the blood and other body fluids of infected people. Several HBV transmission pathways have been identified, such as transmissions from mother to child, through unprotected sexual intercourse, during blood transfusions, *via* organ transplants, or through splashes and wounds caused by cuts and pricks of contaminated objects[7]. HBV, being a blood-borne pathogen, represents a significant occupational risk among healthcare workers (HCWs). The frequencies of infection in HCWs are up to 4-times greater than in individuals who do not work in hospitals[8-10]. Among the 35 million HCWs working globally, approximately 3 million each year have occupational exposure to HBV infection, leading to up to 66 thousand HBV infections (261 deaths)[9,11]. The chain of transmission of HBV is thus maintained from patients to HCWs and *vice versa* as well as to HCW relatives [12]. Vaccination against HBV is recommended in most countries for newborns and high-risk individuals, such as HCWs. Vaccination policies targeting HCWs vary widely according to geographic regions, including the absence of a policy, systematic vaccination, confirmation of vaccine protection, and adherence to maintenance of immunity[10,13-16].

According to high heterogeneity across regions regarding HBV routes of transmission, risk factors of infection, interventions for prevention and immunization among HCWs as well as clinical practice, the global epidemiology of HBV infection in HCWs need to be described. Understanding the seroprevalence, immunization rate, and risk factors for HBV infection in HCWs can provide useful information for decision-making and context-specific interventions to curtail the burden of disease of HBV infection. Therefore, the objective of this systematic review with meta-analysis was to determine the seroprevalence and factors associated with HBV infection and rate of HBV immunization in HCWs.

MATERIALS AND METHODS

Registration

This review was reported following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (Supplementary Table 1)[17]. The protocol for this review was registered in the International Prospective Register of Systematic Reviews (PROSPERO, No. CRD42019137144).

Eligibility criteria

This review included cross-sectional, case-control and cohort (baseline data) studies. Studies in English or French, without geographic restriction, were selected. We included studies using any assay for detecting serological markers of hepatitis B infection. This review considered the following different markers of HBV infection: anti-HBs > 10 IU/mL (total immunity against HBV); anti-HBs (+) and anti-HBc (+) (immunity due to natural infection); hepatitis B surface antigen (HBsAg) (+) and immunoglobulin (Ig) M anti-HBc (+) (acute hepatitis B infection); HBsAg (+) (current HBV infection); and hepatitis e antigen (HBeAg) (+) (current HBV infectivity)[18]. Studies for which the abstract or full text were not available, duplicates, comments, case reports, case series, and studies with less than 10 participants were excluded.

Data sources and search strategy

A search was conducted for articles published from 1970 to 2020 at PubMed and Excerpta Medica Database (Embase). The search terms were related to hepatitis B and HCWs (Supplementary Table 2). To supplement the bibliographic database searches and identify potential additional data sources, we scrutinized the reference list of all relevant articles.

Study selection and data extraction processes

Duplicates identified from the complete list of studies were removed. Titles and abstracts of articles retrieved from electronic literature search were independently screened by four investigators (Mahamat G, Kenmoe S, Ebogo-Belobo JT, and Amougou-Atsama M), and the full texts of those potentially eligible were obtained and further assessed for final inclusion. Data from the included studies was extracted using a Google form by 18 of the study's authors and verified by Kenmoe S. The extracted data were the name of the first author, year of publication, study design, country, country income level, sampling method, timing of data collection, study period, study participant age, male percentage, recruitment setting, HCW category, HBV detection assay, HBV detected markers (HBsAg, HBeAg, anti-HBs, and anti-HBc IgM and IgG), type of sample used for HBV detection, sample size, and number of HBV-positive for each marker. Disagreements observed during study selection and data extraction were resolved by discussion and consensus.

Quality assessment

The tool developed by Hoy and collaborators[19] for cross-sectional studies was used to assess the methodological quality of the included studies (Supplementary Table 3). Discussion and consensus were used to resolve disagreements.

Statistical analysis

The review included HCWs grouped according to WHO guidelines[20]. This classification includes the following as major categories: Health professionals; health associate professionals; personal care workers in health services; health management and support personnel; and other health service providers not classified elsewhere. Prevalence of pooled data was conducted using a random-effects meta-analysis with a Freeman-Tukey double arcsine transformation[21,22]. The I^2 (> 50%), H (> 1) parameters and the Q test P value (< 0.05) were used to indicate significant heterogeneity[21,23]. Subgroup and meta-regression analyses were used to determine sources of heterogeneity. Egger's test (P value < 0.1) and asymmetry of funnel plot were used to indicate publication bias and sensitivity analyses were performed on studies with low risk of bias and cross-sectional studies[24]. R version 3.6.2. statistical software was used to conduct all meta-analyses[25,26].

RESULTS

Study selection

The database search yielded a total of 14059 articles (Figure 1). After removing duplicates, 11575 articles were excluded due to irrelevant titles and abstracts. Of the 1190 articles fully screened 963 were excluded for multiple reasons (Supplementary Table 4). A total of 227 articles met the eligibility criteria. These 227 articles included corresponded to 448 seroprevalence data among HCWs (Supplementary Text 1).

Study characteristics

Most of the prevalence data were at moderate risk of bias (n = 279 prevalence data) (Supplementary Tables 6 and 7). Most of the participants were health professionals. Most prevalence data were reported in high (n = 176) and lower-middle (n = 125) income countries. Most of the prevalence data were from cross-sectional studies (n = 439) with non-probabilistic sampling methods (386), with prospective data collection and analysis (420), and in urban setting (212). The most widely used detection assay was direct ELISA (n = 126) for the detection of HBsAg (n = 292). Almost all the prevalence data reported serological markers of hepatitis in serum (n = 435).

Global seroprevalence of current HBV (HBsAg) infection among HCWs

The seroprevalence of current hepatitis B infection (HBsAg) was assessed in 275 seroprevalence data conducted in 62 countries (Figure 2 and Supplementary Figure 1). The overall seroprevalence of current hepatitis B infections (HBsAg) among HCWs was 2.3% [95% confidence interval (CI): 2.0-2.7].

Global seroprevalence of current HBV (HBeAg) infectivity among HCWs

The seroprevalence of current hepatitis B infectivity (HBeAg) positivity was assessed in three seroprevalence data conducted in three countries (Figure 2 and Supplementary Figure 2). The overall seroprevalence of current hepatitis B infections (HBeAg) among HCWs (HCWs) was 0.2% (95%CI: 0.0-1.7).

Global seroprevalence of acute HBV (IgM anti-HBs + HBsAg) infection among HCWs

The seroprevalence of acute VHB (IgM anti-HBs + HBsAg) infection was assessed in 12 seroprevalence data conducted in seven countries (Figure 2 and Supplementary Figure 3). The overall seroprevalence of acute hepatitis B infection in HCWs was 5.3% (95%CI: 1.4-11.2).

Global seroprevalence of total immunity (anti-HBs > 10 UI/mL) against HBV infection among HCWs

The seroprevalence of hepatitis B immunity (due to natural infection or vaccination) was assessed in 84 seroprevalence data conducted in 29 countries (Figure 2 and Supplementary Figure 4). The overall seroprevalence of total immunity against HBV among HCWs was 56.6% (95%CI: 48.7-63.4).

Global seroprevalence of immunity due to natural HBV infection (anti-HBS + anti-HBc) among HCWs

The seroprevalence of immunity against hepatitis B acquired through natural infection was assessed in 41 studies (57 seroprevalence data) conducted in 22 countries (Figure 2 and Supplementary Figure 5). The overall seroprevalence of immunity to hepatitis B acquired through natural infection among HCWs was 9.2% (95%CI: 6.8-11.8).

Heterogeneity and publication bias

The estimate of these seroprevalence data was associated with substantial heterogeneity current HBV infection (I^2 = 94.1%; 95%CI: 93.6-94.5), current HBV infectivity (I^2 = 92.3%; 95%CI: 80.7-96.9), HBV acute infection (I^2 = 97.9%; 95%CI: 96.9-98.5), total HBV immunity (I^2 = 99.5%; 95%CI: 99.5-99.6), and HBV immunity due to natural infection (I^2 = 96.9%; 95%CI: 96.4-97.3). Egger's test was significant (P < 0.001) for the seroprevalence of current HBV infection (HBsAg) among HCWs, suggesting the presence of publication bias (Table 1). Egger's tests were not significant for the seroprevalence in HCWs of current HBV infection due to HBeAg positivity (P = 0.577), acute HBV infection (P = 0.256), total immunity against hepatitis B (P = 0.509), and immunity due to natural infection (P = 0.853), suggesting the absence of publication bias. Funnel plots confirmed the results of publication bias obtained by Egger's test

Table 1 Summary of meta-analysis results for global seroprevalence of hepatitis B virus serological markers in healthcare workers

	Prevalence % (95%CI)	95% Prediction interval	Studies, *n*	Participants, *n*	[1]*H* (95%CI)	[2]I^2 (95%CI)	*P* value (heterogeneity)	*P* value (Egger test)
Current HBV infection (HBsAg)								
Overall	2.4 (2-2.8)	0-11	275	153326	4.1 (4-4.3)	94.1 (93.6-94.5)	< 0.001	< 0.001
Cross-sectional	2.4 (2-2.9)	0-11.1	271	150516	4.1 (4-4.3)	94.2 (93.7-94.6)	< 0.001	< 0.001
Low risk of bias	1.8 (1.4-2.3)	0-8.2	107	40212	3 (2.8-3.2)	88.8 (87-90.3)	< 0.001	< 0.001
Current HBV infection (HBeAg)								
Overall	0.3 (0-1.7)	0-70.6	3	4408	3.6 (2.3-5.7)	92.3 (80.7-96.9)	< 0.001	0.577
Cross-sectional	0.3 (0-1.7)	0-70.6	3	4408	3.6 (2.3-5.7)	92.3 (80.7-96.9)	< 0.001	0.577
Low risk of bias	0 (0-0.1)	NA-NA	1	3513	NA (NA-NA)	NA (NA-NA)	1	NA
HBV acute infection								
Overall	5.4 (1.4-11.3)	0-37	12	3665	6.1 (5.3-7.1)	97.3 (96.4-98)	< 0.001	0.256
Cross-sectional	5.4 (1.4-11.3)	0-37	12	3665	6.1 (5.3-7.1)	97.3 (96.4-98)	< 0.001	0.256
Low risk of bias	1.9 (0-8.7)	0-48.1	5	1639	6.5 (5.1-8.2)	97.6 (96.2-98.5)	< 0.001	0.824
Immunity against HBV								
Overall	56.6 (49.3-63.7)	2.8-100	84	37622	14 (13.5-14.4)	99.5 (99.5-99.5)	< 0.001	0.763
Cross-sectional	56.3 (48.8-63.7)	2.4-100	80	36311	14.2 (13.8-14.7)	99.5 (99.5-99.5)	< 0.001	0.811
Low risk of bias	65.9 (56.1-75.1)	10.3-100	35	22401	14.7 (14.1-15.4)	99.5 (99.5-99.6)	< 0.001	0.974
Immunity due to natural HBV infection								
Overall	9.2 (6.9-11.8)	0-34.5	57	23002	6.3 (5.9-6.7)	97.4 (97.1-97.8)	< 0.001	0.853
Cross-sectional	9.2 (6.9-11.9)	0-34.6	56	22867	6.3 (5.9-6.7)	97.5 (97.1-97.8)	< 0.001	0.851
Low risk of bias	7 (4-10.8)	0-30.3	20	10408	6.4 (5.7-7.1)	97.6 (97-98)	< 0.001	0.463

[1]*H* is a measure of the extent of heterogeneity, a value of H =1 indicates homogeneity of effects and a value of H > 1 indicates a potential heterogeneity of effects.
[2]I^2 describes the proportion of total variation in study estimates that is due to heterogeneity, a value > 50% indicates presence of heterogeneity.
HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis e antigen; CI: Confidence interval; NA: Not available.

(Supplementary Figures 6-10).

Subgroup analyses and metaregression

Subgroup analysis of current HBV infection in HCWs showed that seroprevalence was higher in cross-sectional studies, low-income countries, WHO Africa region, health management and support personnel, and personal care workers in health services (Figure 3 and Supplementary Table 8). Subgroup analysis of acute HBV infection in HCWs showed that seroprevalence was higher in non-probabilistic studies, prospective studies, upper-middle-income countries, the WHO South-East Asia region, urban areas and health associate professionals. Subgroup analysis of total

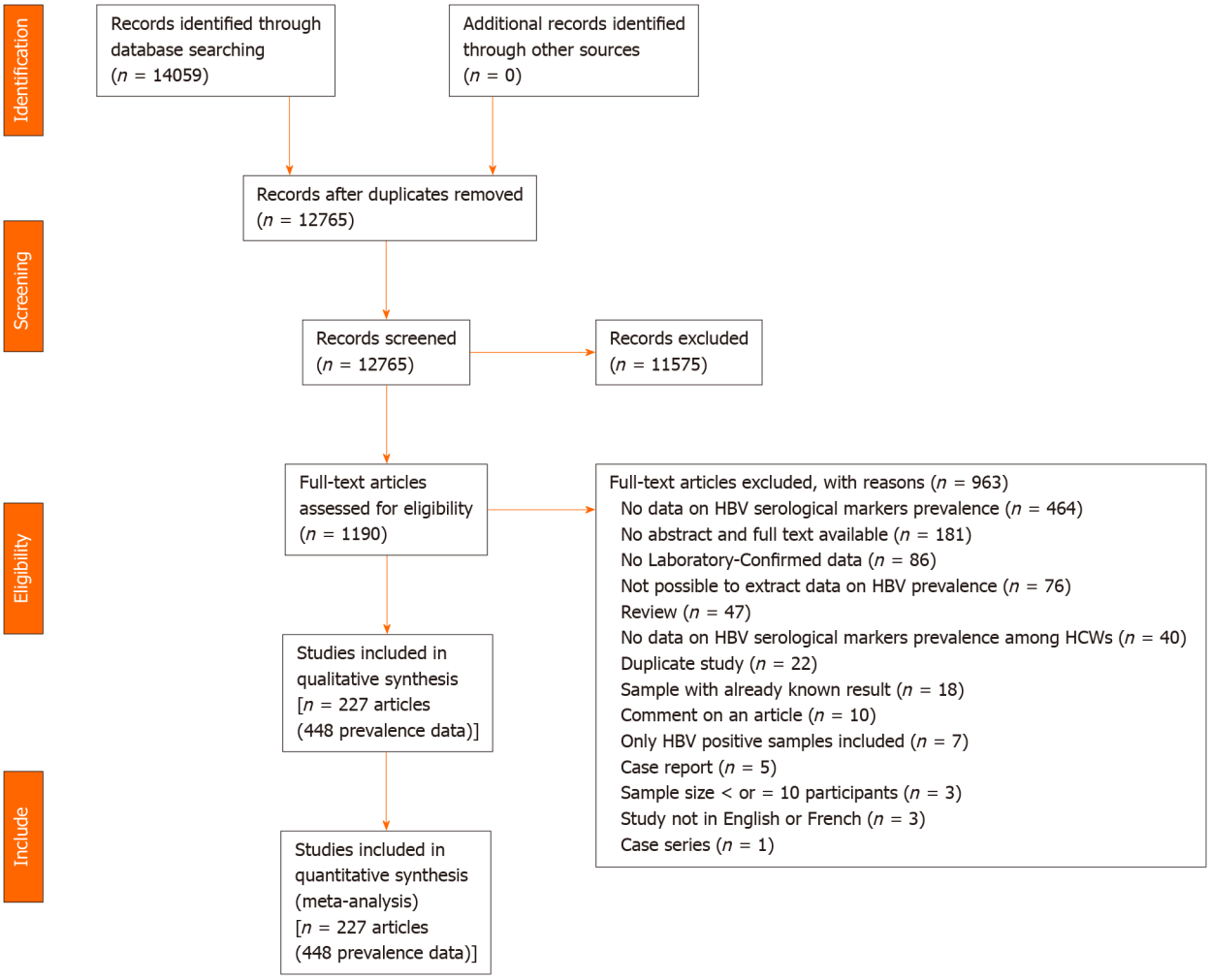

Figure 1 PRISMA flow chart of the included studies.

immunity against HBV in HCWs showed that seroprevalence was higher in retrospective studies, the European, Eastern Mediterranean, and Western Pacific WHO regions, urban areas, and among personal care workers in health services and health associate professionals. Subgroup analysis of immunity against HBV due to natural infection in HCWs showed that the seroprevalence was higher in non-probabilistic studies, retrospective studies, urban areas, and health management and support personnel.

The univariate metaregression allowed the selection of the relevant covariates (Supplementary Table 9). Only the WHO region variable significantly explained the variance observed in estimating the prevalence of current HBV infection and immunity due to natural infection. The variables sampling approach and the HCWs classification significantly explained the variance observed for the estimation of the prevalence of acute HBV infection. No covariates explained the variance observed in the estimate of the prevalence of total immunity to HBV.

DISCUSSION

Our findings showed that the pooled prevalence rates of HBV serological markers among HCWs with current (HBsAg and HBeAg) and acute HBV infections were 2.3%, 0.2% and 5.3, respectively. Our findings also showed that the pooled prevalence rates of total immunity against HBV and immunity due to natural HBV infection were 56.5% and 9.2%, respectively. HBV serological markers varied considerably among categories of HCWs. In the subgroup analysis, the pooled seroprevalence of HBV in HCWs with current infection was highest in low-income countries and particularly in Africa. The pooled seroprevalence of HBV in HCWs with acute infection was higher in upper-middle-income countries, in the South-East Asia and in urban areas. The pooled

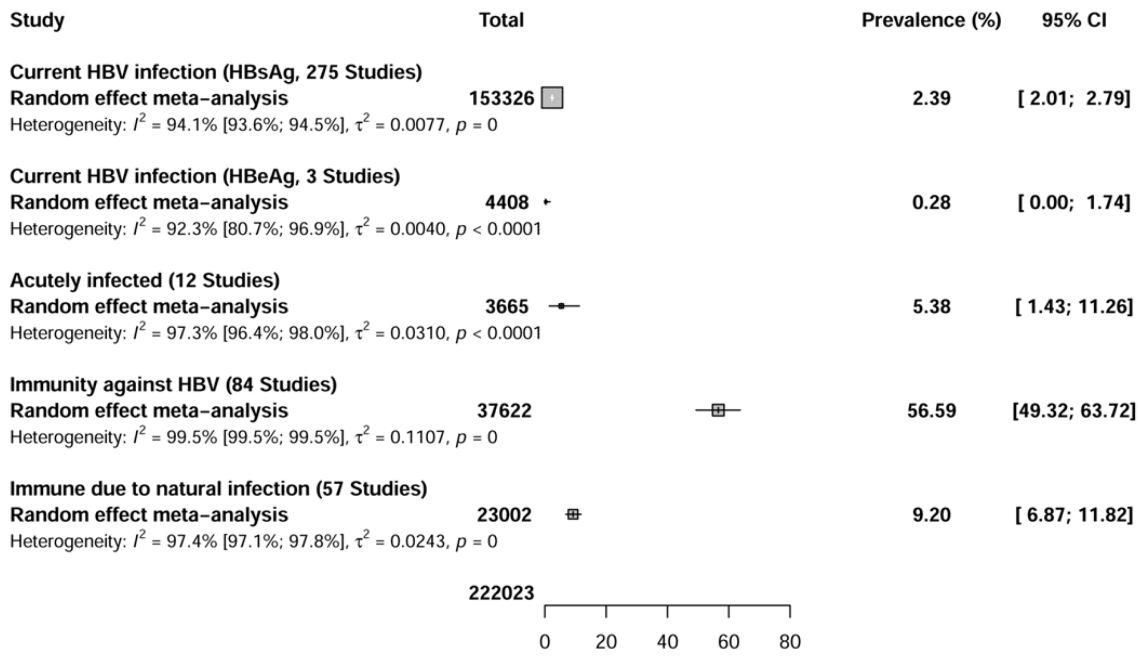

Figure 2 Global seroprevalence of hepatitis B virus serological markers among healthcare workers. CI: Confidence interval; HBV: Hepatitis B virus.

seroprevalence of total immunity against HBV was higher in the Europe, Eastern Mediterranean, Western Pacific, and urban areas. The pooled seroprevalence of immunity against HBV due to natural infection was higher in urban areas.

A previous meta-analysis reported a pooled seroprevalence of current HBV infection (HBsAg) in HCWs of 2.3% in Eastern Mediterranean and Middle Eastern Countries (EMRO) and in the European Union/European Economic regions[27,28]. Our estimated HBV infection seroprevalence, however, presented a strong disparity according to geographic and socioeconomic regions in favor of African regions, South-East Asia and urban areas. These differences may be linked to several factors, including socio-demographic, ethnic, cultural factors, risk factors for transmission, protective factors (heterogeneous vaccination policies, levels of education, availability of preventive measures, and the practice of barrier measures against occupational exposure to blood)[29]. HBV vaccination policies are applied with strong temporal, socio-cultural and economic disparities around the world. Low-resource countries for example are prone to imperfect vaccine policies, including partial coverage of eligible individuals and without any catch-up strategy for adults including HCWs[10,13-16]. This aspect could well explain the high seroprevalence of HBV infections observed in low-income setting in the present review. It is also conceivable that the various detection tests used to search for the serological HBV markers in the present review could be associated with the significant heterogeneity observed. The various occupational categories considered in this review could also be at the origin of the great variability in the observed seroprevalence rates. It has in fact been shown that inexperienced people at the start of training, such as medical students and nurses, were more at risk of occupational contraction of HBV[30]. Nurses who are closer to patients and who are responsible for collecting blood from patients are also at high risk of contracting HBV[31,32]. It should also be noted that dentists and surgeons present a very worrying risk of occupational contamination by HBV, due to their use of sharp objects and procedures that generate aerosols[33,34]. The age and number of years of service (> 5 years) of the health workers have also been associated with a greater risk of contracting HBV infections[35,36]. The number of HCWs per patient as well as the number of hematogenous exposure by HCWs is very variable across the world and could also account for this great heterogeneity observed in the estimates of this review [37]. In resource-limited countries, unlike developed countries, high infection rates are linked to high immunization coverage and the application of the post-exposure prophylaxis policy[38]. The varying dates in countries of immunization policies can also pay dividends. Due to the lack of time restriction in the inclusion criteria for this review, it is highly likely that some participants benefited from universal childhood immunization policies, suggesting different vaccine coverage and hence variable infection rates. In addition, vaccination coverage rates among HCWs vary widely

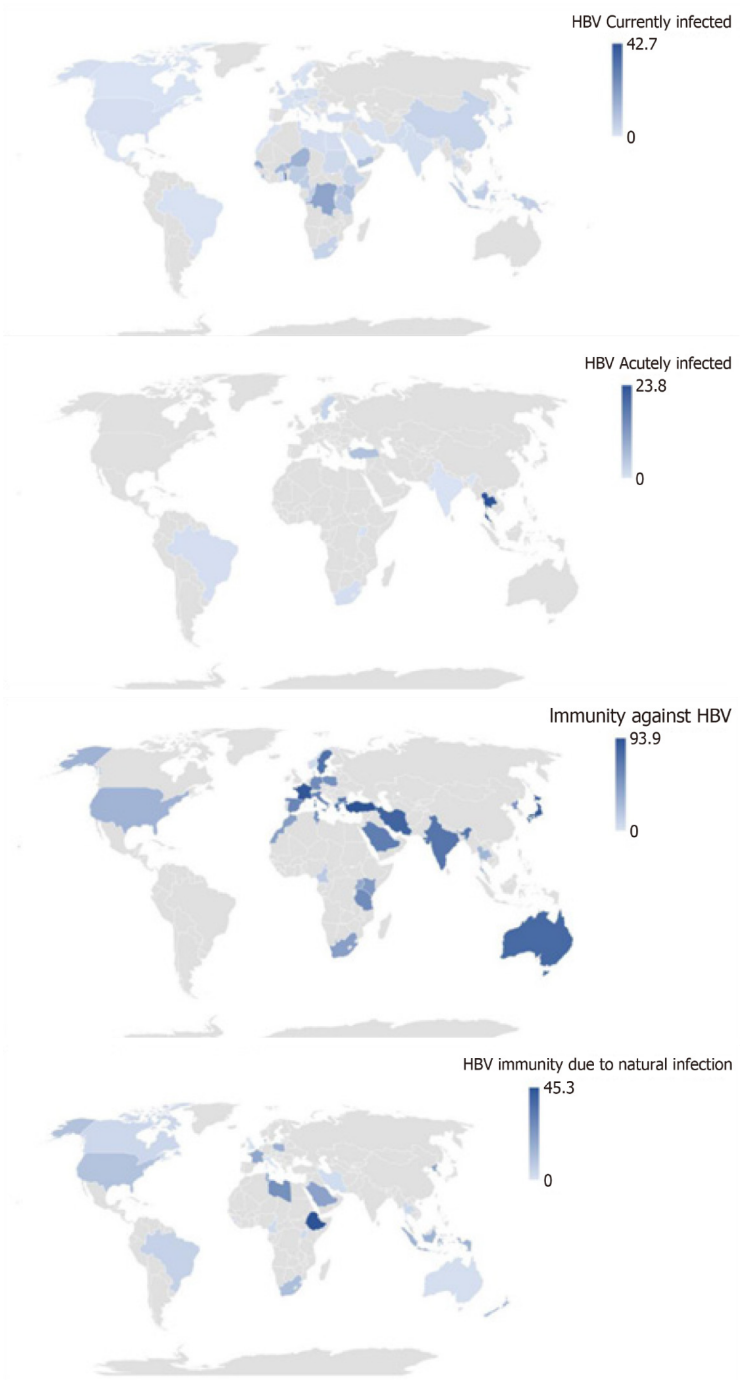

Figure 3 Global seroprevalence of hepatitis B serological markers among healthcare workers.

between countries, ranging from 18% in Africa to 77% in Australia[38]. In this review over half of HCWs had full immunity to HBV and this immunity was highest in urban areas and developed countries, including those in Europe, the Western Pacific, and the Eastern Mediterranean. Recently, a review showed that only a quarter of African HCWs had received the three doses of vaccines recommended for HBV immunization [39]. It is also noted that among this quarter of vaccinated HCWs in Africa, there is still a significant proportion of non-responders who remain at occupational risk of contracting HBV, as reported by other authors[40,41].

Some limitations should be noted for this review. One of the major difficulties of this review was the high variability of the professional categories of HCWs and the difficulty of having an easily applicable definition to group them together in a coherent way. Secondly, we did not consider the contribution of other major risk factors for HBV transmission in assessing the risk of HBV transmission in these HCWs, including sexual behavior or a history of parenteral injections. Also, the prevalence of current HBV infection in this study did not discriminate those with chronic infection

(HBsAg ≥ 6 mo) from those with acute infection. Despite these limitations, one of the strengths of this review lies in the representativeness of all regions of the world. An added value in this review is the concomitant consideration of several serological markers of HBV infection and immunity.

In order to hope to achieve the 2030 goal of eliminating HBV infections, decision-makers should implement training, vaccination and care policies for HCWs who represent a high-risk group of occupational HBV infections. These programs should ideally be subsidized or free to ensure universal access to these measures. Vaccination coverage rates remain low in some regions (Turkey) where the vaccine is free for HCWs[30]. Continuous training of HCWs on the importance of vaccination against HBV, the appropriate use of personal protective equipment, barrier measures against occupational exposure to blood and associated diseases, as well as on proper disposal of sharp objects would be of great benefit in reducing occupational exposure. Training on barrier measures for occupational percutaneous injuries should incorporate safety behaviors, such as the use of puncture-resistant trash cans. In countries with limited resources that bear the heaviest burden of HBV infections, expanded routine immunization programs at birth should also include catch-up vaccinations for high-risk people, such as HCWs. For medical students, to implement systematic vaccination of all HCWs at the start of the professional training or before commencement of duty and verify effective immunization before starting could be more cost effective. For HCWs already in service, an initial phase would be the search for unvaccinated HCWs. For a rational integration of the vaccination program in HCWs, anonymized pre-vaccination anti-HBc screening tests should be carried out beforehand to avoid unnecessary vaccinations. The anti-HBc test should be followed by the HBsAg screening in anti-HBc-positive HCWs. Costly conventional enzyme-linked immunosorbent assay (ELISA) techniques are often unavailable in resource-limited areas, although they bear the heaviest burden of HBV infections[42]. Low cost and easy to use alternative assays with comparable performance to conventional ELISA assays should be made available to resource-limited areas[42-44]. The HCWs eligible to receive the three doses should be those susceptible to HBV infection, negative for the anti-HBc marker. Checks for anti-HBs levels should follow 2 mo to 3 mo after complete vaccination to ensure that the protective titer is achieved (anti-HBs ≥ 10 IU/mL). HCWs not responding to full vaccination should receive additional doses of vaccine. Booster doses could be given periodically (like 10 years if anti-HBs titer is below 10 IU/mL). HBsAg-positive HCWs would benefit from expert guidance for their orientation, rational and appropriate treatment to avoid wastage. Positive HBsAg tests should not disqualify HCWs from their daily practice, although urgent measures should be taken to control their viral load to minimize their risk of transmitting HBV to their patients and to those around them.

CONCLUSION

This systematic review highlights an important burden of HBV infections among HCWs around the world. It also reveals that around half of HCWs are protected against HBV infections worldwide. This protection is mainly attributed to vaccination compared to immunization due to natural infection. The burden of HBV infection is mainly borne by resource-limited countries, particularly Africa, which in parallel also reveals the lowest levels of immunization against HBV.

ARTICLE HIGHLIGHTS

Research background

Hepatitis B infection is a deadly disease that affects and kills more than 1 million people a year. During their work, healthcare workers (HCWs) are exposed to certain direct or indirect risk factors that could lead to hepatitis B virus (HBV) infection. Existing data have shown that HBV infection, depending on markers, is widespread and heterogeneously distributed worldwide among HCWs. Therefore, there is a need to quantify the global proportion of HBV serological markers among HCWs.

Research motivation

HCWs are one of the most vulnerable groups to HBV infection during their routine work, which exposes them to a variety of accidents, *e.g.*, needle stick injuries, exposure

to blood and fluids of HBV-infected patients, *etc*. However, these groups are under-diagnosed in many parts of the world, especially in low-income countries. It remains to be seen how the burden of each marker of hepatitis B infection is distributed worldwide in order to guide future research. We therefore sought to quantify the burden of several serological markers of HBV infection in HCWs. This will enable the development of new strategies to better manage HBV infection in HCWs.

Research objectives

In this review, we aimed to quantify the pooled prevalence rates of serological markers of HBV infection among HCWs. We were able to report these prevalence data among HCWs based on world regions, country income levels, and categories of HCWs. Quantifying these prevalence rates in each region of the world is crucial to improving and/or implementing new strategies for managing HBV infection, as well as guiding future research that will contribute to the elimination of HBV by 2030 and the achievement of Sustainable Development Goal 3.3 related to well-being and good health, specifically ending the AIDS epidemic, tuberculosis, malaria and neglected tropical diseases and combating hepatitis, water-borne and other communicable diseases.

Research methods

To synthesize data from the existing literature on the prevalence of HBV serological markers in HCWs, we followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guideline. We registered the study in Prospero and the search strategy was applied in PubMed and Embase to retrieve observational studies, including cross-sectional, cohort (baseline data) and case-control studies. These studies were selected for eligibility on the Rayyan platform by four investigators (Mahamat G, Kenmoe S, Ebogo-Belobo JT and Amougou-Atsama M) and data extraction was performed by 18 extractors using a Google form questionnaire. The quality of the included studies was assessed by the tool of Hoy *et al*. A random-effects meta-analysis model was used to pool the prevalence of each serological marker in HCWs. Meta-regression and subgroup analyses were used to determine the source of heterogeneity. The statistical software R version 3.6.2. was used to perform all meta-analyses.

Research results

In all, we reported prevalence rates of current infection [hepatitis B surface antigen (HBsAg) and hepatitis e antigen], acute infection (anti-HBs immunoglobulin M + HBsAg), full immunity (anti-HBs > 10 IU/mL), and acquired immunity by natural infection (anti-HBS + anti-HBc) among HCWs of 2.3% and 0.2%, 5.3%, 56.6%, and 9.2%, respectively. Low-income countries, particularly African countries, bear the greatest burden of current infection and have low immunization rates. High-income countries and urban areas are more protected from HBV infection. These results suggest that attention should increasingly be focused on low-income countries and in particular African countries where future research should be directed.

Research conclusions

There is a need to improve awareness, training, screening, vaccination, post-test management and treatment of HBV infection worldwide in order to achieve the World Health Organization goal of eliminating hepatitis B infection by 2030.

Research perspectives

Future research should be directed towards low-income countries, including African countries, where the highest burden of current infection with low vaccination coverage among HCWs has been reported.

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EDITORIAL

Transition of an acronym from nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a global public health concern owing to its substantial contribution to chronic liver diseases. The disease is closely linked to metabolic syndrome (MS), suggesting a common biological pathway and shared disease mechanism for both ailments. Previous studies revealed a close relationship of NAFLD with the components of MS including abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. Hence, a group of experts recently renamed NAFLD as metabolic dysfunction-associated fatty liver disease (MAFLD) in order to encompass a more appropriate pathogenesis of the disease. NAFLD was first named to describe a condition similar to alcoholic hepatitis in absence of significant alcohol consumption. However, knowledge pertaining to the etiopathogenesis of the disease has evolved over the past four decades. Recent evidence endorses NAFLD as a terminology of exclusion and suggests that it may often leads to misdiagnosis or inappropriate management of patients, particularly in clinical practice. On the other hand, the new definition is useful in addressing hepatic steatosis with metabolic dysfunction, which ultimately covers most of the patients with such illness. Therefore, it seems to be helpful in improving clinical diagnosis and managing high-risk patients with fatty liver disease. However, it is imperative to validate the new terminology at the population level to ensure a holistic approach to reduce the global burden of this heterogeneous disease condition.

Key Words: Nonalcoholic fatty liver disease; Metabolic dysfunction-associated fatty liver disease; Redefining; Redefinition of fatty liver disease

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Core Tip: A consensus of experts recently renamed nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease. The new definition is advantageous for improving clinical diagnosis and managing high-risk patients with fatty changes in liver.

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INTRODUCTION

The rising burden of nonalcoholic fatty liver disease (NAFLD) is a global public health concern. This progressive liver disease is a leading cause of chronic hepatic ailments worldwide[1,2]. Recent reports confirm that NAFLD accounts for approximately 8% of the annual 2.14 million global deaths from liver disease[3]. Over the past two decades a substantial elevation in the prevalence of NAFLD has been reported, with strong evidence of a close link between NAFLD and metabolic syndrome (MS)[4]. NAFLD is often found to be associated with the components of MS, such as abdominal obesity, dyslipidemia, hypertension, and hyperglycemia[5]. In addition, the risk factors of NAFLD and MS have also been found to be identical in many studies[1]. Therefore, it has been suggested that both NAFLD and MS follow a common biological pathway as well as a shared disease mechanism. In line with that, a consensus of experts recently renamed NAFLD to metabolic dysfunction-associated fatty liver disease (MAFLD) so that the term could accurately reflect the pathogenesis of the disease[6]. According to the new definition, MAFLD would be diagnosed if there was evidence of hepatic steatosis in addition to any of the following three conditions, overweight/obesity, type 2 diabetes mellitus, or metabolic dysregulation[7]. The expert opinion was that the new definition is superior for diagnosing NAFLD patients with severe liver injury. Moreover, it is more practical to diagnose high-risk patients and evaluate disease progression in clinical settings[8,9].

DISCUSSION

Nonalcoholic steatohepatitis (NASH) was first used nearly four decades ago to describe a condition that mimics alcoholic hepatitis in absence of significant alcohol consumption[10]. Initially, the pathology was found to be linked to obesity or obesity-associated disorders. Subsequently, the disease was renamed NAFLD, referring to the absence of any known etiology of liver disease. In the meantime, a detailed understanding of the etiopathogenesis of the disease has evolved as the link between NAFLD, insulin resistance, and other components of MS was explored. Molecular-level investigations explored the role of multiple genetic and cellular mechanisms in the pathogenesis of NAFLD[11]. Epidemiological studies also revealed a number of social, demographic, and clinical determinants responsible for development of NAFLD [12]. Results of the studies described NAFLD as a heterogeneous condition. However, the archaic NAFLD nomenclature, which is a terminology of exclusion, remained unchanged over the years. The inclusion of alcohol in the name and definition is also problematic. In real-life clinical practice, the features of NAFLD often overlap with the characteristics of patients who consume alcohol. Moreover, there is no accepted method to appropriately measure alcohol intake in clinical facilities. Hence, there remains a possibility of misdiagnosis or inappropriate management of patients. Considering the above context, there has been a proposal to change the name since the beginning of this century. As the disease was found to be closely associated with metabolic dysfunction and insulin resistance, the scientific community proposed several names related to metabolic dysfunction, for example, metabolic steatohepatitis, metabolic fatty liver disease, and metabolic-associated fatty liver[10]. Eventually, a consensus of global experts opted for MAFLD.

Table 1 Potential positive implications and challenges related to transition of nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease

Positive implications	Challenges
Useful in overcoming the dichotomization of NASH and non-NASH in clinical practices	Obtain a global acceptance as some researchers consider the name change premature and inappropriate
Facilitate diagnosis and evaluation of disease progression in high-risk patients	Underestimation of actual prevalence of the disease using the criteria of MAFLD
Improve awareness of physicians, healthcare providers and patients	Further clarification and stratification of the definition to guide decision-making and assess prognosis of the disease
Improve physician-patient communication	Address the patients with fatty changes in liver in absence of metabolic derangements
Improve clinical diagnosis and patient care	Deal with lean or undernourished individuals with hepatic fatty changes
Reduce confusion and stigma regarding the disease	Lack of information regarding genetic risk factors, phenotyping measurements, body fat content, and alterations in gut microbiota in the new definition
Increase public attention and improve health policy actions	Determine the outcome variable of ongoing clinical trials where "improvement in NASH" is one of the endpoints

MAFLD: Metabolic dysfunction-associated fatty liver disease; NASH: Nonalcoholic steatohepatitis.

It is assumed that the new definition would improve clinical diagnosis (Table 1). The term MAFLD annulled two different NAFLD entities, simple steatosis and NASH, and conceptualized the fatty changes in the liver as a disease process. Therefore, the redefinition of MAFLD would help to overcome the dichotomization of NASH and non-NASH, and facilitate the assessment of disease severity in clinical practice[13]. A recent study reported that the switch from NAFLD to MAFLD increased the awareness of physicians regarding the management of the disease[14]. However, changes in nomenclature may have potential implications for ongoing clinical trials in which "improvement in NASH" is an outcome variable. It is possible to redefine the outcomes of clinical trials based on the existing MAFLD framework, but there remains certain disagreement regarding the new terminology and its definition that need to be addressed[15]. The new criteria may underestimate the actual prevalence of the disease, as reported in a recent study[8]. It may also exclude patients without metabolic disturbances. A recent review found that metabolic derangements may be absent in 30% of the patients diagnosed with NAFLD[5]. The new definition is also not clear regarding concomitant liver diseases such as drug-induced, viral or auto-immune liver disease. Apart from individuals with high body mass index, NAFLD has also been reported in lean and nonobese adults. It is assumed that visceral adiposity and differences of metabolic adaptations may play a potential role in the pathogenesis of hepatic steatosis in lean adults[16]. Alterations in gut microbiota can also be a contributing factor in developing NAFLD in lean and undernourished adults[16]. Moreover, there is evidence in support of a significant relationship between a positive family history of metabolic traits and NAFLD, particularly in lean patients with a fatty liver[17]. Individuals with a family history of metabolic traits are likely to develop complications of NAFLD at a younger age[18]. Therefore, body fat content, rate of weight gain, and family history of metabolic traits need to be considered when constructing a new conceptual framework to define MAFLD. It seems that diagnosis of cryptogenic cirrhosis attributable to metabolic derangements would be easier using the new definition of MAFLD, as cryptogenic cirrhosis was found to be associated with obesity and diabetes[19]. Nevertheless, a more insightful opinion is required to establish an accurate definition so that the term incorporates individuals with hepatic fatty changes in the absence of metabolic derangements. Moreover, there should be definitive guidelines regarding inclusion of genetic risk factors, phenotypic measurements, dietary intake, visceral adiposity, and alterations in gut microbiota in the definition.

CONCLUSION

As more than one-fourth of the global population have NAFLD. Emphasis should be given to appropriate understanding of etiopathogenesis of the ailment[20]. To that end, an appropriate term is required so that it can reflect the entire pathophysiology of

the disease and cover the whole population with perturbed accumulation of hepatic fat. The new definition seems to address hepatic steatosis with metabolic dysfunction, which ultimately covers most of the cases with such illness. It is also useful for improving clinical diagnosis and managing high-risk patients with fatty changes in the liver. Therefore, the shift in terminology from NAFLD to MAFLD has already attained global endorsement. However, validation of the new term at the population level is warranted to ensure a holistic approach to reduce the global burden.

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OPINION REVIEW

Non-invasive real-time assessment of hepatic macrovesicular steatosis in liver donors: Hypothesis, design and proof-of-concept study

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Abstract

Macrovesicular Steatosis (MS) is an independent risk factor for adverse post-liver transplant (LT) outcomes. The degree of MS is intimately related to the viability of the liver graft, which in turn is crucial to the success of the operation. An ideal liver graft should have no MS and most centres would find it unacceptable to use a donor liver with severe MS for LT. While a formal liver biopsy is the gold-standard diagnostic test for MS, given the logistical and time constraints it is not universally feasible. Other tests like a frozen section biopsy are plagued by issues of fallibility with reporting and sampling bias making them inferior to a liver biopsy. Hence, the development of an accurate, non-invasive, easy-to-use, handheld, real-time device for quantification of MS would fill this lacuna in the deceased donor selection process. We present the hypothesis, design and proof-of-concept of a study, which aims to standardise and determine the feasibility and accuracy of a novel handheld device applying the principle of diffuse reflectance spectroscopy for real-time quantification of MS.

Key Words: Macrovesicular steatosis; Deceased donors; Liver transplantation; Real-time devices; Diffuse reflectance spectroscopy

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Core Tip: The degree of macrovesicular steatosis (MS) is intimately related to the viability of the liver graft, which in turn is crucial to the success of the liver transplant operation. The development of an accurate, non-invasive, easy-to-use, handheld, real-time device for quantification of MS would fill a lacuna in the deceased donor selection process. We present the hypothesis, design and proof-of-concept study for a novel handheld device for real-time quantification of MS.

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INTRODUCTION

Macrovesicular Steatosis (MS) is an independent risk factor for adverse post-liver transplant (LT) outcomes. The degree of MS is intimately related to the viability of the liver graft, which in turn is crucial to the success of the operation. An ideal liver graft should have no MS and most centres would find it unacceptable to use a donor liver with severe MS for LT. While a formal liver biopsy is the gold-standard diagnostic test for MS, given the logistical and time constraints it is not universally feasible. Other tests like a frozen section biopsy are plagued by issues of fallibility with reporting and sampling bias making them inferior to a liver biopsy. Hence, the development of an accurate, non-invasive, easy-to-use, handheld, real-time device for quantification of MS would fill this much vaunted lacuna in the deceased donor selection process. We present the hypothesis, design and proof-of-concept of a study, which aims to standardise and determine the feasibility and accuracy of a novel handheld device applying the principle of diffuse reflectance spectroscopy for real-time quantification of MS.

AIM

The objective of the present investigation is to apply the principle of diffuse reflectance spectroscopy (DRS) to standardize and determine the feasibility and accuracy of a handheld device for real-time quantification of MS.

PRINCIPLE AND HYPOTHESIS

DRS is an optical measurement method which is based on the principle of tissue illumination and the measurement of reflectance[8]. Briefly, the tissue is illuminated with light from a broadband light source, and after interacting with the tissue the diffusely reflected light is collected and analyzed. By fitting the analyzed data to a mathematical model, tissue characteristics such as its structure and composition can be estimated. Quantification of MS with infrared (IR) spectroscopy directly depends on the absorption of IR light due to vibrational excitation in molecular groups[9]. In liver tissues, the absorption in the visible wavelength range is dominated by bile and hemoglobin, whereas lipid, water and collagen are the main source of absorption in the near-infrared wavelength range. Hence IR spectra is the wavelength of interest for this study. Recent studies on the human liver show that the absorption of light around 1200 nm is dominated by the lipid and this can be used for the assessment of steatosis [9-11].

We hypothesize that the broadband light source can be replaced with a narrow band light emitting diode (LED) of 1200 nm and the spectrometer with a highly sensitive photodetector. Using the absorption characteristics, a calibration curve can be determined based on the fat content on the liver; allowing for the development of a mathematical model and a real-time quantitative analysis of MS. We also hypothesize that once the difference in absorbance spectrum between normal and MS liver is

established, the optical device can be miniaturized further. This novel optic-based handheld device for MS detection will retain its accuracy whilst being portable and affordable as well.

DEVICE SETUP AND METHOD

A handheld device was designed and developed with a single infrared LED (IR-LED)-photodetector (PD) arrangement coupled through a fibre optic reflection probe bundle. One end of the reflection probe was coupled to a LED, and the other end to a highly sensitive photodetector. These optoelectronic components were placed in a custom-made plastic block to avoid ambient noise or cross-coupling between the LED and PD. The optoelectronic circuitry comprised of a 5 V linear voltage regulator followed by a constant current circuit using two bipolar junction transistors to drive IR-LED and a trans-impedance amplifier circuit for the PD to convert photocurrent into photo-voltage. This circuit was powered by a 9 V battery placed within the handheld device (Figure 1A). The obtained photovoltage was then transmitted to a low-power system on a chip microcontroller *via* a buffer integrated circuit[12]. The device has an LED display that shows voltage response corresponding to the diffused reflectance data from the liver (Figure 1B).

The measurement was carried out with the handheld device employing a mathematical model. With the device powered on, the fiberoptic reflection probe was placed on the diffuse reflectance standard (WS-1 ocean optics, United States) and the initial voltage value made note of. This was taken as the reference value; the probe was then placed on the test sample to record its voltage value. An algorithm was formulated to calculate the resultant fat absorbance value (A_t) with this reference (V_r) and test (V_t) voltage values from the below equation.

$$A_f = -\log_{10}\frac{V_t}{V_r}$$

Proof-of-concept

For a practical assessment of the above hypothesis, an initial proof of concept analysis was done using 50 abattoir retrieved large animal livers, with varying percentage of fat (Figure 2). Calibration of the device was initially done with 100% fat and normal liver. The results from fat and normal liver were compared to determine the fat composition. Absorbance data was normalized by taking the closest valley to 1300 nm to improve its sensitivity towards estimation of fat percentage [13,14].

$$\text{Normalized absorbance} = 1 - \frac{a_1 \times \lambda_2}{a_2 \times \lambda_1}$$

The above equation was used to calculate the normalized absorbance value. This was done by taking the ratio of absorbance responses (a1, a2) at two wavelengths (λ1 and λ2) and subtracting it from 1. The specific absorption spectrum of fat peaked at approximate 1200 nm and the normal liver had a Gaussian response at 1200 nm (Figure 3A). Figure 3B shows the calculated absorbance response of fat and liver was noted to be 0.3203 ± 0.09 and 0.058 ± 0.01 respectively. The absorbance values obtained were evaluated against the gold standard biopsy results of these animal livers.

STUDY DESIGN

It is an observational study where the point-of-care device is used to assess MS in a non-invasive manner. The study is to be conducted at organ retrieval centres across the city of Chennai, India. The study design is presented in Figure 4.

Calibration cohort

Initial calibration of the device is to be conducted on 50 livers. Fifteen live liver donors will be assessed for levels of MS. Ten recordings with the device per liver will be noted across the right lobe. As a standard unit protocol, all live liver donors undergo an intraoperative liver biopsy which will be used for comparison. 100% fat as a baseline calibration will be used by analyzing the excised falciform ligament from each of these patients. 35 livers in the real-world deceased donor situation will be analyzed using the device to correlate the estimated MS content with a standard biopsy estimation.

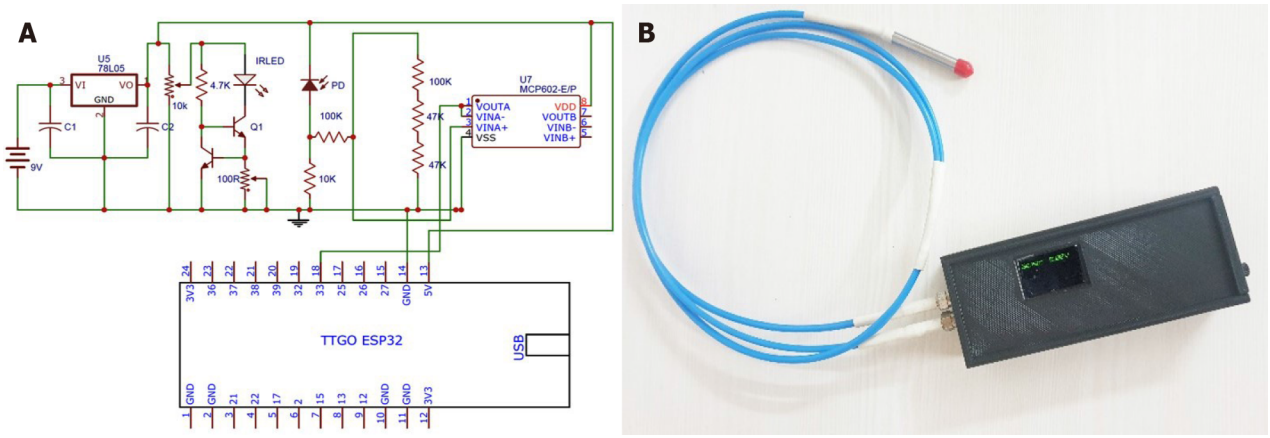

Figure 1 Principle and set-up of the hand-held real time device to measure macrovesicular steatosis. A: Optoelectronics circuit, B: Handheld point of care device.

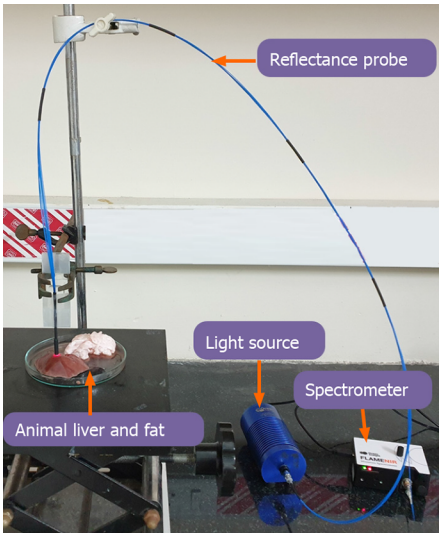

Figure 2 Proof-of-concept study using the prototype model of the device.

These observations will enable the development of a calibrated algorithm based on the reflectance for MS. Optimum conditions for use, including lighting, temperature, distance from the liver, will also be standardized.

Validation cohort

Analysis will be performed on 50 deceased donor livers to test and evaluate the accuracy of the developed algorithm and the point-of-care device.

Inclusion criteria

For the calibration cohort, all living liver donors will be included. The standard selection criteria for these living donors are include: (1) Age 18-50 years; (2) ABO compatible blood group with the recipient; (3) No comorbidities, or 1 comorbidity; (4) Liver attenuation index ≥ +6; (5) Body mass index < 30 kg/m^2; (6) Graft to recipient weight ratio > 0.8; (7) Functional liver remnant volume > 30%; (8) Anatomically suitable for donation; and (9) Any other donor who beyond the above criteria but approved for donation based on the decision of the multi-disciplinary team meeting.

The deceased donors include all brain-dead donors consented for organ donation: (1) Adults between 18 years and 75 years of age; and (2) Donation after brain death. For the validation cohort, all brain-dead donors consented for organ donation will be included: (1) Adults between 18 years and 75 years of age; and (2) Donation after brain death.

As the device analyses the fat content of the donor liver, no specific recipient-based inclusion criteria were defined. Donors of all recipients who underwent the LT

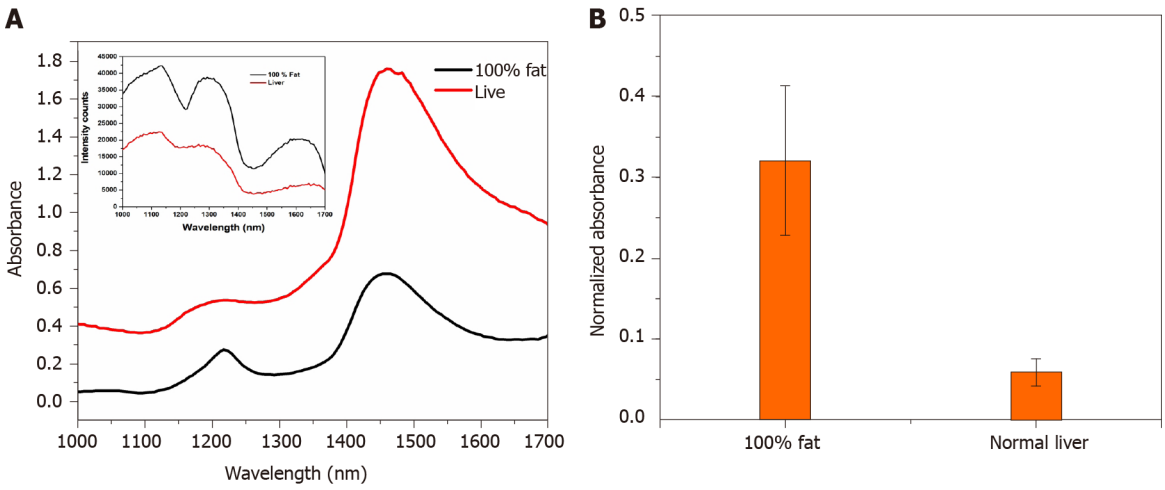

Figure 3 Comparison between large animal liver retrieved from abattoir and 100% fat. A: Absorbance spectrum of abattoir retrieved large animal liver and 100% fat (inset: intensity spectrums of liver and 100% fat); B: Calculated absorbance response of abattoir retrieved large animal liver and 100% fat.

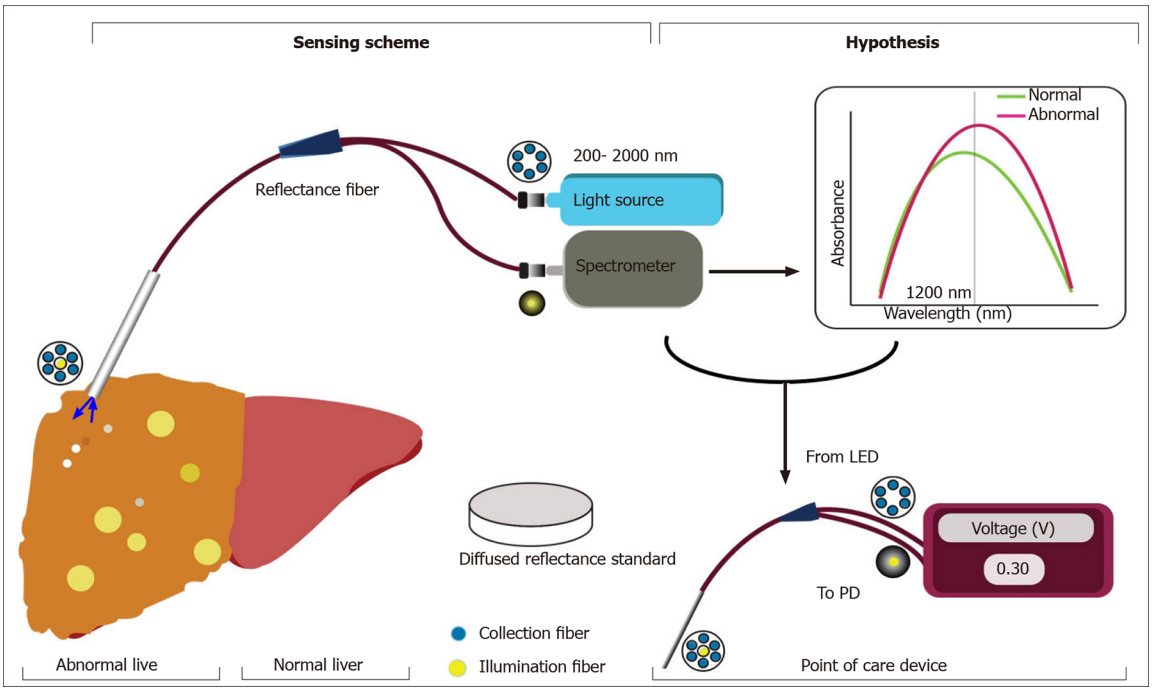

Figure 4 Schematic representation of the proposed study design, experimental setup, and hypothesis towards the development of handheld device. It consists of reflectance probe bundle with home-made plastic block to house light emitting diode, photodetector, optoelectronic circuitry, and display. PD: Photodetector.

operation and recipients of all etiologies were included.

Exclusion criteria

(1) Paediatric deceased donors; (2) Donation after cardiac death; (3) Donations where a frozen section/standard biopsy could not be performed; and (4) Discarded organs.

Concerns and untested variables

Liver with underlying fibrosis, cholestasis, sinusoidal obstruction syndrome (blue color) and those which could possibly bias the spectral analyses.

Ethics, informed consent, safety, and registration of trial

The study will be conducted in accordance with the principles of the Declaration of Helsinki and "good clinical practice" guidelines. Approval from the institutional ethics committee has been obtained. As a testimonial to its bona fide nature, the study has

also been registered with the Clinical Trials Registry of India, National Institute of Medical Statistics, Indian Council of Medical Research, India. CTRI No: CTRI/2021/01/030223.

STATISTICAL ANALYSIS

Statistical analysis will be performed with the SPSS V.20.0. To compare specific variables, the extended χ^2 test will be used. For non-parametric analysis of continuous distributed variables, the Mann-Whitney *U* test and the Kruskal-Wallis test will be used. $P < 0.05$ is considered statistically significant.

DISCUSSION

The need for a quick, portable, efficacious and economical device to diagnose MS is evident by the number of proof-of-concept studies available in this regard[7,9,11,15]. DRS as a diagnostic modality has been used in endoluminal studies of upper and lower gastrointestinal endoscopies[16,17]. Using the absorption and scattering patterns of biological tissues, DRS allows for accurate differentiation of polyps and subendothelial pathology. Reports on the use of DRS in the identification of MS in murine and porcine liver models show promising results[10,11]. Clinical studies are however sparse and those attempted involve using a micro-spectrometer placed directly over the liver graft[8,10,15]. Nonetheless, there are several drawbacks to these devices. The micro-spectrometers require a sophisticated optical setup, which included an optical spectrometer and other expensive optical components. In addition, due to concerns of sterility, a spectrometer cannot be used on multiple patients. Moreover, these devices require network access, without which the diagnostic algorithm may not be useful. Put together these devices have proved cumbersome to the organ-retrieving surgeon.

To overcome the pitfalls of these prototype models, our device uses IR light guided *via* an optical fibre, and the diffuse reflections are obtained from the tissue sample by measuring the steady-state spectrum. The broadband light source is replaced with a narrow band LED and the spectrometer with a photodetector. Once the algorithm is standardized this optical setup can be miniaturized further, and linked to the internet allowing for remote viewing by the concerned teams.

To push the envelope further, should our device be validated in the current study, we propose that there is potential to link our device with a smartphone application incorporating the algorithm and make use of the current generation of high-resolution smartphone cameras. This would allow for a real-time high-resolution image along with MS percentage to be remotely transmitted using the mobile network to the concerned senior members of the transplant team.

CONCLUSION

We hypothesize that once validated, our device can potentially prove to be an invaluable apparatus at the hands of the organ retrieving surgeon. It will be non-invasive, portable (hand-held), economical, provide real-time readings of the percentage of MS with image reference and be efficaciously handled by junior surgeons, while not requiring any special network capabilities apart from the presence of the now ubiquitous smartphone. This will dramatically ease the currently available circuitous and subjective process of determining MS and decision making in selecting deceased donor organs for LT. Nonetheless, ours is a hypothesis and initial proof-of-concept study which requires real-world validation across multiple centres and in a large cohort of patients before it can become an integral part of the liver retrieval algorithm.

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REVIEW

Impact of COVID-19 pandemic on liver, liver diseases, and liver transplantation programs in intensive care units

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Author contributions: Omar AS and Kaddoura R have made substantial contributions to the outline of the article; Omar AS was responsible for the abstract, introduction, impact of COVID-19 on liver transplantation programs and implications and future directions; Kaddoura R was responsible for prevalence and consequences of COVID-19-associated liver injury, pre-existing liver disease in COVID-19-associated liver injury and the tables/figure; Hanoura S wrote pathogenesis of liver injury in COVID-19; Orabi B wrote drug-induced liver injury in patients with COVID-19, drug-drug interactions between immunosuppressive therapy and COVID-19 agents and impact of COVID-19 on CVS and HCC; all authors revised and approved the final manuscript.

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Abstract

Emerging worldwide data have been suggesting that coronavirus disease 2019 (COVID-19) pandemic consequences are not limited to the respiratory and cardiovascular systems but encompass adverse gastrointestinal manifestations including acute liver injury as well. Severe cases of liver injury associated with higher fatality rates were observed in critically ill patients with COVID-19. Intensive care units (ICU) have been the center of disposition of severe cases of COVID-19. This review discusses the pathogenesis of acute liver injury in ICU patients with COVID-19, and analyzes its prevalence, consequences, possible drug-induced liver injury, and the impact of the pandemic on liver diseases and transplantation programs.

Key Words: COVID-19; Critical care; Drugs; Liver; Liver transplantation; Outcome; Severe liver injury

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Core Tip: In this manuscript, liver dysfunction is seen more in patients with more severe disease upon presentation. It is difficult to separate the independent effect of viral infection from various treatment modalities, including antibiotics and experimental antiviral drugs that are used in these patients.

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INTRODUCTION

More than a year ago, the global pandemic started from its epicentre in Wuhan. In coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the lung is the main organ targeted by the virus[1]. The organism exhibits a wide range of severity and a diverse disruption of extra-pulmonary systems, including gastrointestinal, renal, cardiac[2,3], hepatic[4], and even multi-organ damage[2,5]. Moderate or severe symptoms have been reported in almost 20% of all COVID-19 patients, while 5% progress into critical stages of the disease[6].

The rate of intensive care unit (ICU) admission due to COVID-19 is quite variable, ranging from 3% to 100% in literature[7]. The liver could be affected in COVID-19 through several mechanisms, including virus-related liver cell injury, disorganized immune response, drug-induced liver injury (DILI) and ischemic liver dysfunction in the settings of multisystem organ failure[8]. The reported rate of COVID-19-induced liver injury ranged from 14.8% in one study[9] and up to 74% in another[10]. In a case series of critically ill patients with COVID-19, liver injury was frequent but transient and non-severe[11]. Patients may not be equally affected by the pandemic, certain patient populations are potentially more vulnerable. Immunocompromised patients and patients with cirrhosis are probably more susceptible to worse outcomes after SARS-CoV-2 infection[5]. The data in literature on how chronic immunosuppression can influence COVID-19 outcomes is scarce[6]. This minireview will discuss the pathogenesis of acute liver injury in ICU patients with COVID-19, focusing on its prevalence, consequences, DILI, and its impact on existing liver diseases and liver transplantation programs.

PATHOGENESIS OF LIVER INJURY IN COVID-19

Liver injury in COVID-19 can be related to the direct cytopathic effect of the virus, DILI, uncontrolled immune reaction, or sepsis[12]. SARS-CoV-2 ribonucleic acid has been detected in blood and stool samples of COVID-19 patients who presented with diarrhoea, indicating the liver's probable involvement in the disease pathogenesis[13,14]. It has been suggested that there is a considerable expression of angiotensin-converting enzyme 2 (ACE2) receptors in cholangiocytes, where SARS-CoV-2 binding may adversely affect liver function. Moreover, COVID-19 may worsen the underlying chronic liver disease(s) (CLD), leading to hepatic decompensation or acute-on-chronic liver failure and increasing the risk of mortality, particularly in critically ill patients[12,15-17]. However, in severe COVID-19, liver damage is more likely due to the inflammatory cytokine storm[12,18] rather than the direct cytopathic effects of the virus[12].

The progression of SARS-CoV-2 infection has been divided into four phases: Upper and lower respiratory tract infection, usually treated as outpatients, COVID-19 associated lung injury, usually treated as inpatients, systemic inflammatory response syndrome (SIRS), and systemic failure. Liver involvement is often observed in the latter phases but can also occur in the earlier ones. In SIRS, pro-thrombotic factors accumulate due to bone marrow and liver acute phase response causing thrombosis, whereas in the last phase, multi-organ vascular dysfunction and cytokine storm occur in view of the ongoing interaction between the lung and systemic inflammation[19].

Hypoxic hepatitis (HH), known as shock liver or ischemic hepatitis, is an acute liver injury resulting from liver hypoxia[20]. The extensive complex vascular supply together with high metabolic efficacy results in a liver vulnerable to circulatory disturbances. Critically ill patients with circulatory or respiratory manifestations which may influence liver perfusion are at higher risk of HH[3,21,22]. The mechanism by which SARS-CoV-2 infection leads to HH is not fully understood. Multiple theories have been postulated, including hypoxemia developed due to COVID-19 pneumonia [2] and systemic stress caused by SIRS[19]. Both may provide a route to a compensatory decrease in peripheral and splanchnic blood flow, resulting in decreased hepatic blood flow leading to hepatocellular hypoxia[23]. Reperfusion injury is mediated by the generation of reactive oxygen species when ischemic hepatocytes are re-exposed to oxygen, leading to cell injury *via* lipid peroxidation[24]. Waseem and Chen[21] defined the diagnostic criteria for HH as circulatory or respiratory failure with a dramatic but transient rise in serum aminotransferases activity when excluding other causes of liver cell necrosis, especially viral or drug-induced hepatitis[21]. A visual summary of liver injury in COVID-19 is presented in Figure 1.

PREVALENCE AND CONSEQUENCES OF COVID-19-ASSOCIATED LIVER INJURY

The liver injury induced by COVID-19, including its pattern and severity, has not been uniformly defined or well characterized[25,26]. Some definitions reported in the literature, including DILI, are presented in Table 1. Secondary liver injury was the most common, being the first occurrence[3]. Liver injury has been reported as the elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels[25,26]. Thus, the liver injury appears to be of a hepatocellular (56%) rather than cholestatic (24%) or mixed (19%) pattern[3,25-28], while jaundice is uncommon[3]. Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) as markers of bile duct injury have not increased significantly in the respective studies[3,25]. However, not all liver function tests (LFTs) have been strictly reported[29].

Liver injury in COVID-19, manifested as changes in LFTs, is usually mild and transient[26,30-33] and does not require treatment[30,32]. However, severe cases have been reported[26,30,32]. Mild, moderate, and severe injuries were reported in 45%-65%, 21%, and 6.4% of the cases, respectively[25-27]. In one study, mild elevation in LFTs levels was reported in 90% of patients admitted to hospital with marked elevation during hospital stay[34]. Elevation in AST levels is more common than ALT and other LFTs[29,30]. In one report, the acute liver injury occurred on day 17 after the onset of symptoms[28]. In patients with severe COVID-19, the elevation in the transaminases and bilirubin levels was at least double that in patients with mild and moderate disease[33]. Elevation in GGT levels was more noticeable in severe cases, while ALP levels usually remained normal in both mild and severe cases[35]. Variable and inconsistent degrees of LFTs abnormalities, ranging from 3.75% to more than 50% of all patients, have been described[5,25,33,36]. A meta-analysis found a pooled incidence of elevated liver enzymes by 23.1%[37]. Although some studies did not show a statistical difference in abnormal LFTs between patients with severe and non-severe disease[37,38] or between survivors and non-survivors[39], many other studies have consistently shown elevated LFTs to be more prevalent in fatal or severe disease[1,2,14,28,34,40-43] in up to 58%-78% of cases[40,44,45].

Patients with LFTs abnormalities had a more severe inflammation[25-27] and degree of organ dysfunction[27]. At least two meta-analyses have confirmed the association between liver injury and the severity of COVID-19[46,47]. Liver injury had prognostic implications in patients with COVID-19. Liver injury or abnormal LFTs were associated with increased risk of ICU admission[25,27,48,49], intubation[25,49], mechanical ventilation need[27], acute renal injury, vasopressor use[25,27], long hospital stays[27], mortality[25,27,28,37,48,49], and composite of ICU admission and mortality[27,50]. Tables 2 and 3 present selected liver injury-related markers and clinical outcomes of non-survivors[39,43,44,51-55], or patients with severe disease[1,2,9,28,34,40,42,56-63], including those admitted to ICU due to COVID-19[1,2,57].

PRE-EXISTING LIVER DISEASE IN COVID-19-ASSOCIATED LIVER INJURY

Underlying CLD in patients with COVID-19 have been reported in several studies and

Table 1 Reported definitions for liver injury in coronavirus disease 2019

Term	Definition(s)
Liver disorder	Serum ALT or AST > 2 × ULN, TB > 2 × ULN, ALP ≥ 2 ULN[75]
Liver injury or acute liver injury	ALT and/or AST above 3 × ULN, ALP, GGT, and/or TB above 2 × ULN[9,34]
	ALT and/or AST ≥ 2 × ULN, with TB ≥ 2 × ULN and/or INR ≥ 1.7[70]
	ALT levels above 3 × the ULN[28]
Mild liver injury	ALT above the ULN and below 2 × the ULN[25]
Moderate liver injury	ALT between 2-5 × the ULN[25]
Severe liver injury	ALT above 5 × the ULN[25]
	Any elevation of enzymes above 3 × the ULN and bilirubin above 2 × the ULN[5]
Liver test abnormalities	Elevation of the following serum liver enzymes: ALT > 40 U/L, AST > 40 U/L, GGT > 49 U/L, ALP > 135 U/L, and TB > 17.1 μmol/L[34]
De novo LFTs abnormality	The occurrence of abnormal LFTs in patients with normal LFTs at admission[27]
LFTs elevation	Increase in serum liver enzyme levels above the ULN[27,28]
Mild LFTs elevations	Elevation 1-2 times above the ULN[25,34]
Hepatocellular or hepatocyte type	The pattern of abnormal LFTs with predominantly elevated ALT and AST[27]
	Patients with raised ALT and/or AST more than 3 × the ULN[34]
	AST/ALT activity is higher than the ALP/GGT activity, with liver enzyme activities calculated by multiples of their ULN[34]
Cholestatic or cholangiocyte type	Pattern of abnormal LFTs with predominantly elevated ALP and GGT[27]
	Patients with raised ALP or GGT 2 × the ULN[34]
	ALP/GGT activity was higher than the AST/ALT activity, with the liver enzyme activities calculated by multiples of their ULN[34]
Mixed type	Mixed pattern when the extents of AST/ALT and ALP/GGT are similar[27]
	A combination of both ALT/AST elevated more than 3 × the ULN and ALP/GGT twice the ULN[34]
Drug-induced liver injury	Any elevation in liver enzymes or TB after the initiation of the drug in the absence of identified common causes of liver disease[5]

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio; LFTs: Liver function tests; TB: Total bilirubin; ULN: Upper limit of normal.

ranged from 2% to 11%[30,36,64], up to 19% in one study[65]. Pooled prevalence of pre-existing CLD in one meta-analysis was 3%[66], which was comparable to that of another meta-analysis (3.6%)[5]. The latter reported pooled prevalence of CLD of 3.9% and 4.7% among severely infected patients and the non-survivors, respectively[5]. Compared with patients without underlying liver diseases, the odds ratio (OR) of developing severe disease was 0.81 [95% confidence interval (CI): 0.31-2.09, $P = 0.67$] [67]. The presence of underlying liver disease was associated with increased the risk of mortality and hospitalization, before {[risk ratio (RR): 2.8, 95%CI: 1.9–4.0, $P < 0.001$]; (RR: 1.7, 95%CI: 1.2-2.0, $P < 0.001$)} and after propensity matching [(RR: 3.0, 95%CI: 1.5-6.0, $P = 0.001$); (RR: 1.3, 95%CI: 1.1-1.6, $P = 0.006$)], when compared to those without liver diseases, respectively[68].

The presence of CLD was also found to be an independent predictor for ICU admission (adjusted OR 1.77, 95%CI: 1.03-3.04, $P = 0.04$) and mechanical ventilation need (adjusted OR 2.08, 95%CI: 1.20-3.60, $P = 0.0092$)[65]. The reported etiologies of the pre-existing liver diseases before COVID-19 included chronic viral hepatitis B and C, alcoholic and metabolic liver disease, cirrhosis of any cause, and others[5,26,31]. Liver cirrhosis is the end-stage of these liver-related diseases[31]. In one study ($n = 363$), 19% of patients had a pre-existing liver disease with the predominance of non-alcoholic fatty liver disease (NAFLD) (79.7%). Compensated cirrhosis, decompensated cirrhosis, and viral hepatitis B and C accounted for 8.7%, 4.3%, 2.9%, and 8.7% of all patients, respectively[65]. In contrast, the reported rates in one meta-analysis of 107 studies ($n = 20874$) were, CLD/cirrhosis in 61.1%, NAFLD in 19.5%, hepatitis B in 17.8%, and hepatitis C in 0.73% of patients[5].

Table 2 Reported data on survivors versus non-survivors in coronavirus disease 2019

Ref.	*N* (all) *n* (non-survivors)	Age (year)	Male	Pre-existing CLD	Type of liver disease	Elevated LFTs on admission (%)	LFTs levels on admission. ALT/AST/ALP/GGT (U/L)/TB (μmoL)	Selected complications or clinical outcomes
Cao *et al* [51]. China	*N* = 102 (*n* = 17)	53 *vs* 72	47.1% *vs* 76.5%	2.4% *vs* 5.9%	-	ALT: NR *vs* 41.1%	ALT: NR *vs* 40	ALI: 24.7% *vs* 76.5%; ARDS: 5.9% *vs* 88.2%; Shock: 3.5% *vs* 41.1%; MV: 2.4% *vs* 70.6%
Chen *et al* [52]. China	*N* = 274 (*n* = 113)	51 *vs* 68	55% *vs* 73%	-	HBV surface antigen positivity	ALT: 19% *vs* 27%; AST: 16% *vs* 52%	ALT: 20 *vs* 28; AST: 25 *vs* 45; ALP: 64 *vs* 76; GGT: 28 *vs* 42; TB: 8.4 *vs* 12.6	ALI: 2% *vs* 9%; ARDS: 52% *vs* 100%; Shock: 0% *vs* 41%; MV: 82% *vs* 16%
Chen *et al* [53]. China	*N* = 55 (*n* = 19)[1]	72 *vs* 77	50% *vs* 84.2%	2.8% *vs* 5.3%	-	ALT: 19.4% *vs* 31.6%; AST: 50% *vs* 73.7%	ALT: 40 *vs* 44;AST: 55 *vs* 78	MV: 30.6% *vs* 68.4%
Du *et al* [54]. China	*N* = 85[2]	65.8	72.9%	5.9%	-	ALT: 16.5%; AST: 32.9%; TB: 35.3%	ALT: 72.9; AST: 94.4; TB: 18.4	ALI: 35.3%; ARDS: 74.1%; Shock: 81.2%; MV: 93%[3]
Wu *et al* [42]. China	*N* = 84 (*n* = 44)[4]	50 *vs* 68.5	77.5% *vs* 65.9%	3.5%[5]	-	-	ALT: 35 *vs* 39; AST: 38.5 *vs* 37; TB: 11.6 *vs* 14.5	MV: 57.5% *vs* 97.8%[3]; Others reported as association
Yang *et al* [55]. China	*N* = 92[2]	69.8	53.3%	3.3%	-	-	ALT: 27; AST: 31; TB: 13.6	ALI: 16.5%; ARDS: 80.2%; MODS: 15.4%
Yang *et al* [39]. China	*N* = 52 (*n* = 32)	51.9 *vs* 64.6	70% *vs* 66%	-	-	-	TB: 13.1 *vs* 19.5	ALI: 30% *vs* 28%; ARDS: 45% *vs* 81%; MV: 35% *vs* 94%
Zhang *et al*[44]. China	*N* = 82[2]	72.5	65.9%	2.4%	-	ALT: 30.6%; AST: 61.1%; TB: 30.6%	ALT: 26; AST: 72; TB: 13.6	Hepatic damage: 78%; Liver-associated death: 1.2%; MV: 40.2%
Zhou *et al* [43]. China	*N* = 191 (*n* = 54)	52 *vs* 69	59% *vs* 70%	-	-	ALT: 24% *vs* 48%	ALT: 27 *vs* 40	ARDS: 7% *vs* 93%; Shock: 0% *vs* 70%; MV: 2% *vs* 100%[3]

[1]Patients ≥ 65 years subgroup (55 of 203 patients).
[2]Reported fatal cases only.
[3]Invasive and non-invasive mechanical ventilation.
[4]Subgroup of patients who developed acute respiratory distress syndrome (ARDS) after admission and those who progressed from ARDS to death (total patients = 201).
[5]Reported for all patients.
ALT: Alanine aminotransferase; ALI: Acute liver injury; ALP: Alkaline phosphatase; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; CLD: Chronic liver disease; GGT: Gamma-glutamyl transpeptidase; HBV: Hepatitis B virus; LFTs: Liver function tests; MODS: Multiple organ dysfunction syndrome; MV: Mechanical ventilation; N and n: Number of patients; NR: Not reported; TB: Total bilirubin.

Hepatitis B virus co-infection may subject COVID-19 patients to an exacerbated liver injury[30] and a more severe disease[69]. Acute liver injury in COVID-19 patients with hepatitis was significantly higher than that in patients without chronic hepatitis (15.0% *vs* 7.0%, $P < 0.001$)[70]. Patients with NAFLD, renamed as metabolic-associated fatty liver disease[26,31], had a significantly higher likelihood of abnormal LFTs, longer viral shedding time, and higher rate of COVID-19 progression (OR: 6.4, 95%CI: 1.5-31.2), compared to those without NAFLD[71]. NAFLD was significantly associated with ICU admissions (adjusted OR: 2.30, 95%CI: 1.27-4.17, $P = 0.03$) and mechanical ventilation need, (adjusted OR: 2.15, 95%CI: 1.18-3.91, $P = 0.02$) but not with mortality [65]. Furthermore, NAFLD in younger patients (< 60 years) was associated with the prevalence of severe COVID-19 (adjusted OR: 2.67, 95%CI: 1.13–6.34, $P = 0.03$)[72]. COVID-19 patients with liver cirrhosis were found to be at increased risk of mortality compared with those without the disease (RR: 4.6, 95%CI: 2.6–8.3, $P < 0.0001$)[68,71]. Multivariate analysis showed that liver cirrhosis was an independent predictor for mortality (adjusted OR: 12.5, 95%CI: 2.16-72.5, $P = 0.009$) but not for ICU admission or mechanical ventilation need[65].

Table 3 Reported data on critically ill, intensive care units, or severe coronavirus disease 2019 patients

Ref.	*N* (all), *n* (severe disease). Patient population	Age (year)	Male	Pre-existing CLD	Type of pre-existing CLD	Elevated LFTs on admission (%)	LFTs levels on admission. ALT/AST/ALP/GGT (U/L)/TB (μmoL)	Selected complications or clinical outcomes
Arentz *et al*[56]. United States	*N* = 21. Critically ill	70	52%	4.8%	Cirrhosis	-	ALT: 108; AST: 273; ALP: 80; TB: 0.6 mg/dL	ALI: 14.3%; Severe ARDS: 57.1%; MV:71%; Death: 52.4%
Cai *et al* [34]. China	*N* = 318 (*n* = 85)[1]. Non-severe *vs* severe	47. All patients	47.5%. All patients	5%. All patients	ALD, NAFLD, HVB	ALT: 6.4% *vs* 21.1%; AST: 0.68% *vs* 18.8%; GGT: 5.1% *vs* 29.4%; TB: 1.2% *vs* 7%	-	MOF: 0% *vs* 11.7%
Cai *et al* [9]. China	*N* = 298 (*n* = 58). Non-severe *vs* severe	41 *vs* 62.5	44.1% *vs* 67.2%	8.3% *vs* 13.7%	NAFLD: 3.3% *vs* 10.3%; ALD: 3.3% *vs* 1.7%; HBV: 1.7% *vs* 1.7%	-	ALT: 20 *vs* 26.8; AST: 26 *vs* 36; ALP: 61 *vs* 58; GGT: 21 *vs* 35.2; TB: 10.9 *vs* 11.2	ALI: 9.6% *vs* 36.2%; Discharge: 93.3% *vs* 75.9%; Hospital-stay: 19 d *vs* 27 d; Death: 0% *vs* 5.2%
Du *et al* [57]. China	*N* = 109. Non-ICU *vs* ICU	72.7 *vs* 68.4	65.5% *vs* 70.6%	3.4% *vs* 0%	-	ALT: 13.8% *vs* 19.6%; AST: 49% *vs* 43.1%	ALT: 21.6 *vs* 27; AST: 32 *vs* 40	Invasive MV: 0% *vs* 64.7%; Hospital-stay: 12.5 d *vs* 15.9 d
Guan *et al*[40]. China	N = 1099 (*n* = 173). Non-severe *vs* severe	45 *vs* 52	58.2% *vs* 57.8%	2.4% *vs* 0.6%	HBV	ALT: 19.8% *vs* 28.1%; AST: 18.2% *vs* 39.4%; TB: 9.9% *vs* 13.3%	-	ARDS: 1.1% *vs* 15.6%; MV: 0% *vs* 38.7%; Discharge: 5.4% *vs* 2.9%; Hospital-stay: 11 d *vs* 13 d; Death: 0.1% *vs* 8.1%
Huang *et al*[2]. China	*N* = 41 (*n* = 13). Non-ICU *vs* ICU	49 *vs* 49	68% *vs* 85%	4% *vs* 0%	-	AST: 25% *vs* 62%	ALT: 27 *vs* 49; AST: 34 *vs* 44; TB: 10.8 *vs* 14	ARDS: 4% *vs* 85%; Shock: 0% *vs* 23%; Invasive MV: 0% *vs* 15%; Discharge: 75% *vs* 54%; Death: 4% *vs* 38%
Lei *et al* [28]. China	*N* = 5771 (*n* = 1186). Non-severe *vs* severe	55 *vs* 59	45.1% *vs* 55.3%	1.2% *vs* 2.1%	Viral hepatitis Cirrhosis	-	ALT: 23 *vs* 26; AST: 22 *vs* 31; ALP: 65 *vs* 63; TB: 10.3 *vs* 10.6	Reported as association not absolute values
Li *et al* [58]. China	*N* = 548 (*n* = 269). Non-severe *vs* severe	56 *vs* 65	45.2% *vs* 56.9%	1.1% *vs* 0.7%	HBV	ALT: 22.3% *vs* 24.1%; AST: 23.3% *vs* 43.4%; TB: 2.3% *vs* 6.4%	-	ALI: 15.8% *vs* 23%; ARDS: 9.7% *vs* 68%; MV: 4% *vs* 34.2%[2]; Discharge: 72.9% *vs* 31.7%; Death: 1.1% *vs* 32.5%
Mo *et al* [59]. China	*N* = 155 (*n* = 85)[3]. General *vs* refractory	47 *vs* 61	44.3% *vs* 64.7%	2.9% *vs* 5.9%	-	-	ALT: 20 *vs* 28; AST: 32 *vs* 37	Critical case: 4.3% *vs* 40%; MV: 0% *vs* 41.2%; Others reported as association
Wan *et al* [60]. China	*N* = 135 (*n* =40). Mild *vs* severe	44 *vs* 56	54.7% *vs* 52.5%	1% *vs* 2.5%	-	AST: 16% *vs* 37.5%	ALT: 21.7 *vs* 26.6; AST: 22.4 *vs* 33.6; TB: 8.6 *vs* 9.8	ARDS: 1.1% *vs* 50%; Shock: 0% *vs* 2.5%; Discharge: 10.5% *vs* 12.5%; Death: 0% *vs* 2.5%
Wang *et al*[1]. China	*N* = 138 (*n* = 36). Non-ICU *vs* ICU	51 *vs* 66	52% *vs* 61.1%	3.9% *vs* 0%	-	-	ALT: 23 *vs* 35; AST: 29 *vs* 52; TB: 9.3 *vs* 11.5	ARDS: 4.9% *vs* 61.1%; Shock: 1% *vs* 30.6%; Invasive MV: 0% *vs* 47.2%
Wu *et al* [42]. China	*N* = 201 (*n* = 84)[4]. Non-ARDS *vs* ARDS	48 *vs* 58.5	58.1% *vs* 71.4%	3.5%[5]	-	-	ALT: 27 *vs* 35; AST: 30 *vs* 38; TB: 10.5 *vs* 12.9	MV: 0% *vs* 78.6%[2]; Others reported as association
Zhang *et al*[61]. China	*N* = 221 (*n* = 55). Non-severe *vs* severe	51 *vs* 62	44% *vs* 63.6%	1.8% *vs* 7.3%	-	-	ALT: 22 *vs* 32; AST: 27 *vs* 51; TB: 9.6 *vs* 11.4	ARDS: 0% *vs* 87.3%; Shock: 0% *vs* 27.3%; MV: 1.2% *vs* 74.6%[2]; Discharge: 21.1% *vs* 12.7%; Death: 0% *vs* 21.8%
Zhang *et al*[62]. China	*N* = 140 (*n* = 58). Non-severe *vs* severe	51.1 *vs* 64	46.3% *vs* 56.9%	5% *vs* 6.9%	Fatty liver and abnormal liver function	-	-	-

Zheng *et al*[63]. China	N = 161 (*n* = 30). Non-severe *vs* severe	40 *vs* 57	50.4% *vs* 46.7%	3.1% *vs* 0%	-	ALT: 6.1% *vs* 16.7%; AST: 7.6% *vs* 40%; TB: 4.6% *vs* 10%	ALT: 19.3 *vs* 23.9; AST: 23.4 *vs* 31.6; TB: 10.7 *vs* 12.7	-

[1]Total number of patients is 417 and 318 is the number for patients with liver injury (for which the comparison between severe and non-severe disease was done).
[2]Invasive and non-invasive mechanical ventilation.
[3]Reported patients with refractory and critical illness and ≥ 10 d of treatment in hospital.
[4]Subgroup of patients who developed acute respiratory distress syndrome (ARDS) after admission and those who progressed from ARDS to death.
[5]Reported for all patients.
ICU: Intensive care units; ALD: Alcoholic liver disease; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ALI: Acute liver injury; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; CLD: Chronic liver disease; GGT: Gamma-glutamyl transpeptidase; HBV: Hepatitis B virus; LFTs: Liver function tests; MOF: Multiorgan failure; MV: Mechanical ventilation; N and n: Number of patients; NAFLD: Non-alcoholic fatty liver disease; TB: Total bilirubin.

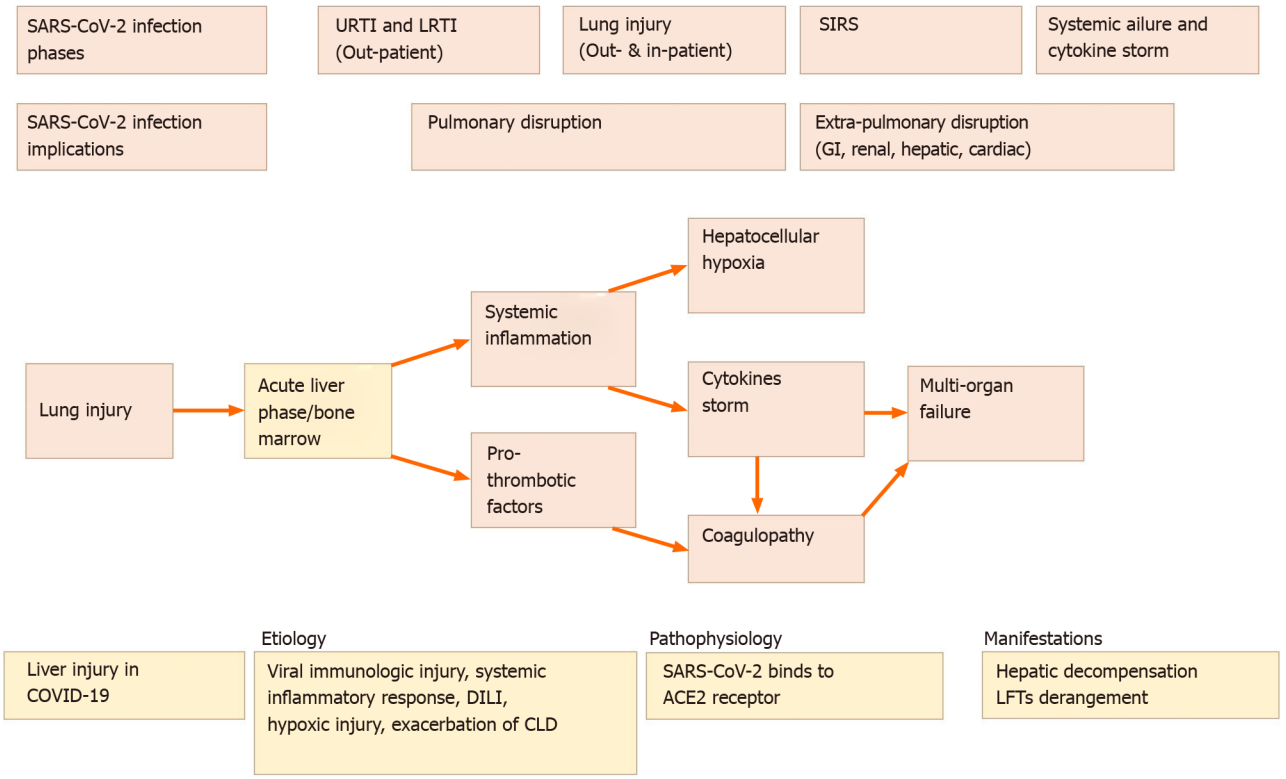

Figure 1 Pathogenesis of liver injury in coronavirus disease 2019. ACE2: Angiotensin-converting enzyme 2; COVID-19: Coronavirus disease 2019; CLD: Chronic liver disease(s); DILI: Drug-induced liver injury; GI: Gastrointestinal; LRTI: Lower respiratory tract infection; LFTs: Liver function tests; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; SIRS: Systemic inflammatory response syndrome; URTI: Upper respiratory tract infection.

DILI IN PATIENTS WITH COVID-19

Numerous medications that are currently used to treat SARS-CoV-2 infection carry the risk of hepatoxicity. Given that many medications are being used in combination, the interpretation of the commonly seen raised liver transaminases in patients with COVID-19 can be biased. While the efficacy of these medications towards improving COVID-19's morbidity and mortality is still to be proven, their safety should be monitored closely[73]. A retrospective study aimed to investigate adverse drug reactions (ADRs) in 217 COVID-19 patients using a hospital pharmacovigilance system in China found that 82 patients experienced 94 ADRs, with 13.8% of them were categorized as liver disorders. A multivariate analysis showed that the occurrence of ADRs has been associated with the length of stay (OR: 2.02, 95%CI: 1.03–3.96, P = 0.04), number of drugs used in hospital (OR: 3.12, 95%CI: 1.60–6.27, P = 0.001) and underlying diseases (OR: 2.07, 95%CI: 1.02–4.23, P = 0.045)[73]. In a prospective study using pharmacovigilance system in Spain, patients with COVID-19 had a higher incidence of hepatitis as a serious ADR than that in non-COVID-19 patients (45.1% *vs* 23.7%)[74]. In a meta-analysis of 10 studies, DILI in COVID-19 was reported in 25.4%

of the total patients[5]. Therapies that have been implicated in hepatotoxicity included remdesivir, lopinavir/ritonavir, oseltamivir, hydroxychloroquine, paracetamol[5], tocilizumab[74], in addition to antibiotics, non-steroidal anti-inflammatory drugs, herbal medications, and interferon[34]. In a retrospective, observational cohort study (n = 1827), the use of lopinavir/ritonavir, hydroxychloroquine, remdesivir, and tocilizumab was associated with statistically significant abnormal ALT and AST levels (*i.e.*, > 5 × upper limit of normal)[75].

Data on DILI's clinical significance have not been consistent. Sun *et al*[73] reported 18.1% of ADRs to be of serious severity, with 82.3% of them related to liver injury[73]. Ramírez *et al*[74] reported a mortality rate of 30.5% in COVID-19 patients with serious ADRs compared with 3.9% in non-COVID-19 patients with serious ADRs[74]. However, Kulkarni *et al*[5] concluded that remdesivir and lopinavir/ritonavir DILI was not life-threatening[5]. A systematic review and network meta-analysis of 110 studies reported no association between a regimen or an agent with non-cardiac severe adverse events[76]. In a multicenter and retrospective study (n = 565) on hospitalized COVID-19 patients, *de novo* LFTs abnormality was noted with tocilizumab (82% *vs* 52%; P = 0.009) and lopinavir/ritonavir (64% *vs* 48%; P = 0.045). Moreover, there was a trend towards an increased composite endpoint of death or transfer to ICU associated with *de novo* LFTs abnormality with an incidence of 14% *vs* 5% (P = 0.069)[27]. Although published data regarding the incidence, severity and clinical significance of DILI have not been consistent, it warrants close monitoring of LFTs. Table 4 summarizes the reported DILI of selected therapies against COVID-19[5,27,34,74,75,77-95].

DRUG-DRUG INTERACTIONS BETWEEN IMMUNOSUPPRESSIVE THERAPY AND COVID-19 AGENTS

Calcineurin inhibitors (CNIs), such as cyclosporine and FK506 (tacrolimus), antimetabolite drugs, such as mycophenolate mofetil (MMF), mycophenolic acid, and corticosteroids are commonly used for immunosuppression after liver transplantation (LTX)[96]. Some centres adopted dose modifications based on expert opinion with many uncertainties regarding the best approach for combination therapies and immunosuppressive agents against COVID-19. In two large academic centers in New York City including 90 patients with solid organ transplant, antimetabolite drugs doses were reduced or held in 88% of patients, steroids in 7%, and CNIs in 18%, with no reported acute rejection cases at 20-day follow-up[6]. In a prospective European study of 57 liver transplant patients with COVID-19, immunosuppression therapy doses were reduced in 39% of patients and discontinued in 7%. Reduction or continuation of therapy did not affect mortality, while the discontinuation effect was not assessed[97]. Drug interactions between COVID-19 medications and immunosuppression therapy were also considered. For instance, lopinavir-ritonavir combination interacts with CNIs and MMF, it is not recommended to be used with steroids[98] and has been reported to interact with mechanistic target of rapamycin (mTOR) as well[18]. Moreover, tocilizumab may decrease CNIs plasma concentration, unlike remdesivir which does not interact with the immunosuppressive drugs[98]. Hydroxychloroquine has been reported to interact with CNIs and mTOR[18]. Relevant recommendations included checking for drug interactions[18,98], dose reduction of steroids, CNIs and MMF[98,99], switching mTOR to CNIs[18], switching MMF and CNIs to steroids, and withdrawal of agents such as CNIs and MMF in severe COVID-19[99]. Monitoring of immunosuppressive drug levels should be warranted when possible[98,99]. The European Society of Clinical Microbiology and Infectious Diseases advised not to reduce the doses of immunosuppressive drugs in liver transplanted patients and raised the importance of considering vaccination with *Streptococcus pneumonia* and influenza vaccines[100].

IMPACT OF COVID-19 ON LIVER TRANSPLANTATION PROGRAMS

General measures

The unprecedented disturbance created by the COVID-19 pandemic has impacted different sectors of health care systems worldwide. For instance, elective services were cancelled or postponed while lifesaving transplant programs, including those for a liver transplant, have been continued. However, the non-lifesaving transplant services

Table 4 Reported effects of selected coronavirus disease 2019 therapies on liver

Medication (class)	Pattern of liver injury	Evidence
Corticosteroids (Anti-inflammatory agent)	Acute liver injury[77]	Multicenter cohort study (n = 774); COVID-19 with ARDS: Incidence of ALI versus control (18.3% *vs* 9.9%; P = 0.001)[77]
		Meta-analysis; critically ill COVID-19 patients: No association with serious adverse effects[78]
		RECOVERY trial: No reported serious ADRs or DILI[79]
Favipiravir (RdRp inhibitor)	Abnormal LFTs[80]	RCT (n = 150); mild-to-moderate COVID-19: Abnormal LFTs versus control 6.8% *vs* 2.7%)[80]
	Elevation of transaminases levels[81]	RCT; moderate COVID-19: Elevated ALT and AST were reported[81]
Hydroxychloroquine (Antimalarial agent)	Liver toxicity is not common[82]. Elevation of transaminases levels[74,75,82-84]	Retrospective study (n = 153): Elevation in AST (11%) and ALT (9%)[82]
		RCT (n = 504); mild-to-moderate COVID-19: Elevation in ALT or AST elevation 10.6% in HCQ plus azithromycin, 9% in HCQ, and 3.5% in control arm (P = 0.008)[83]
		Systematic review: Elevations of LFTs was transient[84]
		Recovery trial: No reported DILI[85]
Interferon	-	Data on safety in COVID-19 patients is scarce
Lopinavir/ritonavir (Protease inhibitor)	Rise in liver function parameters[5,27,34,74,86]	RCT (n = 199): Elevated AST versus control (2.1% *vs* 5.1%), elevated ALT (1.1% *vs* 1 %), elevated TB (3.2% *vs* 3 %)[86]
	Hyperbilirubinemia[5,34]	Meta-analysis: DILI in 37.2% of patients (as hyperbilirubinemia followed by elevation of transaminases)[5]
Remdesivir (RdRp inhibitor)	Not well established. Elevation of transaminases levels[5,75,87-89]. Elevation of TB levels[88]. Hypoalbuminemia[88]	Case series: Elevated aminotransferases in 23 % discontinuation in 4% of patients[87]
		RCT (n = 237) in severe COVID-19: Elevated TB versus placebo (10% *vs* 9%) and AST (5% *vs* 12%), hypoalbuminemia (13% *vs* 15%). Discontinuation in 1% of patients[88]
		Open-label, phase 3 trial: Elevated ALT (5%-6%) and AST (7%-8%)[89]
		Meta-analysis: Pooled incidence of DILI of 15.2%[5]
		Meta-analysis: No difference as compared to placebo in liver enzymes elevation[90]
Tocilizumab (Humanized recombinant monoclonal antibody)	Elevation of transaminases levels[27,75,91-94]. Liver injury as early as 24 h with a 40-fold increase in transaminases that normalized in 10 d[91]	Case series; 7 severe COVID-19 patients: Up to 4.5 folds elevated baseline ALT and AST. Transaminases normalized in 3 wk[92]
		Retrospective study (n = 1827): AST > 5 × ULN in 69.1%, and ALT > 5 × ULN in 72.1% of patients[75]
		Observational study (n = 104): Minor increase of AST, ALT (P < 0.001) and GGT (P = 0.003; no safety concerns on follow up[93]
		RCT (n = 243): ALT elevation versus placebo (5% *vs* 4.9%), AST elevation in 3.7%[94]
		RCT (n = 130); moderate or severe COVID-19: No increase in hepatitis risk[95]

COVID-19: Coronavirus disease 2019; ADRs: Adverse drug reactions; ALI: Acute liver injury; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase, ALI: Acute liver injury; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; DILI: Drug-induced liver injury; GGT: Gamma-glutamyl transpeptidase; HCQ: Hydroxychloroquine; LFTs: Liver functions tests; RCT: Randomized controlled trial; RdRp: RNA-dependent RNA polymerase; TB: Total bilirubin; ULN: Upper limit of normal.

were frequently delayed, exposing patients to emergency situations[101]. LTX is the most common solid organ transplantation procedure after the kidney, with a global rate of 3.7 *per* million population[102]. The indication of LTX in the acute phases of liver diseases includes acute liver cell failure, metabolic liver diseases, advanced

complicated cirrhosis, and CLD associated with systemic complications[103]. Elective LTX indications include advanced cirrhosis associated with deteriorating synthetic function, renal function, and the related complications[104]. The general precautions before LTX currently comprise a COVID-19 testing for both donors and recipients awaiting transplant and consenting for the possible hazard of acquiring nosocomial COVID-19[100]. The standard method of COVID-19 testing is through a naso-pharyngeal swab or intraoperative bronchoalveolar lavage. Viral load should be measured in positive cases. The transplant team should be adequately screened, and the risk of exposure identified[101]. Hollander and Carr[105] advised on the use of telemedicine, such as virtual clinics or *via* phone calls, to minimize both healthcare providers and patient's exposure to COVID-19[105]. The success of telemedicine in the Chinese territory during the peak of the pandemic could be transposed to future networking to use information and communication technology extensively during the care of patients with COVID-19[106].

ICU care of liver transplant patients in the era of COVID-19

Strict infection control measures are required in the post-operative care of LTX patients to prevent nosocomial infections that include COVID-19[107]. During the admission of LTX patients, they should be directed to separate rooms away from the general wards, and strict disinfection and isolation practices should be in place. Medical and surgical rounds should be minimized, and laboratory testing and radiological studies should be reduced to the least required[108]. Acquiring symptoms suggestive of COVID-19 in a LTX patient should prompt urgent evaluation with the relevant investigations[105]. Other challenges prompted by the COVID-19 pandemic include the increased demand for ICU beds, requiring health care practitioners to work in a dynamic way to maximize ICU bed utilization[109]. During the pandemic, the settings required for ICU should include separate units equipped with high-efficiency particulate air filters[110]. The goals of ICU disposition for LTX patients comprise neurological monitoring, hemodynamic monitoring and support, early weaning from the mechanical ventilator, preventing nosocomial infections and graft-related complications and enhancing early graft recovery[111]. Some institutes screen for COVID-19 in LTX recipients[112]. Simple and effective measures could be implemented to shorten the ICU stay for LTX patients through fast-track procedures, including operating room early extubation, reduction of ventilation time, and direct transfer from the recovery room to surgical wards[113,114]. Transplant services constantly demand resources, which have become extremely limited with the emergence of the COVID-19 pandemic due to staff shortage, saturation of the ICU, and drainage of supplies. Exceptional scarcity of donors and demand for organs also aggravate this problem[115,116].

Transplantation outcome during COVID-19 pandemic

Reports regarding the outcomes of LTX patients have been inconsistent. Although early reports have not found more severe or worse outcomes among immunosuppressed patients[117], subsequent data showed that solid organ transplant recipients diagnosed with COVID-19, including LTX, seemed to be at increased risk of severe disease, morbidity, and poor outcomes[6,118], such as high mortality with an in-hospital mortality rate of 29%[119]. Bossini *et al*[120] reported a higher rate of acute respiratory distress syndrome (ARDS) and death among patients who received solid organs[120], while others reported similar outcomes in COVID-19 patients with and without solid organ transplant[121]. In a multi-centre study of ICU patients after solid organ transplant, the rate of ARDS, duration of mechanical ventilation, vasopressors requirements, and death were similar between groups[122].

Vaccination considerations

It was reported that in liver transplant recipients, COVID-19 infection was not associated with increased mortality. However, these patients are subjected to severe disease, as evidenced by a higher rate of both ICU admission and mechanical ventilation use[123]. The European Association for the Study of the Liver (EASL) suggested a particular form of judging the vaccination decision based on patient's morbidities[124]. The immune response to COVID-19 vaccination could be lower in LTX patients when compared with healthy subjects. Poor response to vaccination is affected by age, renal function, and enhanced immune suppression[125].

IMPLICATIONS AND FUTURE DIRECTIONS

The COVID-19 pandemic has been presented as an unprecedented global health care crisis, causing significant setbacks among various health care services including the management of CLD. Besur *et al*[35] reported that screening for CLD, its complications and regular follow up visits were deferred which affected slowing or reversing the progression of CLD and worsened the prognosis of patients with CLD. Late identification of CLD complications such as hepatocellular carcinoma (HCC) could also affect the clinical outcomes in these patients. Social distancing measures have put CLD patients at risk of malnutrition, reduced mental health capacity, and decompensation [35]. The evidence associating acute liver injury with poor patients' outcomes and increased severity of COVID-19 is growing, and more research is necessary to further explain the relation between liver biomarkers changes and patients' outcomes in COVID-19[126]. Various factors influence the course of COVID-19, and there is a need for international collaborative registries to clarify the full spectrum of the disease. The registries, Surveillance Epidemiology of Coronavirus (COVID-19) Under Research Exclusion (SECURE-Cirrhosis) and Coronavirus (COVID-19) in liver disease reporting registry (COVID-HEP) were established to report data on patients with liver disease. The last report published in August 2020 by SECURE-Cirrhosis and the EASL supported COVID-Hep, reported 158 deaths (31%) among patients who had cirrhosis and developed SARS-CoV-2 infections[127]. When this article was written, the latest update from both COVID-Hep and SECURE-Cirrhosis registries reported 1341 cases that included 645 cases with cirrhosis, 205 liver transplant recipients and 270 deaths as of February 12, 2021[128].

COVID-19 pandemic has disrupted various healthcare services worldwide, limiting the services offered to urgent and emergent cases. These changes in services, clinician behaviour and re-organization of hospital activities can indirectly affect morbidity and mortality[129]. delaying or halting diagnostic and therapeutic services for diseases with a high global burden such as cardiovascular diseases can contribute to long-term and indirect adverse health outcomes. For example, cardiac diagnostics procedures, stress tests[129], emergency department (ED) and hospital admissions, procedures and treatments were markedly declined during the pandemic year as compared with that of the previous years[130]. In 909 inpatient and outpatient centres from 108 countries, the rate of cardiac diagnostic procedures decreased by 42% and 64% as of March and April 2020, respectively, with the highest reduction of 78% observed for the stress tests, as compared with March 2019.[130]. A further 22% reduction was noted in low and low-middle income countries, which might be attributed to inaccessible personal protective equipment and telehealth services[130].

In United Kingdom, a cross-sectional study conducted in nine hospitals compared the hospitals' cardiovascular activity data between October 2019 and May 2020 with the respective weeks in 2018 and 2019. There was a marked decline in ED attendances, admissions and hospital procedures and treatments[129]. Patients with other chronic diseases which require close follow up have been negatively affected as well. A cross-sectional study of six referral centres in France showed that in 2020 significantly fewer patients with HCC were referred to the multidisciplinary tumour board (P = 0.034) and fewer received the first diagnosis of HCC (P = 0.083) compared with 2019[131]. Therapy optimization and frequency of follow-up visits were also affected by the global pandemic in response to social distancing and re-allocation of services towards fighting COVID-19. A delay in therapy modification for more than one month was noted in 21.5% *vs* 9.5% of patients during 2020 compared with 2019 (P < 0.001), respectively[131]. In patients with hepatitis C virus who were following up for HCC, there was significant reduction in their scheduled visits, *i.e.*, by before 75%, 63.0%, and 49.1% in March to May 2020, respectively, compared with 97% before February 2020 [132]. Surgical interventions for HCC have significantly declined or stopped across many centers in the world due to increased risk of blood transfusion, ICU stay, prolonged hospitalization and developing COVID-19 after surgery[133]. In a national survey by the Italian Association for the Study of the Liver, HCC treatment was affected; where surgical treatment was reduced in 44% and suspended in 44% of the participating centres, while the loco-regional treatment was reduced in 34% and suspended in 8% of the centres[134].

CONCLUSION

The pathogenesis and characteristics of COVID-19-related multifactorial liver injury

can be explained by multiple mechanisms. The knowledge about the full spectrum of SARS-CoV-2 infection is being accumulated, given the novelty of the disease and the constantly reported new data. Liver dysfunction is commonly seen in patients presenting with the severe form of COVID-19. Various therapeutic options used for COVID-19 can lead to DILI and contribute to the exacerbation of the existing liver injury. It is challenging to identify the causal factor in the settings of infection, sepsis, and/or hypoxia, especially when the liver enzymes abnormalities are non-specific. The underlying liver disease has not been linked with poor outcomes. Hospitalized patients or those with liver comorbidities should be monitored closely. Patients with COVID-19 and LTX must maintain strict infection control and monitor drug interactions while maintaining immunosuppressive therapy at regular doses. Future research would help explain liver injury associated with SARS-CoV-2 infection and design specific guidelines for the management of COVID-19 in these patients.

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REVIEW

In the era of rapid mRNA-based vaccines: Why is there no effective hepatitis C virus vaccine yet?

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Abstract

Hepatitis C virus (HCV) is responsible for no less than 71 million people chronically infected and is one of the most frequent indications for liver transplanta-tion worldwide. Despite direct-acting antiviral therapies fuel optimism in controlling HCV infections, there are several obstacles regarding treatment accessibility and reinfection continues to remain a possibility. Indeed, the majority of new HCV infections in developed countries occur in people who inject drugs and are more plausible to get reinfected. To achieve global epidemic control of this virus the development of an effective prophylactic or therapeutic vaccine becomes a must. The coronavirus disease 19 (COVID-19) pandemic led to auspicious vaccine development against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus, which has renewed interest on fighting HCV epidemic with vaccination. The aim of this review is to highlight the current situation of HCV vaccine candidates designed to prevent and/or to reduce HCV infectious cases and their complications. We will emphasize on some of the crossroads encountered during vaccine development against this insidious virus, together with some key aspects of HCV immunology which have, so far, ham-pered the progress in this area. The main focus will be on nucleic acid-based as well as recombinant viral vector-based vaccine candidates as the most novel vaccine approaches, some of which have been recently and successfully employed for SARS-CoV-2 vaccines. Finally, some ideas will be presented on which methods to explore for the design of live-attenuated vaccines against HCV.

Key Words: Hepatitis C virus; Vaccine candidates; Nucleic acid-based vaccines;

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Core Tip: Hepatitis C virus (HCV) remains a global health burden despite the successful introduction of direct-acting antiviral therapies. In order to achieve global control of HCV epidemic a vaccine is necessary. Its development has faced many hurdles, reason why it is still elusive. Herein, we describe all the challenges during HCV vaccine research, focusing on HCV immunology and emphasizing on current vaccine candidates, particularly nucleic acid-based as well as recombinant vector-based vaccines. We also highlight the impact of severe acute respiratory syndrome coronavirus-2 vaccine race on the renewed interest on HCV vaccine production. Finally, we present ideas on live-attenuated vaccine approaches against HCV.

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INTRODUCTION

Hepatitis C virus infection and the need for a vaccine

Hepatitis C virus (HCV), discovered in 1989[1], represents an important health burden. In 2015, the World Health Organization (WHO) estimated that there were at least 71 million people chronically infected with HCV, which represents a global prevalence of approximately 1%[2]. Additionally, around 400000 deaths occurred from infection complications.

Infections with HCV cause both acute as well as chronic liver disease in 60%-80% of the cases. Chronicity is associated with the development of cirrhosis (15%-30%) and hepatocellular carcinoma (HCC)[3]. Liver damage resulting from this infection makes it one of the most frequent indications for liver transplantation worldwide[4-8].

The problem of HCV infections worldwide has led the WHO to propose the elimination of viral hepatitis as a public health burden by 2030[2]. However, in order to achieve this goal, big scale interventions are needed, such as screening testing, effective treatment and hopefully vaccination, the latter still non-existing for HCV.

Access to widely available screening tests is uncommon and is hindered by economic reasons, particularly given the fact that new HCV infections are mainly asymptomatic[9]. This leads to an underestimation of the disease prevalence and does not contribute to the eradication goal. Concerning treatment, the development of interferon-free (IFN-free) regimens based in direct-acting antivirals (DAAs) has revolutionized HCV therapy. These antivirals have significantly increased response rates (up to 98%) and greatly reduced treatment duration to only 8-12 wk of oral treatment. DAAs have generated optimism on the global control front, and some consider that this pathogen can now be effectively controlled solely by means of antiviral therapy[10,11]. However, there are some limitations and obstacles to keep the virus in check, in particular, the cost and practical aspects of treatment access, which is uneven among different countries and leaves underdeveloped regions without treatment[11]. Additionally, resistance to DAAs emerged concomitantly with their development and implementation. Resistance-associated substitutions have been detected both before as well as during and after treatment with DAAs[12]. Another interesting aspect to consider is that eliminating HCV infection with DAAs does not eradicate the risk of developing liver cancer. Also, protective immunity is usually insufficient after natural or treatment-induced viral clearance, thus, the possibility of reinfection remains[13]. Together, these facts make HCV elimination in high-risk groups a very challenging task and the need for an effective prophylactic vaccine remains the greatest uncovered medical problem in the hepatitis C field[14]. Vaccination against HCV infection would reduce public healthcare resources by avoiding expensive DAA-based regimens or medical treatments for any liver or metabolic

complications derived from long-term infections[15-17], especially in low- or middle-income countries, where HCV prevalence is still moderate-high and access to diagnosis and treatment uneven and costly[18].

Proper immune responses are able to clear HCV acute infections, preventing the progression to chronicity (in 20%-40% of infected individuals). This fact suggests that vaccination could be a reasonable goal[19] provided we grasp a better understanding of immune responses against HCV in order to develop different vaccine candidates that allow for appropriate protection.

Global epidemic control will only be possible if the number of new HCV infections is reduced alongside with an increased number of cured patients[11,14]. However, a recent report showed that almost 60% of 91 surveyed countries had, in 2016, higher rates of infection than cures, making the goal of HCV elimination as a health burden by 2030, difficult to achieve[20].

For all the reasons previously mentioned, safe and effective prophylactic and/or therapeutic vaccines are necessary for the global control of HCV epidemic[11,21-24]. Indeed, no infectious disease has been controlled and eradicated with antimicrobial treatment, while it has in fact been possible by vaccination[10]. Furthermore, effective vaccination strategies widely available have been the only unfailing method to keep viral transmission at bay by providing herd immunity[25]. Modelling studies have indicated that, even with the introduction of new DAA treatments, only a quasi-eradication of HCV would be possible[26,27], highlighting the need for a vaccine against HCV.

Two extraordinary and unique situations that took place during this last year have fueled optimism on vaccine development against HCV. First, the Nobel Prize in Physiology or Medicine 2020 for the discovery of HCV which was awarded last October [28]. Three distinguished researchers, Harvey J. Alter, Michael Houghton and Charles M. Rice, received the prize for their contribution in identifying the etiological agent of the hepatitis formerly known as non-A non-B, and enabling the development of screening tests and antiviral drugs for its treatment. All of them expressed their hopes for a future vaccine against hepatitis C in their Nobel lectures, and Charles M. Rice specifically stated that he hoped we can learn from all the efforts that are being put into developing coronavirus disease 19 (COVID-19) vaccines[29]. This last state-ment refers to the second event from last year that has renewed interest on HCV vaccines: The COVID-19 pandemic and the remarkable development of several vac-cines to fight it. In the same line, in June 2020, the National Institutes of Health (NIH) opened a grant opportunity for the design of vaccines against HCV assigning USD 8 million to this aim[30].

This review focuses on different vaccine candidates designed to prevent or diminish HCV infection cases, and summarizes all the pitfalls encountered during vaccine development against this virus, including some key aspects of HCV immunology. We make special emphasis on nucleic acid-based vaccines as well as recombinant viral vectors and provide information on severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccines as examples of approaches that might be important in HCV vaccine development.

Prophylactic vs therapeutic vaccines

Vaccine candidates with two different goals have been considered to control HCV epidemic: Prophylactic and therapeutic (primary and secondary prevention, respectively). The most widespread use of vaccination has always been to prevent a particular disease (prophylactic vaccination)[31] by building immunity in an individual prior to the first encounter with the pathogen, and thus becoming immune to a particular illness. On the other hand, therapeutic vaccination is meant to induce immune responses against a disease that is already in course in a given individual[32].

As we will later discuss in detail, the challenges for designing an effective prophylactic vaccine are vast (HCV variability and diversity, limited animal models and a complex immunological response). Many preventive vaccines against other viral patho-gens are able to induce neutralizing antibodies (nAbs) that correlate with protection, which seems to be difficult to achieve for HCV[14]. Nevertheless, even a low efficacy prophylactic vaccine might be useful to decrease the epidemic impact in high-risk populations by reducing the number of new infections[33-35].

Therapeutic vaccines against HCV have great potential to aid in controlling chronic infections by increasing curing rates or reducing therapy duration[36]. In this new DAA era, sustained virological response (SVR) rates are extremely high (above 98%) and treatment duration has already been shortened compared to classic dual therapy (pegylated IFN-α plus ribavirin). However, there are difficult to treat patients (with active HCC or severe liver decompensation, those experiencing multiple DAA

treatment failures, or those infected with HCV genotype 3)[37] for which this therapeutic approach would be beneficial. These vaccines would boost HCV-specific T cell responses and would help in three different ways: (1) Preventing viral relapse if therapeutic vaccines were to be administered in conjunction with DAA therapy; (2) Maximizing early viral clearance and thus increasing SVR rates by first employing a therapeutic vaccine followed by the antiviral treatment; and (3) Producing partial control of HCV infection just by means of therapeutic immunization and thus reducing viral load[38]. Despite promising results in decreasing viral titers, rebounds have been observed, most likely due, either to immune escape or the inability of properly inhibiting viral replication or eliminating most of HCV-infected hepatocytes[21].

Expected outcome of effective vaccine candidates

In general, effective vaccine candidates should stimulate generation of nAbs and a proper cellular immune response. In order to design vaccines that elicit protective immunity against HCV, it is of utmost importance to consider the virus tropism (mainly hepatocytes), transmission route (parenteral transmission through contaminated blood) and pathogenesis[39].

A vaccine that induces immune responses similar to those produced by individuals which have successfully cleared the virus after an acute HCV infection, might prove valuable[19]. As we will discuss in the next section, vigorous responses of broadly cross-reactive CD4+, CD8+ T cells to conserved epitopes[40-42], as well as nAbs contribute to HCV spontaneous clearance[43,44].

ADAPTIVE IMMUNE RESPONSE IN HCV INFECTION

Approximately 20%–40% of HCV-infected patients clear the virus spontaneously, while the rest develop a persistent infection that will result in severe fibrosis, cirrhosis and HCC[3,45]. Thus, it is essential to understand the immune protection induced during acute infections in patients that achieved spontaneous viral clearance in order to determine the immune parameters that a successful vaccine has to reach.

Multiple evidences in human and animal models have demonstrated the undoubted association of spontaneous viral clearance with a broad, sustained HCV-specific T cell-mediated immunity (CMI) to conserved HCV non-structural proteins[46,47] and nAb targeting conserved regions of viral envelope glycoproteins E1E2[48].

As will be detailed below, both arms of the immune response are primed during HCV infection, but the characteristics vary depending on whether an acute infection is spontaneously resolved or if it evolves to chronicity.

Cellular immune protection

While HCV-specific CD8+ T cells are the main effector cells, the outcome of infection depends on eliciting efficient virus-specific CD4+ T cell responses[49]. These cells are the central regulators of adaptative immunity providing help for priming CD8+ T cell response as well as antibody response during viral infections. The breadth of the T cell response is a key determinant to spontaneously clear HCV. High numbers of CD4+ and CD8+ T cells targeting different epitopes were observed in individuals who resolved acute infections in comparison to those who evolve to chronicity[42,50,51]. These cells are multi-specifically targeting both structural and non-structural HCV proteins[46,52,53]. However, CD8+ T cells targeting non-structural proteins are immunodominant and associate with spontaneous clearance[54].

The strength of the CMI is also important for HCV infection outcome. Indeed, a robust HCV-specific CD8+ T cell response is associated with the resolution of acute HCV infection[55]. In an acute infection, cytotoxic T lymphocytes (CTLs) have cytolytic and noncytolytic functions which mediate viral eradication[56]. They traffic from the lymph nodes to the liver, where they recognize HCV-antigenic peptides loaded on human leukocyte antigen class I in infected hepatocytes. These infected cells can be lysed through the action of perforins and granzymes, or, killed *via* Fas/FasL interactions that activate the caspase cascade and end up in the apoptosis of the target cell. The noncytolytic function occurs without destroying infected cells, where viral replication is inhibited by cytokines released by CTLs which generate an antiviral environment.

Broad specific CD4+ T cells are detected during the acute phase regardless of the final outcome. However, these cells undergo an early decrease in frequency and breadth in persistent HCV infection compared to patients who clear the infection spontaneously[57]. Thus, spontaneous resolution is associated with a CD4+ T cell

response significatively stronger in comparison to persistently, or chronically infected individuals[58,59].

In chronic infections, the limited functionality of specific CD4+ T cells due to the lack of proliferative capacity and cytokines production[59-61] leads to a dysregulated CD8+ T cell response which facilitates the emergence of escape viral variants[62]. Dysfunctional CD8+ T cells are unable to control the viral load and become exhausted because of the persistent exposure to HCV epitopes which have not mutated[63]. Thus, these exhausted T cells undergo a progressive loss of their cytotoxic activity, proliferative capacity and proinflammatory cytokines production[64,65]. However, it is of note, that the cytolytic activity, and in particular the Fas/FasL dependent function, are associated with HCV immunopathology. Fas expression is up-regulated in hepatocytes of an infected liver whereas FasL is expressed in CTLs. This leads to liver damage by apoptosis of both infected and bystander hepatocytes, and subsequent liver fibrosis development[66].

Humoral immune protection

During acute HCV infection antibodies are produced and target epitopes in both structural and non-structural proteins, however, the envelope glycoproteins E1 and E2 are the main targets of the humoral immune response. Located at the N-terminal end of E2, the hypervariable region 1 (HVR1) is an immunodominant motif[67], which is the most variable region of the HCV genome[68]. Mutation in neutralizing epitopes allow the virus to escape from isolate-specific nAbs[69-71].

Early studies reported that nAbs developed against HCV target the HVR1 region of E2, however these nAbs were isolate-specific[67,69]. Thus, diverse studies have identified monoclonal antibodies (mAbs) that target conserved sites across multiple HCV genotypes located on either linear[72,73] or conformational[74,75] epitopes on E2 ectodomain.

Analyzing sera from different patients who were infected with the same HCV isolate showed that 43% of those who resolved their infections had nAbs against the main HVR1 variant, whereas these antibodies were present only in 13% of patients who evolved to chronicity[76]. Interestingly, plasma isolated from HCV-infected patients immediately prior to clearance has a better capacity to neutralize HCV strains from different genotypes compared to acute infection plasma from patients who subsequently evolve to persistence[77,78]. Furthermore, analysis from patients who cleared HCV infection showed detectable level of nAbs at earlier time points in comparison with acute infections that proceed to chronicity[79]. Chronic infections have been associated with a delayed cross-reactive nAbs response[43,77,78,80]. Although cross-reactive nAbs elicited during chronicity are not able to clear the infection, these have been associated with reduced liver fibrosis[81].

Despite the high genetic diversity of HCV, it was possible to isolate broadly neutralizing human Abs (bNAbs) from HCV-infected individuals, capable of neutralizing diverse HCV genotypes targeting relatively conserved regions on envelope glycoproteins[48,75,82]. These bNAbs have shown to be protective against infection in animal models of HCV[75] and are capable of abrogating established HCV infection in a humanized transgenic mouse model[48]. These findings underscore the protective role of the antibody response.

Evidence of protective immunity against HCV reinfection

The resolution of the initial HCV infection does not lead to sterilizing immunity so patients who previously controlled the primary HCV infection can be infected again [83]. However, differential rates of reinfection and/or chronicity have been reported among people who inject drugs (PWIDs) with the same risk of exposure, being reduced in people previously infected in comparison with people without previous infection[84]. Resolution is achieved in about 80% of HCV-reinfected patients[85].

Reinfection was characterized by a significant reduction in duration and magnitude of viremia compared with the primary infection and it was also shown to protect against persistence[85]. Moreover, clearance of reinfection was associated with an earlier and higher frequency of broadened T cells secreting IFN-γ as compared to primary infection[86-89] and an early induction of nAbs[85,90].

Long-lived memory HCV-specific CD4+ and CD8+ T cells are detected in the peripheral blood in humans following spontaneous resolution of the primary infection for up to 20 years[89,91]. CD4+ T cell depletion before reinfection leads to viral persistence even in the presence of functional CD8+ T cells which evidences the protective role of memory T cells upon re-exposure to HCV. While CD8+ T cells are the main effector cells in viral control, CD4+ T cells are essential for CD8+ T cell function and prevent viral escape within epitopes targeted by CD8+ T cells.

CHALLENGES FOR DEVELOPING ANTI-HCV VACCINES

A number of difficulties have hindered the development of vaccines against HCV throughout the years (Figure 1). Despite all the knowledge acquired on the biology of this virus in recent years, a full understanding of key aspects of its pathogenesis and the host's immune response remains elusive. Taking into account the correlate of protection, an effective vaccine needs to be able to prime both arms of the adaptative immune response. Thus, vaccination has to induce an early and sustained expansion of specific CD4+ and CD8+ T cell response. Alongside cellular immunity, cross-reactive nAbs need to be elicited to provide protection against different variants and genotypes.

In this section we will go over the most important challenges on the design and validation of an effective vaccine against HCV.

Lack of economic incentive

Despite the fact that vaccines are great tools to prevent diseases, usually they are not as profitable as are drugs and other health services, and therefore investing in vaccine development is less appealing for the pharmaceutical industry[92]. Additionally, the development of vaccines with two different aims (prophylactic and therapeutic) would probably be expensive, and including prime/boost vaccination strategies may result impractical[19]. On another front, most newly infected individuals are PWID which mainly belong to populations with limited financial resources. This represents another discouraging aspect for companies interested in vaccine development[19].

From an economic perspective, though, there is well-reported evidence that vaccines are, in the long run, the most cost-effective public health measure after access to clean water[93,94]. A vaccine to fight HCV will, most likely, not be an exception.

Viral genetic diversity and variability

HCV is an enveloped virus with a single-stranded positive RNA genome which has a single open reading frame (ORF) flanked by non-coding regions at both ends (5′ and 3′). For these features, it is classified as the prototype member of the Hepacivirus genus within the *Flaviviridae* family[95]. The ORF codes for a polyprotein of around 3000 amino acids which is co- and post-translationally processed into three structural (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B)[96].

Mutation is a key mechanism contributing to HCV genetic diversity and it is mainly driven by the error prone NS5B RNA-dependent RNA-polymerase[97]. HCV has an approximate mutation rate of 10^{-5} mutations/nucleotide/replicative cycle[98,99], a characteristic which together with big population sizes, short generation times, and high replication rates generates the intra-host circulation of a complex population of closely related genome variants, usually termed as viral quasispecies[100,101]. Of utmost importance is the N-terminus of the envelope protein E2[67]. It contains the HVR1 region of about 30 amino acids which exhibits a huge variation among different isolates, and it is the most variable region of the entire HCV genome[68]. Even though most HCV-infected individuals develop nAbs against the virus, this high variability represents a problem as it allows the virus to escape immunologic surveillance and prevents the development of vaccines that induce cross-reactive nAbs[21]. Thus, a major challenge for the development of a broadly reactive vaccine for the control of HCV infection is identifying conserved neutralizing epitopes outside of HVR1.

Notably, mutations within HVR1 have also been associated with resistance to cross-neutralizing antibody response even if their epitopes are conserved, which highlights again the difficulties in achieving HCV neutralization as HCV could persist even in the presence of an antibody response to conserved epitopes[102,103]. This finding suggests that the neutralizing capacity of an antibody should not only consider the degree of conservation of its epitope.

Mutation rates coupled with the selective pressure exerted by the host's immune system has steered HCV diversification into 8 genotypes and 90 subtypes[104,105]. HCV strains from different genotypes differ by 30% in their nucleotide positions within the coding region, whereas subtypes exhibit 15% nucleotide variation[106]. Genotypes 1 and 3 are the most prevalent worldwide (accounting for 49.1% and 17.9% of diagnosed cases, respectively), and are most frequently found in developed countries[107].

The quasispecies dynamic as well as the resulting viral diversity confers HCV an amazing ability to adapt which in turn implies the possibility to escape from different therapeutic or preventive approaches such as antiviral drugs or vaccines[108-112].

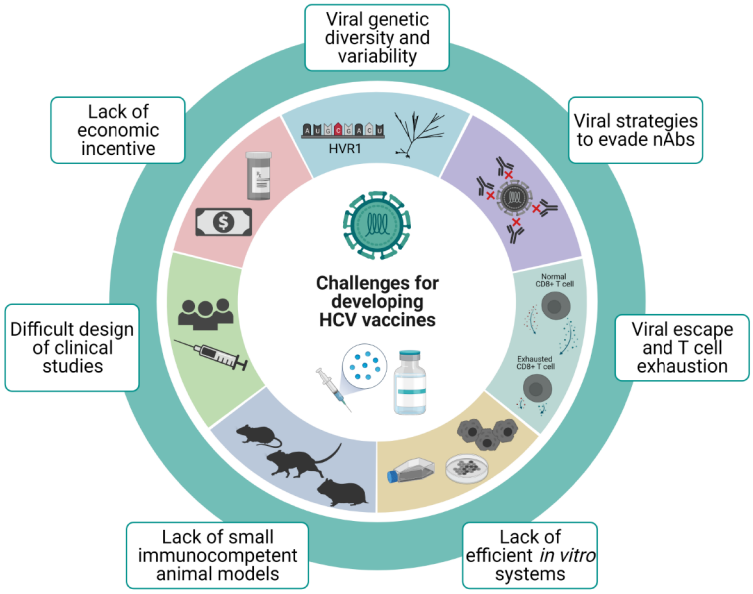

Figure 1 Challenges in hepatitis C virus vaccine research. Graphical representation of all the hurdles yet to be overcome in order to develop effective vaccines against hepatitis C virus. Image created with BioRender.com. HCV: Hepatitis C virus. HVR1: Hypervariable region 1.

Thus, T cell-based vaccines intended to induce broadly reactive immune responses by targeting more conserved regions/proteins of the virus are desirable if the aim is to protect against new infections and/or persistence[11,21].

Viral strategies to evade neutralization by antibodies

Viral entry to host cells and viral interactions with different host factors could theoretically be blocked by nAbs targeting HCV envelope glycoproteins E1 and E2. However, the virus has evolved several mechanisms which affect the host´s ability to neutralize the virus. One of the mechanisms has been described extensively above (genetic diversity, particularly in HVR1 region), yet there are a number of other strategies employed by this virus to evade neutralization: (1) Glycosylation of structural proteins; (2) Cell-to-cell transmission; (3) Interfering antibodies; (4) Association with lipoproteins; (5) Antibody decoy; (6) Flexible conformational epitopes; and (7) Enhancing of viral entry.

Glycosylation of structural proteins: This feature reduces their immunogenicity as they are recognized as selfstructures. This is an important mechanism used by HCV to escape host humoral immune response. Glycans act by masking antigenic sites targeted by nAbs, interfering sterically with antibody neutralization[113]. Indeed, the deletion of N-glycans leads to an increase in E1E2 immunogenicity and can induce a more potent antibody response against HCV[114-116]. Glycan shift is another mechanism to induce neutralization resistance through glycosylation. Single point mutations which result in deleting a glyco-sylation site or generating a new glycosylation site in another part of the protein could facilitate viral resistance to neutralization. It has been reported that a new glycosylation site arose after incubating for 5 d a cell-culture derived HCV with nAbs obtained from mice. As a result, those broadly nAbs showed a decrease in their efficacy[117].

Cell-to-cell transmission: It is another mechanism for viral dissemination, which avoids the extracellular compartment and favors escaping host humoral immune responses[118,119].

Interfering antibodies: When non-nAb bind to sequences in the C-terminal region of HVR1, they disrupt the recognition of conserved epitopes by antibodies with neutralizing capability. Indeed, the remotion of interfering antibodies in chronic patients and vaccinated chimpanzees increases virus susceptibility to neutralization highlighting the role of interfering antibody in viral escape[120]. Similarly, when HVR1 was removed, enhanced and broad cross-neutralizing activity was observed[121,122].

Association with lipoproteins: HCV circulates in the blood in association with triglyceride-rich lipoproteins and low-density lipoproteins forming hybrid lipoviral

particles, which are a hallmark of infectious HCV particles. Several host-derived factors play a role in evading antibody neutralization. Lipoproteins such as apolipoprotein E contribute to humoral immune escape by hiding relevant neutralization epitopes in E2 protein, preventing them to be exposed during HCV assembly and maturation, hence, abrogating antibody neutralization[123,124].

Antibody decoy: Interestingly, *in vitro* studies have reported that HCV-infected cells release E2-containing exosomes that act as antibody bait making HCV virions less susceptible to neutralization[125].

Flexible conformational epitopes: The capacity of some conserved neutralizing epitopes in E2 to adopt different conformations when complexed with diverse antibodi-es contributes to evade neutralization by antibodies. This conformational flexibility must be taken into account during vaccine design[126].

Enhancing of viral entry: It has been shown that host mutations that alter the interaction of serum components like high-density lipoprotein with scavenger receptor BI enhance viral entry to the cell[127]. This, in turn, protects the virus against humoral response as the time window in which nAbs can bind and act is reduced[128,129]. Fofana *et al*[130] (2012) also showed that mutations in the E2 glycoprotein, conferred viral escape to humoral responses by altering the use of the T cell receptor CD81[130].

Despite these challenges, it has been possible to isolate broadly cross-neutralizing mAbs with the ability to block HCV infection of various genotypes and thus, protect against heterologous viral infection[75,131-134]. These findings suggest that a prophylactic vaccine against HCV may indeed be achievable.

The elucidation of the crystal structure of E2 has provided a better insight into different antigenic domains and regions that allow a rational vaccine design. A study showed that epitopes within E2, exhibiting moderate or conserved variability, were efficiently targeted by bNAbs[135,136]. Unfortunately, despite the relative conservation of some bNAbs epitopes, escape mutations have been identified[137,138].

Escape mechanisms from T cell responses: Viral escape and T cell exhaustion

Several studies have evidenced the key role of cellular immunity in the clearance of infection. An effective vaccine has to induce a rapid recall of the memory T cell responses that is associated with reduced viraemia and a higher likelihood of spontaneous resolution. However, the virus has developed different mechanisms to lead to an inefficient cellular response even when re-exposed with homologous virus: (1) Viral escape T cell recognition; and (2) T cell exhaustion.

(1) Escape mutations within major histocompatibility (MHC) class I-restricted HCV epitopes represent the main mechanism used by HCV to evade CTL responses and thus it is associated with persistence. Unlike CD8+ epitopes, escape mutations within targeted CD4+ T cell epitopes are not common, suggesting that CD4+ T cells failure mechanisms cannot be completely explained by viral escape[139]. Escape mutations occur early in infection and they are rare during long-term chronic infection, possibly due to the lack of T cell-mediated selective pressure[140]. Interestingly, escape variants show an impaired replicative fitness[141,142] and this contributes to limiting the variability within some epitopes[143,144]. As a consequence, the ideal target for T cell-based vaccines are conserved epitopes less likely to mutate because of viral fitness cost [141,142]. Another effect of escape variants results in impaired recognition by T cells receptors and thus prevents CD8+ T cell recognition. Moreover, CD8+ T cells from infected patients with genotype 4 were not able to recognize epitopes from other genotypes[52]. This finding highlights the challenging task of choosing vaccine targets that protect against multiple HCV genotypes. Hence, identifying conserved epitopes recognizable by specific CD8+ T cells is a key point to develop efficient T cell-based vaccines.

(2) T cell exhaustion: While T cell-based vaccines likely provide protection against chronic virus infections, they also have the potential to generate immunopathology following subsequent virus infection. This is illustrated by the fact that during chronic infection an impaired HCV-specific CD8+ T cell response develops, known as T cell exhaustion. This phenotype is associated with the inability of the immune system to control viraemia during chronic infection. These exhausted T cells undergo a progressive loss of their ability to proliferate, to secrete cytokines (such as IFN-γ), and to be cytotoxic[64,65].

Long-lived memory T cell response is only induced following spontaneous clearance and it can provide some protection. However, individuals who cannot maintain such long-lived memory T cell response due to T cell exhaustion are not

protected upon re-exposure.

One of the major challenges for immunogenic T cell vaccines refers to the recovery of T cell immunity through vaccination in people with persistent HCV infection. Kelly *et al*[145] (2016) demonstrated that when an HCV T cell vaccine based on chimpanzee adenoviruses (ChAd3) are given to patients with chronic disease, the immune response is not able to restore T cell function[145]. Failure to respond to this vaccine approach may be the result of T cell exhaustion, as vaccination is stimulating memory responses that were induced early in infection but that ended up partially dysfunctional following viral exposure[145].

Lack of efficient in vitro systems

An essential step in vaccine research is the evaluation of antibodies generated as a result of natural infections or experimental immunizations, as well as the evaluation of vaccine candidates. For those purposes using different *in vitro* and animal models becomes a must[23].

As we will exemplify in a later section on vaccines against SARS-CoV-2, the generation of live-attenuated and/or inactivated whole virus vaccines has been possible against a number of different viruses (measles, mumps, rubella, rotavirus, hepatitis A virus, poliovirus, among others), however this strategy is not achievable to generate HCV vaccines. Since HCV was discovered[1], and only until recently, research has been thwarted by the inability to culture the virus both *in vitro* and *in vivo*[23, 146].

As for *in vitro* models, propagating HCV in cultured cells remained limited for several years since inoculation of patient sera or plasma in different cell lines resulted in limited or no viral replication[147]. The first report of efficient replication came from working with HCV subgenomic replicons (where the structural region was replaced by a neomycin-encoding gene)[148]. However, the challenge was to generate an *in vitro* system that was able to produce infectious HCV particles at high titers that would allow further research[23]. The production of cell-culture derived viral particles (HCVcc) was only achieved in 2003 with the discovery of a genotype 2a isolate (strain JFH-1) derived from a Japanese patient with a fulminant hepatitis[149,150]. Transfecting replicon HCV RNA from isolate JFH-1 into human hepatoma-derived Huh7 cells resulted in efficient RNA replication without the need of any adaptive mutations[150, 151]. Nevertheless, despite this breakthrough, efforts to replicate this with other isolates corresponding to different genotypes were only partially succe-ssful. On the one hand, some of these cloned full-length RNAs were able to produce infection *in vivo* (in chimpanzees), but on the other hand, even in the presence of multiple adaptive mutations, they failed to produce infectious viral particles in cell culture, despite some being able to efficiently replicate (details on the history of HCV cell culture systems are thoroughly reviewed elsewhere[147,152-154]).

Further studies on HCVcc led to the discovery of more permissive cell clones derived from Huh7 cells (*e.g.*, Huh7.5 and Huh7.5.1)[155,156] as well as to the generation of inter- and intragenotypic recombinant genomes that are able to recapitulate the complete HCV life cycle and produce high titers of infectious particles *in vitro.* These recombinants have been shown to be optimal *in vitro* models to study the neutralization ability both of mAbs as well as of sera from infected patients[82,157-160]. They have also been used to characterize antibody escape mutations[71,137,161]. Additionally, reporter and flag-tagged JFH-1-based genomes (J6/JFH1) have been generated[162-164] and used in vaccine development[165], the latter in particular to facilitate large-scale purification of viral particles[163]. However, the most important aim in this field would be to efficiently grow any virus derived from HCV infected patients, which unfortunately has not yet been achieved[153]. For now, we depend on the constructs described above as well as a few full-length consensus clones, which have been developed after a lot of research effort and had to be designed including numerous adaptive mutations[166-170], therefore, not quite resembling natural circulating isolates. In spite of the setbacks, all these constructs have the potential to be employed for producing inactivated whole-virus vaccines.

Another *in vitro* approach to assess the neutralizing ability of sera and mAbs, in addition to HCVcc, relies on the generation of HCV pseudoparticles (HCVpp). These are generated by cotransfecting HCV E1 and E2 genes together with a retroviral packing and reporter system[171]. Due to the struggles imposed by the generation of different HCVcc derived viral particles, HCVpps were actually developed earlier[172, 173] but continue to be used in vaccine research nowadays[157,174-176].

Lack of small immunocompetent animal models

Humans are the natural hosts of HCV, and in order to test the efficacy and safety of vaccine candidates in pre-clinical studies, *in vivo* animal models are needed. Foremost, *in vivo* studies on pathogenesis of HCV chronic infections have been problematic since HCV only infects humans and, under experimental conditions, also chimpanzees. The first and most successful immunocompetent animal model has indeed been the chimpanzee. However, ethical concerns and its inclusion on the United States Fish and Wildlife Service's Endangered Species have led to a ban in its use for biomedical research[177]. Even before this prohibition, the continued use of these animals faced many issues such as high costs, small cohort sizes which made statistically significant results difficult to achieve, and the inability to genetically manipulate chimpanzees. Furthermore, it would require the need to have special and expensive facilities to breed and keep them under study[178].

Small animal models are frequently very useful tools to test potential vaccine candidates, but, since HCV does not infect rodents, a lot of effort has been devoted into developing strategies to adapt mice to evaluate HCV vaccines. This led to the use of chimeric humanized or transgenic mice with humanized livers[179] or expressing human CD81 and occludin[180], two cellular proteins that HCV uses as receptors for cell entry. However, mouse models are difficult to produce, and most are immunocompromised, which makes them inappropriate to study virus-host interactions and immune responses. Additionally, they do not exhibit cirrhosis or HCC[181]. In spite of this, genetically humanized fully immunocompetent inbred mice expressing human orthologs of HCV entry factors were developed[182], which have allowed the study of viral entry, yet not the full viral cycle. To address the latter, Chen *et al*[183] (2014), developed an immune-competent humanized mice model that is capable of developing persistent HCV infections and hepatopathological manifestations[183], yet the mice stock are outbred and genetically not well defined. More recently, Keng *et al*[184] (2016) were able to establish a new humanized mouse model including human hepatocytes as well as human immune system[184], which was able to recapitulate HCV infection and immunopathogenesis[181], although low levels of B cells were detected when compared to clinical settings.

For the difficulties in getting broad access to small immunocompetent mouse models, alternative experimental non-human primate models have been explored. However, no signs of infection were detected (for a detailed review see Ploss and Kapoor[178], 2020), with the exception of tree shrews (now classified in a separate order Scandentia, but previously designated as small squirrel-like primates) which can become symptomatic and even progress to chronicity[185]. Despite this encouraging finding, keeping these animals in captivity is a difficult task, and additionally they are genetically diverse for being an outbred species, which again poses issues to be widely used in HCV biomedical research[178].

Altogether, this shows us the difficulty we face when we need animals that can be employed for vaccine development but also to study HCV-associated pathogenesis. An alternative could be the use of substitutes and analogue viral models that can be propagated in mice lab strains and that appear to share basic immunological features with HCV. Recently, the discovery of non-primate hepaciviruses has raised interest since they can be used as analogues of HCV infection[23]. A rodent Hepacivirus discovered in Norway rats[186] has been shown to establish high-titer liver infections when inoculated in immunocompetent mice, and thus, provides insight into hepatic immune responses[187]. However, the main drawback of this model is the limited sequence homology to HCV[186]. On the other hand, equine hepacivirus (eqHV), formerly known as non-primate Hepacivirus, is the closest relative of HCV and both species share some important features such as the level of E1E2 glycosylation or the presence of miR-122 seed sites in their 5′ non-coding regions (2 sites in HCV and 1 site in eqHV)[188,189]. These approaches of using alternative and analogue viral models for vaccine development is extremely valuable, yet it is worth acknowledging that different mammalian immune systems might respond in different ways and this should be taken into consideration at the moment of interpreting data[23].

Difficulty in designing clinical studies

The design of clinical studies for HCV vaccine candidates poses its own hurdles. It must be considered that, in order for an effective vaccine to be validated, it should be tested in populations at risk for HCV infection[11,36]. This is an issue in developed countries where HCV infection incidence is low other than in PWID populations. Targeting this group of patients has ethical concerns and practical difficulties to be overcome[190]. Despite this, there are a few studies which have been successful in

identifying, enrolling and monitoring PWID before developing an acute HCV infection [191,192], the latest completed phase I/II clinical trial with outcome results was able to enroll 548 active intravenous drug users (ClinicalTrials.gov Identifier: NCT01436357 [193])[194]. On the other hand, large studies could be conducted where incidence is higher, such as some developing countries. However, logistical problems may arise due to the large number of patients needed and their appropriate follow up, specifically to detect acute cases of hepatitis, which usually course without any symptoms[36].

APPROACHES TO DESIGN VACCINE CANDIDATES FOR HCV

There are several traditional and newer approaches in vaccine development, and most of them have been explored for the design of HCV vaccine candidates (Figure 2), albeit the majority only directed at genotype 1.

Traditional vaccine approaches include whole-organisms vaccines containing either inactivated whole or live attenuated viruses. Live attenuated vaccines are potent in inducing CMI and humoral immunity and have been successful for many viral infections because they resemble what occurs naturally. Nevertheless, they have the potential risk of reverting to virulent wild-type strains. In contrast, inactivated viruses are noninfectious but have the downside of being less immunogenic than attenuated viruses. Therefore, when inactivated whole viruses are developed as vaccine candidates, they often include adjuvants and/or booster injections in order to enhance the immunogenicity[195].

Newer methods involve the use of one or more genes of the virus of interest to be incorporated into the genome of a nonpathogenic organism for amplification. In this way, mainly three different approaches have been developed: Subunits vaccines (by purifying the protein/s of interest generated in the heterologous organisms), DNA vaccines (usually by isolating a plasmid containing the gene/s of interest), and recombinant viruses (by using the entire host virus as a live vector)[195].

The latest method successfully explored has been the use of RNA-based vaccines, whose development is faster than other technologies, easily scalable, and of lower cost to manufacture. These characteristics have been essential to the development and recent authorization for emergency use of some of the vaccines currently available to control the COVID-19 pandemic[196].

In this section we will go over some of the vaccine candidates explored against HCV, and we will delve into nucleic acid-based and recombinant viral vector approaches.

Inactivated whole virus (HCVcc)

This traditional approach of inactivated virus was only feasible after the development of cell culture systems, with all the challenges that they impose even nowadays. This is partly the reason why there are only a few pre-clinical studies assessing the immunogenicity of inactivated HCVcc as vaccine candidates[197,198]. Both studies have shown the induction of humoral immune responses in chimeric mice[198] as well as in a non-human primate model[197]. The latter also elicited T cell responses. These findings are promising, but there are still some developmental challenges to overcome if this approach is to be considered for clinical trials, such as production in serum-free culture conditions and scalable and cost-efficient downstream processes. Fortunately, there are a few studies which have addressed these difficulties, and have shown that high titer serum-free HCVcc is possible for different intra and intergenotypic recombinants based on JFH-1 isolate[199] and that more efficient downstream processes based on ultracentrifugation and chromatography can be applied[200]. Nevertheless, the challenge of generating high titers of HCVcc of the most widespread genotypes and subtypes still remains.

Recombinant subunits and synthetic peptides

Recombinant E1/E2 proteins were the first prophylactic vaccine candidates being tested since they are the major targets for nAb, in particular HVR1 region within E2. They were shown to be able to induce the generation of nAb in chimpanzees[201], yet only one candidate reached clinical trials in 2007 (ClinicalTrials.gov Identifier: NCT00500747[202]). Results of the phase I trial in healthy volunteers showed the vaccine was well-tolerated at different doses used, and that it was able to induce antibody production[203,204].

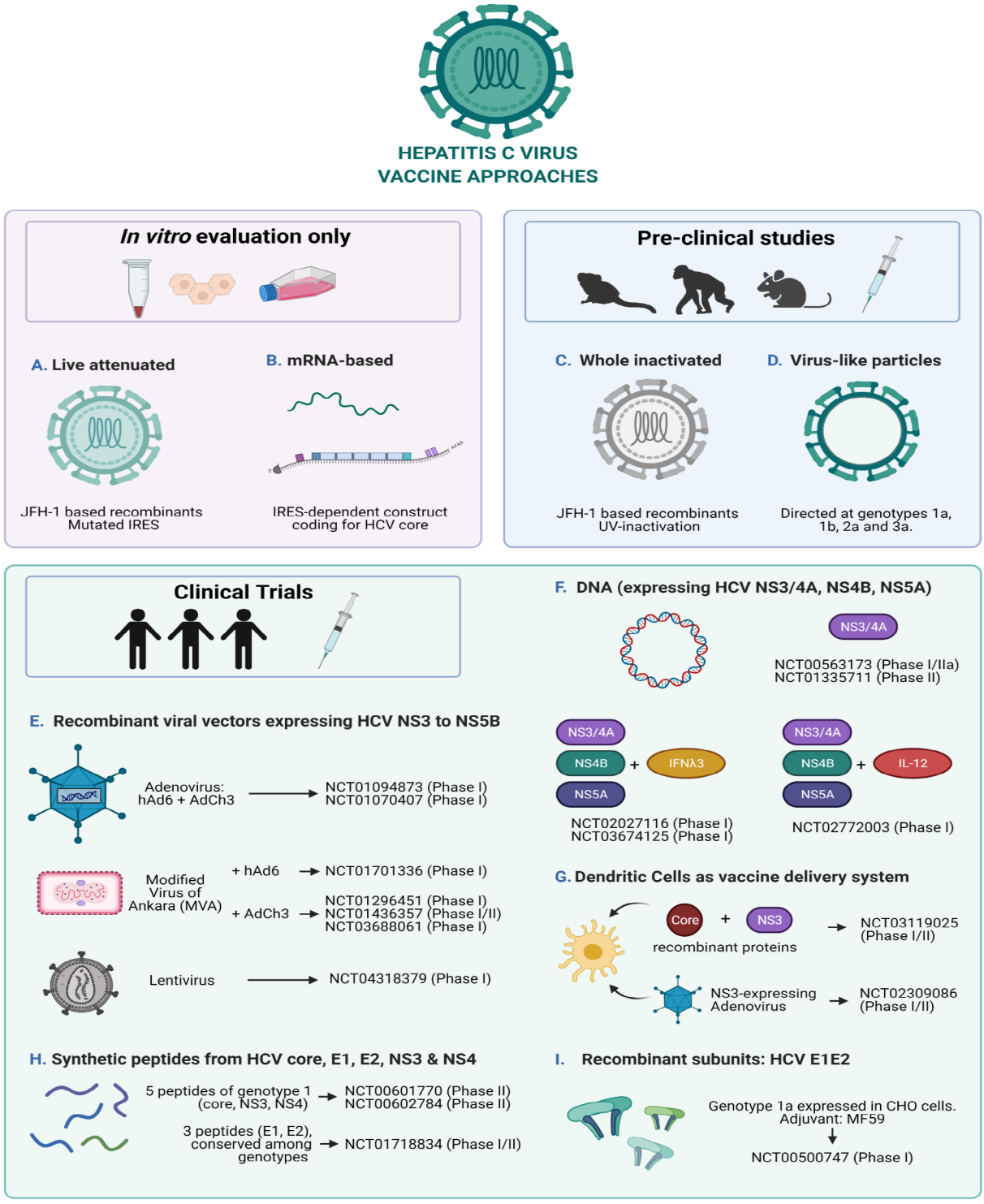

Figure 2 Summary of all hepatitis C virus vaccine approaches explored to date. The studies are divided in three categories depending on the highest stage of research achieved: *In vitro* evaluation only (in lilac background), pre-clinical studies in different animal models (in light blue background) and clinical trials in healthy volunteers and/or chronically hepatitis C virus-infected patients (in green background). For each approach (A to I) key characteristics on the vaccine candidates are provided. In addition, for all the technologies that have reached clinical trials, the ClinicalTrials.gov Identifier and the phase of the trial are indicated. Image created with BioRender.com.

Whereas recombinant E1E2 vaccines were designed to elicit humoral immune response, synthetic peptide vaccines are more attractive since they can be designed to prime both arms of the immune response. Some peptide combinations targeting both cytotoxic lymphocytes and CD4+ T cell epitopes (core, NS3, NS4) have entered clinical trials. Results for the phase 2 trial NCT00602784[205] have shown that the peptide vaccine IC41 can trigger T cell responses in relapse patients after dual therapy, yet viral clearance was not achieved[206]. Unfortunately, humoral response was not analyzed. The results of the other studies remain to be published (ClinicalTrials.gov

Identifier: NCT01718834[207] and NCT00601770[208]).

Of interest, computational identification of B and T cell epitopes has been explored as an alternative for the rational design of effective vaccine candidates. By means of different immune-bioinformatic and population dynamics simulation approaches, many predicted epitopes in E2, NS3/4A, NS5A and NS5B have been identified[209-212]. These approaches provided valuable information and *in silico* screening methods for highly conserved immunogen candidates with the putative ability to block escape mutations (for a detailed review please see[213]). These computational designs can help speed up vaccine development at the experimental stages by rationally selecting the most promising epitopes for subunit vaccine *in vitro* and *ex vivo* evaluation.

Virus-like particles

Virus-like particles (VLPs) are particles that resemble a virion but do not contain the viral genome, rather they are generated by the auto assembly of structural proteins in a manner that is genome-independent. In this way, the particle is similar to the native virus but it lacks the ability to replicate and for vaccine candidates is a very attractive technology since they are more immunogenic than soluble proteins and can prime both arms of the immune response[214].

The rationale behind this type of vaccines is supported by the successful development of vaccines against hepatitis B virus and human papilloma virus, currently commercially available[23]. Unfortunately, despite having shown promising pre-clinical results[215,216], to the best of our knowledge, HCV VLPs have not yet reached clinical trials.

Recombinant vector-based vaccines

The use of live recombinant viral-based HCV vaccines as a genetic immunization approach has shown to be powerful for eliciting CMI[217]. For this purpose, different modified viruses are used as vectors to carry HCV genetic information[19].

Adenoviral vectors are the most widespread used in the vaccine developing industry. They are attractive models for different reasons: Adenoviral genomes are well characterized and are relatively easy to modify into replication-defective viruses, most human adenoviruses cause mild infections, they infect a broad number of cell types (dividing and non-dividing), they can be grown to high titers in tissue culture, and by deleting essential genes, genetic information of interest can be inserted[218]. The most frequently used in immunization studies is the human adenovirus serotype 5 (hAd5), which is included in at least 12 of the vaccines against SARS-CoV-2 that are currently on clinical trials and in one that already had authorization for emergency use (Sputnik V vaccine)[219,220]. Despite their benefits, individuals might exhibit preexisting anti hAd5 Abs, which could diminish the immune response to vaccines based on this viral vector. For this reason, less frequent serotypes such as hAd24, hAd6 or hAd26 have been employed in pre-clinical and clinical studies of vaccine candidates against different viruses[221-223]. Additionally, adenoviruses that infect chimpanzees (AdCh3) have been tested in conjunction with hAd6, both carrying HCV non-structural proteins NS3 to NS5B of genotype 1b, yet despite reaching clinical trials, they have only been evaluated in phase I studies (ClinicalTrials.gov Identifiers: NCT01094873[224] and NCT01070407[225]). The reason for not continuing these studies seemed to be the inability to restore CMI, and as a result, a non-significant effect on HCV viral load was observed[145].

In light of these drawbacks, another viral vector has been employed in prime/boost vaccination strategies against HCV: The Modified Virus of Ankara (MVA), an attenuated poxvirus strain which is immunogenic and safe since it lacks several immunomodulatory genes[226]. MVA vector together with hAd6, both expressing HCV non-structural proteins NS3 to NS5B have entered phase I clinical trials to evaluate the combination as a therapeutic vaccine to be used in conjunction with dual therapy (ClinicalTrials.gov Identifier: NCT01701336[227]). Even though the study is complete, no results have been disclosed, presumably due to the newer DAA treatments which have completely substituted classical therapy. The most promising trials currently in phase I and II use the combination of ChAd and MVA vectors harboring HCV NS3-NS5B genomic regions. A phase I study in healthy volunteers showed promising results in terms of eliciting T cell responses (ClinicalTrials.gov Identifier: NCT01296451[228])[229]. Unfortunately, a phase I/II study in PWID population showed that this vaccination strategy was not effective for preventing chronic infections since T cell exhaustion was not reversed (ClinicalTrials.gov Identifier: NCT01436357[193])[194,230]. These results highlight the need for a vaccine strategy that stimulates both humoral and T cell immunity[23,231]. However, attempts to enhance CMI without the need of boosting the generation of Abs, have been

addressed in pre-clinical studies on non-human primates by fusing the HCV non-structural antigen to MHC class II-associated invariant chain[232]. The results showed enhanced and accelerated CD8+ T cell responses and paved the way to reach clinical trials. At the time of writing this manuscript, there is an actively recruiting phase I clinical trial (ClinicalTrials.gov Identifier: NCT03688061[233]) that seeks to enroll 25 healthy participants to assess the safety and immunogenicity of HCV prime/boost vaccination with both ChAd and MVA vectors expressing HCV non-structural antigens fused to a class II-invariant gene. Results from only 15 individuals seem promising, largely mimicking pre-clinical studies, but more participants are still needed and assessment of durability of the enhanced CMI needs to be further addressed[234].

The most recent vector-based therapeutic vaccine candidate entering phase I clinical trials is a lentiviral based HCV immunotherapy (HCVax) which aims to evaluate both the safety and the immune response in chronic HCV patients (ClinicalTrials.gov Identifier: NCT04318379[235]). Last generation lentiviral vectors are safer than first generation ones (previously used for gene therapy) and like adenoviral vectors, are capable of infecting both dividing and nondividing cells, and since they integrate into the host's genome, expression of the transgene can be long-term, a characteristic which makes them attractive as vaccine strategy[236].

Nucleic acid-based vaccines

Nucleic acid based-vaccines present numerous advantages over traditional vaccine approaches: (1) No issues associated with misfolding of proteins in recombinant protein vaccines or with high manufacture costs; (2) No infectious risks that might be associated with live-attenuated or inactivated whole virus vaccines; (3) They are able to activate both arms of the immune response (humoral and cellular); (4) The expression of antigens resembles natural epitopes; (5) In a single injection, multiple genes can be delivered; and (6) If multiple doses are needed, unlike the use of recombinant virus-based vaccines, there is no risk of anti-vector immunity[39,237,238].

DNA-based vaccines have been in the picture for nearly 40 years now[239]. They usually consist of purified plasmids which harbor sequences of interest that are expressed under the control of a eukaryotic promoter for a robust expression in mammalian cells. They are inexpensive, easy to manufacture, and also important, stable at room temperature. All of which are features that make them an ideal technology in vaccine research, as distribution and access could be granted effortlessly even to developing countries[39].

RNA vaccines have been explored for around 25 years, beginning with studies of self-amplifying RNA vectors (modified RNA from viruses) as well as mRNA pulsed into dendritic cells (DCs)[240,241], and have been largely assessed for tumor vaccination[242]. They share some features with DNA vaccines, but they do not need to enter the nucleus to translate the genetic information into antigen proteins, which represents an advantage over DNA immunization since the barrier of the nuclear envelope is removed, and thus, their efficacy is higher[238]. However, RNA is more labile than DNA, which might yield less robust vaccines than DNA-based formulations due to RNA shorter shelf life, reason why modified nucleosides have been used to enhance stability and therefore induce a higher antigen production[238], as it is the case of the COVID-19 mRNA Vaccine (nucleoside modified)[243].

The first approach for delivery of nucleic acid-based vaccines, was direct injection of naked DNA plasmid or mRNA (transdermally or intramuscularly), however, efficiency seemed to be very low, in part due to the negative charge of these molecules. There-fore, several delivery methods were developed to improve uptake and immunogenicity in different organisms: (1) Gene gun: DNA is loaded on the surface of tungsten or gold particles and then fired at target cells; (2) Electroporation: Transient pores in cell membranes are created by electrical impulses allowing DNA delivery inside the cell; and (3) Nanoparticles: Non-viral vectors made up from lipids, inorganic molecules and polymers can safely carry DNA and RNA into a cell by encapsulating the negatively charged nucleic acid, preventing its digestion by endonucleases and facilitating intracellular release[36,238].

DNA-based vaccines

Multiple pre-clinical studies in different animal models have been performed throughout the years to assess the efficiency of several DNA-based formulations against HCV to elicit immune responses. Nevertheless, only a few have entered phase I or II clinical trials.

The use of core as antigen, directly injected as naked DNA plasmid intramuscularly (IM) or intraperitoneally (IP) into different mice models, has evidenced a weak immunogenic capacity in terms of humoral response but strong CMI, even though at least 2 doses 2-4 wk apart were administered[244-248]. Using the same delivery method and injection scheme, HCV core and E2 sequences were fused to immunogenic proteins (hepatitis B surface antigen or gD protein from herpes simplex virus type-1) to address the weak Ab response, and both arms of the immune response were detected in mice as well as in rats[249-251]. Others have attempted to evaluate if different localizations of HCV antigens within the cell and the CpG content of the plasmid backbone might influence the Ab response. Results indicated that membrane-bound and secreted E2 forms as well as the addition of immunostimulatory CpG motifs elicited a better humoral response in mice[252]. Low doses of IFN-α have also shown to augment CTL response after DNA immunization with a plasmid encoding HCV core protein in mice models[253].

Targeting structural proteins in DNA-based formulations employing injection of naked plasmid as the delivery method was thoroughly tested in animal models but the vast majority failed to enter clinical trials. With the increasing knowledge on immune correlates during acute infections, it became clear that non-structural proteins are the target of CMI during acute resolutions, and that other delivery methods such as electroporation or gene gun rendered broadly reactive CTL responses[254].

As a consequence, DNA-based vaccines encoding HCV non-structural proteins have become widely used approaches. Transdermal gene gun injection of DNA plasmid encoding NS3/4A proteins into mice has shown high titers of Abs and the ability to prime CD4+ T helper cells[255] and also a CD8+ T cells that were able to clear HCV protein-expressing hepatocytes and persist up to 12-18 mo after immunization[256, 257]. When NS3 DNA vaccine was co-administered with interleukin-12 as adjuvant, strong immunogenicity was also displayed in murine models[258]. Several other adjuvants have also been employed in NS3-based DNA vaccination in order to enhance their potency (for a detailed review see Sepulveda-Crespo *et al*[231] 2020). In addition, constructs encoding a codon-optimized NS5A injected IM into mice, in combination with *in vivo* electroporation, were also able to prime specific T cell responses[259]. Two clinical trials in chronic HCV patients (naïve to treatment, infected with genotype 1) have entered phase I/IIa and phase II to evaluate a potential therapeutic vaccine based on a plasmid encoding NS3/4A (ChronVac-C) (ClinicalTrials.gov Identifier: NCT00563173[260] and NCT01335711[261]). Results have shown that high doses of ChronVac-C were able to activate HCV-specific T cell responses which led to a transient reduction in viral loads[262]. When 8 of the 12 patients enro-lled also received dual therapy after the vaccine doses, 6 were able to achieve SVR, which might indicate that immunization had a beneficial effect on the response to therapy. However, these results seem irrelevant at present with the advent of DAA treatments.

Even though pre-clinical results were promising, full-length NS3 protein exhibits immunosuppressive effects and it is possibly involved in the development of HCC due to its enzymatic activity which deregulates the normal functions of the host cells[263]. Even though DNA immunization renders antigen expression only transiently, and the adverse effects possibly caused by NS3 enzymatic activity would be marginal, alternative plasmids for DNA vaccination encoding modified NS3 sequences have been tested in animal models. Ratnoglik *et al*[264] (2014) showed that vaccinating mice with a non-enzymatic version of NS3 (with its catalytic site and NTPase/RNA helicase domains mutated to abrogate their functions) induced strong CMI, indicating that mutations in this protein do not seem to interfere with its immunogenicity[264]. Additionally, a plasmid with a truncated form of NS3, only encoding immunogenic epitopes (1095–1379 amino acids positions), succeeded in eliciting a strong Ab response after repeated intra-dermal inoculation in mice[265]. However, none of these candidates has reached clinical trials.

These findings seem to indicate that immunizing only with DNA-based formulations coding for NS3/4A or NS5A might not be sufficient to control viremia in HCV-infected patients, despite encouraging pre-clinical results in animal models.

In addition to NS3/4A or NS5A plasmid vaccination, IM injections followed by electroporation of constructs encoding NS3 to NS5B into *Rhesus macaques* and chimpanzees, in multiple-dose boosting schemes, evidenced HCV-specific effector CD4+ and CD8+ T cells and effector memory-like CTLs after immunization[266,267]. More recently, studies in mice have shown that adding a plasmid expressing cytokine IFN-λ3 (formerly known as IL28B) to the immunization with plasmids expressing NS3/4A, NS4b and NS5A provided significant immunoadjuvant activity[268]. These encouraging results led to a phase I clinical trial to evaluate the safety, tolerability and immunogenicity of this strategy in chronic hepatitis C patients infected with HCV

genotypes 1a or 1b, which had previously exhibited treatment failure to dual therapy alone or in combination with DAAs (ClinicalTrials.gov Identifier: NCT02027116[269]). The vaccination strategy comprised a combination of 3 plasmids each encoding NS3/4A, NS4B or NS5A (formerly known as VGX-6150) and a fourth plasmid encoding IFN-λ3 as an efficacy enhancer (the mixture of 4 plasmids has been renamed to GLS-6150). Three different doses were tested in a prime-vaccination scheme of 4 doses every 4 wk, and then a booster immunization at week 36, all injected IM followed by electroporation. Results of this trial have been recently published and they showed that GLS-6150 is safe and was overall well tolerated with no serious adverse events identified[270]. More importantly, vaccination increased the HCV-specific T cell responses, although, surprisingly, RNA viral titers did not decrease. Therefore, considering the reinfection possibility of patients who achieved SVR after DAA treatment, a new phase I clinical trial is ongoing in order to assess immunogenicity of GLS-6150 in this population and in healthy volunteers (ClinicalTrials.gov Identifier: NCT03674125[271]). Another clinical trial employing DNA vaccination of plasmids encoding NS3 to NS5A (INO-8000) but with the co-administration of a different adjuvant (interleukin-12) is currently active as a phase I study in chronically HCV infected patients (genotype 1) (ClinicalTrials.gov Identifier: NCT02772003[272]) which highlights the potential of these approaches including immunostimulatory molecules as adjuvants. The main takeaway of these approaches is that, the addition of more nonstructural genes as well as the co-administration of immunostimulatory adjuvants, might still be insufficient to clear an established infection. The question remains if they might be useful to prevent reinfections.

Therefore, as an alternative, heterologous prime/boost vaccination strategies have also been explored in mice, in which immunization with DNA-based vaccines is followed by immunization with viral vectors such as MVA to enhance response levels [273]. Even though results provided proof-of-concept that 2 different HCV vaccine technologies can improve immunogenicity when used in combination, to the best of our knowledge, so far, no clinical trial has tested this approach.

RNA-based vaccines

As will be detailed in the section about vaccines against SARS-CoV-2, several mRNAs-based vaccine candidates have been intensely explored in clinical trials, in particular to fight the COVID-19 pandemic. However, so far none have been approved for human use, with the exception of some of the vaccines currently in phase 3 clinical trials which are undergoing assessment for WHO emergency use listing and prequalification[274-277] (ClinicalTrials.gov Identifier: NCT04368728[278] and NCT04713553 [279]–Pfizer/BioNTech SE, ClinicalTrials.gov Identifier: NCT04470427[280] and NCT04649151[281]–Moderna TX, Inc).

On the contrary, with the exception of using mRNA to transfect DCs (which will be discussed in the next section), there have been no pre-clinical or clinical trials using mRNA-based vaccines against HCV. Interestingly, Sharifnia *et al*[282] (2019) have proposed for the first time that an RNA-based vaccine against HCV could be feasible since after *in vitro* generation of an mRNA coding for the core protein, they were able to detect core protein in monocyte-derived DCs which were previously transfected with this construct[282]. Unfortunately, no further animal studies were performed to assess the immunogenicity of this approach.

DCs as vaccine delivery system

DCs are one of the most potent antigen-presenting cells needed to induce and maintain immune responses. Given their fundamental roles, DC-based vaccination strategies have been given special attention, in particular for cancer immunotherapy [283]. However, different approaches have also been explored in HCV vaccination both in pre-clinical studies as well as in clinical trials[284]. Strategies involve loading DCs with HCV core, NS3 or NS5 proteins[285,286], pulsing them with HCVpp[287], transfecting them with DNA[288] or mRNA[289], or transducing them with adenoviral vectors expressing HCV non-structural proteins[290-293].

Two recently concluded phase I/II clinical trials have enrolled chronically HCV-infected patients (HCV genotype 1b) to evaluate the safety and clinical efficacy of therapeutic vaccination using autologous DCs. Despite employing different strategies (autologous DCs loaded with recombinant HCV core and NS3 proteins *vs* transduced with a recombinant adenovirus encoding NS3), both studies revealed similar results in terms of immunogenicity and ability to reduce viral titers: T cell responses were generated albeit weakly, and these were insufficient to clear the virus or reduce viral loads[286,293] (ClinicalTrials.gov Identifier: NCT03119025[294] and NCT02309086[295]). These findings are somewhat discouraging since in order to design better

vaccination strategies, attention will have to be placed on enhancing CMI so as to, at least partially, reduce viral titers.

IS THERE A POTENTIAL USE OF ATTENUATED VIRUSES AS VACCINE CANDIDATES AGAINST HCV?

As with whole inactivated virus vaccines against HCV, the limited *in vitro* culture systems have hampered studies on attenuated vaccines. In particular, attenuation has been achieved by serial passaging of a given virus in non-primate cells, which leads to the emergence of mutations that have low fitness in human cells. Yet HCV does not replicate efficiently in non-human cells, which poses problems for the identification and production of attenuated strains. Additionally, there is also the risk of causing an infection after the use of these types of vaccines, which in principle, limits their potential use[11,14]. However, it is worth noting that live-attenuated viral vaccines are licensed for human use for prevention of several viral diseases such as dengue, hepatitis A, measles, mumps, varicella, yellow fever and gastrointestinal disorders caused by rotaviruses[296]. Therefore, if properly designed, this technology offers safe and effective vaccines.

Considering the issue of identifying attenuating mutations in non-human cultures, an alternative is to detect mutations occurring naturally within the human host, present only as minority variants within the quasispecies, and exhibiting an attenuated phenotype.

HCV, as many members of the *Flaviviridae* family (all except for those within the Flavivirus genus), translate its polyprotein in a CAP-independent manner by recruiting the ribosome directly to the internal ribosome entry site (IRES), which is found in the 5′ non-coding region[297]. IRES structure and sequence are essential to its function, and any change can affect the translation process[298,299]. Therefore, investigating on mutations that might affect this process may enable an alternative approach for the design of live-attenuated vaccines against HCV. In this regard, our group has identified several mutations within the IRES of HCV isolates from chronically infected patients of genotype 1a and 3a, that are present in very low frequencies within the viral population, and that have evidenced a significant decrease in viral translation efficiency *in vitro*[300]. Studies in cell culture, using full-genome chimera replicons based on JFH-1 strain are underway in order to assess both translation efficiency as well as viral fitness.

It is important to mention, that one of the initial vaccines designed to fight polio was a formulation with poliovirus (PV) strains where, through successive passages in non-human cells, mutations were selected along the whole genome[301]. Of those, a mutation within PV IRES which drastically diminishes the translation efficiency, is the main responsible for the attenuated phenotype[302]. Unfortunately, live-attenuated PV vaccines have shown to be genetically unstable, and some of the mutations that confer the attenuated phenotype can reverse during replication in humans, causing rare cases of vaccine-associated paralytic poliomyelitis[303]. Thus, if the aim were to design a safe live-attenuated HCV vaccine with mutations in the IRES region, perhaps additional approaches would need to be considered so as to minimize the chances for reversion or enhancing the resulting immune response. One such approach could be constructing a bicistronic vector co-expressing an antiviral protein (for example IFN-β), which has already been proven effective to limit viral spread and to induce antiviral immunity in animal models when assessing a Flavivirus vaccine candidate[304].

On the other hand, a rational synthetic design of attenuated strains might be a new and achievable approach to employ based on the newest infectious replicons that harbor almost the entire genome sequence from non-JFH-1 strains, covering in this way most of the circulating HCV genotypes. This strategy has been successfully developed and tested in mice for other RNA viruses such as Influenza A virus and Coxsackievirus[305]. It consisted of engineering codons that were more prone to generate a Stop mutation after a single nucleotide change in as many positions as possible, without changing the amino acid identity. This strategy proved that the synthetic and rational generation of self-limiting vaccines is possible in different RNA viruses and thus, could represent an alternative way of generating HCV attenuated vaccines as well, provided that the issues with *in vitro* scaling-up production can be overcome in the near future.

LESSONS LEARNT FROM ANTI-SARS-CoV-2 VACCINES

COVID-19, caused by the SARS-CoV-2[306], has become a major health concern all over the world and has spawned challenges to develop safe and effective antiviral drugs and vaccines for preventive use. Vaccine development is a complex and time-consuming process, that typically requires years of research and testing before reaching the clinic. But in 2020, in an unprecedented effort due to the synergy between academia, researchers, and pharmacists, added to financial support and guided by cumulative knowledge from many years of scientific work, scientists were able to produce safe and effective coronavirus vaccines in record time[307]. Coronavirus vaccine types include inactivated vaccines, nucleic acid vaccines, adenovirus vector - based vaccines, and recombinant subunits vaccines. Up until February 18th researchers were testing 70 vaccine candidates in clinical trials, and 20 have reached the final stages of testing. Over 10 have been approved for emergency use in several countries around the word. Among these, it seems important to highlight the Emergency Use Authorization for 2 highly effective mRNA COVID-19 vaccines from Pfizer-BioNTech and Moderna. This is the first time that mRNA-based vaccines have ever been approved for human use, and marks a critical milestone for achievement in both science and public health[275,308,309]. As previously mentioned, mRNA vaccines trigger immune responses by transfecting synthetic mRNA encoding viral antigens (in this case spike protein or protein motifs) into human cells. Once the nucleic acid enters the cytosol of the cell, the mRNA vaccine temporarily induces the cell to produce specific viral antigens coded by the mRNA[308,310]. The major breakthroughs of these two vaccines were: (1) The mRNA modifications and purification process to reduce the innate immune response and to improve mRNA stability; and (2) The effective intracellular delivery to facilitate cellular uptake of mRNA and to protect it from RNase degradation.

These RNA vaccines generate powerful antibody responses to the SARS-CoV-2 coronavirus, but they have not proven to be as good as the AstraZeneca/Oxford vaccine (adenoviral vector vaccine) at stimulating $CD8^+$ T cells. Recently animal studies suggest that a combination of an RNA coronavirus vaccine and a adenoviral vector vaccine (AstraZeneca/Oxford vaccine) could strengthen immune response by rousing $CD8^+$ T cells in mice better than either vaccine alone[311,312]. This preliminary data should be confirmed in upcoming clinical trials.

Thus, what can we learn about SARS-CoV-2 impressive vaccine development? Firstly, that when there is interest and resources, the development and production times of a vaccine can be significantly reduced. Secondly, that mRNA vaccines have a high potency, ability for rapid development, and cost-efficient production. Thirdly, that preliminary data suggests that mixing COVID vaccines technologies boosts the immune response at a cellular level.

Is it possible, therefore, to apply all the knowledge gained from COVID-19 vaccines to accelerate HCV vaccine development? Unfortunately, only partially. As mentioned in the section about challenges, many hurdles remain since HCV biology and immunology differ greatly from that of SARS-CoV-2. However, the so far unexplored possibility of an HCV mRNA-based vaccine could certainly benefit from the experiences and developments in the field of RNA-based vaccines against SARS-CoV-2.

CONCLUSION

HCV is an insidious virus, which, since its discovery, has caused enormous difficulty to be kept under control. The successful introduction of DAAs has become a milestone in keeping the epidemic in line, however it has proven to be insufficient to achieve global eradication of this virus and all the health complications derived from the infection. Therefore, numerous approaches have been explored in order to design an effective vaccine, either prophylactic or therapeutic. Unfortunately, to date, none of these attempts have rendered a viable vaccine for human use. Several drawbacks have hampered its development, among which, to our understanding, one of the most difficult to override is T cell exhaustion, the main cause of therapeutic vaccines failure. However, many other challenges related to a still incomplete understanding of HCV immunology remain to be overcome. Noteworthy among these, is the insufficiency of CMI to control infections and the need for a joint humoral response, as well as the necessity for characterization of better epitopes for nAbs. An approach that might prove effective in the future, is the use of heterologous prime/boost vaccination,

where two different technologies can be employed to enhance the immune responses. Additionally, we believe that ongoing efforts to develop improved and more suitable *in vivo* systems should be a priority, since many of the successful pre-clinical studies have possibly failed in clinical trials due to the differences in immunopathology between the used animal models and humans. All of the hard work that has enabled the rapid and effective development of vaccines against SARS-CoV-2 should be taken as an example of what can be achieved if the interest and the efforts are focused on tackling a health burden. In particular, the advances on mRNA-based vaccine technology, which so far has not been explored in HCV vaccine candidates, would be a good starting point if the aim is to explore alternatives not investigated so far. Additionally, different methodologies which have been shown to be efficacious against other RNA viruses, are available for the design of live-attenuated strains as vaccines against HCV. Following this line of thought, and likely fueled both by the success of COVID-19 vaccines[313] and by the Nobel Prize in Physiology or Medicine 2020 (awarded to three scientists for the discovery of HCV)[28], last year, the NIH opened a grant opportunity for projects concerning HCV vaccine design[30]. As a result, it is expected that more research will be focused on this subject in the upcoming years, and hopefully, auspicious findings will follow. This renewed interest in funding HCV vaccines might be what is needed to achieve HCV global eradication, as has been proposed by the WHO a few years ago. Allocating funds for this purpose boosts the research area that has been left behind in terms of breakthroughs that can be effectively translated to public health benefits.

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REVIEW

Pediatric non-cirrhotic portal hypertension: Endoscopic outcome and perspectives from developing nations

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Abstract

Non-cirrhotic portal hypertension (NCPH) forms an important subset of portal hypertension in children. Variceal bleed and splenomegaly are their predominant presentation. Laboratory features show cytopenias (hypersplenism) and preserved hepatic synthetic functions. Repeated sessions of endoscopic variceal ligation or endoscopic sclerotherapy eradicate esophageal varices in almost all cases. After variceal eradication, there is an increased risk of other complications like secondary gastric varices, cholangiopathy, colopathy, growth failure, especially in extra-hepatic portal vein obstruction (EHPVO). Massive spleno-megaly-related pain and early satiety cause poor quality of life (QoL). Meso-Rex bypass is the definitive therapy when the procedure is anatomically feasible in EHPVO. Other portosystemic shunt surgeries with splenectomy are indicated when patients present late and spleen-related issues predominate. Shunt surgeries prevent rebleed, improve growth and QoL. Non-cirrhotic portal fibrosis (NCPF) is a less common cause of portal hypertension in children in developing nations. Presentation in the second decade, massive splenomegaly and patent portal vein are discriminating features of NCPF. Shunt surgery is required in severe cases when endotherapy is insufficient for the varices. Congenital hepatic fibrosis (CHF) presents with firm palpable liver and splenomegaly. Ductal plate malformation forms the histological hallmark of CHF. CHF is commonly associated with Caroli's disease, renal cysts, and syndromes associated with neurological defects. Isolated CHF has a favourable prognosis requiring endotherapy. Liver transplanta-tion is required when there is decompensation or recurrent cholangitis, especially in Caroli's syndrome. Combined liver-kidney transplantation is indicated when both liver and renal issues are present.

Key Words: Extrahepatic portal vein obstruction; Non-cirrhotic portal fibrosis; Portosystemic shunt surgery; Congenital hepatic fibrosis

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Core Tip: The review discusses the natural history, endoscopic outcome, and management of non-cirrhotic causes of portal hypertension in children, especially in resource constraint developing nations. Extrahepatic portal vein obstruction is the most common cause of portal hypertension in developing countries. Endoscopic variceal ligation and sclerotherapy effectively eradicate the esophageal varices. Other complications require shunt surgery that ultimately reverses portal hypertension. Non-cirrhotic portal fibrosis has favourable outcomes in terms of variceal bleeding and mortality. Isolated congenital hepatic fibrosis (CHF) has a relatively good outcome. Liver transplantation is required when CHF is associated with Caroli's disease, recurrent cholangitis, and decompensation. The presence of significant renal disease requires combined liver and kidney transplantation.

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INTRODUCTION

Portal hypertension refers to a pathological increase in portal pressure. Direct measurement of portal pressure is clinically impractical and cumbersome. The indirect way of estimating of portal pressure is by the measurement of the hepatic venous pressure gradient (HVPG), which is the difference between hepatic venous wedge pressure and free hepatic venous pressure[1]. When the blood flow in the hepatic venous channels is obstructed by a catheter, the proximal static column of blood in the hepatic veins communicates with the hepatic sinusoids reflecting sinusoidal pressure. Normal HVPG is between 1 to 5 mmHg[2]. HVPG ≥ 10 mmHg is defined as clinically significant portal hypertension[3]. HVPG > 12 mmHg predisposes to variceal rupture. Non-cirrhotic portal hypertension (NCPH) refers to the conditions where causes other than liver cirrhosis are responsible for portal hypertension. Causes of NCPH are extrahepatic portal vein obstruction (EHPVO), non-cirrhotic portal fibrosis (NCPF) and congenital hepatic fibrosis (CHF). NCPH is different from cirrhosis in various aspects. Unlike cirrhosis, NCPH has normal synthetic functions (hypoalbuminemia, coagulopa-thy), but mostly presents as variceal bleed and splenomegaly[1,4]. The incidence of decompensation and mortality following a variceal bleed is much lower in NCPH as compared to cirrhosis[5]. NCPH is overall uncommon in the West. Issues in developing countries are unique. This review discusses the endoscopic and outcome perspectives of NCPH in children.

EHPVO

Pathophysiological implications

Acute portal vein thrombosis in children is an event that is usually unrecognized and on most occasions, the etiology is unknown. It is perceived that an innocuous insult to the portal vein takes place in infancy or early in childhood. A preceding febrile illness, intra-abdominal infection, or dehydrating illness is usually followed by subtle abdominal pain or transient ascites which may have been forgotten or undetected. In retrospect, a search into the child's past history is often unyielding and perplexing for the physician. Following this event of portal vein thrombosis, the thrombus begins to organize. To bypass the obstruction, multiple hepatopetal collaterals form in 6-20 d to compensate for the high-volume flow from the splanchnic system draining into the liver. A well-established portal cavernoma forms in 3 wk[1,4]. This "temporary adjustment" by the body is however insufficient to decompress the high portal pressures. As a result, varices, hemorrhoids, collaterals, and spontaneous shunts form between the portal and systemic circulation. As evident from the series by Orloff *et al* [6], EHPVO involves portal vein alone in 70%, portal vein and splenic vein in 20%, portal vein and superior mesenteric vein (SMV) in 5%, and all three veins in 10%[6]. A liver biopsy will show mild periportal fibrosis with no signs of hepatocyte injury[7].

Clinical features

In developed nations, the mean age of presentation is around three years even before the variceal bleed[8]. However, in developing nations, EHPVO predominantly presents as variceal hemorrhage mostly from esophageal varices (77%-84%). The rest present as non-bleeders with isolated splenomegaly (16%-23%)[9-11]. The reason for presentation as variceal bleed in third world countries is due to delay in diagnosis and poor referral systems. The age of presentation is 6.3-9.3 years with a mean number of 1.8-3.1 bleeding episodes *per* child at the time of presentation[11,12]. Antecedent febrile illness and respiratory tract infection (Valsalva maneuver) tends to rupture the varices. Bleeding is worsened by ingestion of non-steroidal anti-inflammatory drugs (*e.g.*, ibuprofen, diclofenac). Long-standing gastroesophageal reflux also predisposes to erosions over the varices. Episodes of variceal bleeding are recurrent and tend to increase in frequency and severity with age. The presence of postural signs (dizziness, syncope, prostration) and hypotension indicates significant blood loss[11]. Clinical examination reveals isolated splenomegaly without any stigmata of chronic liver disease. The liver may be palpable if the patient is in cardiac failure due to anemia (post-bleeding). Splenic size may acutely decrease just after a massive hemorrhage (to compensate for the volume loss) and resume pre-bleeding size soon after blood transfusion.

Massive splenomegaly causes a dragging sensation, left upper quadrant pain, and early satiety[1]. Though hypersplenism is common, symptoms related to the same (symptomatic anemia, spontaneous skin bleeds) are less common in adults and rare in children (5%)[13]. Chronic dragging sensation and apprehensions of rupture of a massive spleen may preclude them from contact sports. Massive bleeding may be accompanied by diuretic-responsive transient transudative ascites in 4%-18% cases[12,14]. Jaundice is seen in advanced EHPVO due to symptomatic portal cholangiopathy (5%-19%) resulting from obstruction of extrahepatic bile ducts (compression by collaterals or ischemic biliary strictures) but it is extremely rare in children[15-17]. Unscreened blood transfusion in the past may cause chronic hepatitis B or C infection manifesting later with frank liver disease. Growth retardation (stunting and wasting) occurs in up to 33%-54% children[18,19]. Portal colopathy is a complication that presents with bleeding *per* rectum from anorectal varices and mucosal changes in the colon but is less commonly seen in children[20]. Small bowel ectopic varices are rare yet cause a considerable diagnostic dilemma.

Growth failure and quality of life

Duration and severity of portal hypertension determine the growth of the child. A pediatric series on EHPVO showed that growth retardation (stunting and wasting) occurs in 54% of children[19]. The theories proposed for the same are (1) Malabsorption due to portal enteropathy; (2) Deprivation of hepatotropic factors due to poor portal supply to the liver; (3) Chronic anemia; and (4) Growth hormone resistance as shown by increased levels of growth hormone and decreased levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3. Menon *et al*[21] had observed that after shunt surgery there was an improvement in height velocity in 76% of EHPVO children[21]. The study supported the portal enteropathy hypothesis as a reason for growth retardation. In a prospective study in which adequate nutritional intake was ensured, anthropometry, fasting growth hormone, and insulin-like growth factor 1 were compared between 22 well-nourished patients with EHPVO with growth retardation and 35 age-matched well-nourished controls. Insulin-like growth factor scores were significantly lower in patients (-1.48 ± 0.88) than in controls (-0.49 ± 1.09, $P < 0.001$), whereas basal growth hormone was significantly higher in patients (4.60 ± 3.70 mIU/L) compared to controls (2.66 ± 0.82, $P < 0.01$)[18]. Improvement in growth parameters seen at 12 and 24 mo after meso-Rex bypass, is possibly due to restoration of blood supply to the liver[22].

Poor health-related quality of life (QoL) and school performance is contributed by anemia and various social stigmata. EHPVO children have growth retardation and protuberant abdomen as compared to their peers in school. They also have minimal hepatic encephalopathy causing behavioural issues. QoL scores do not show much improvement on variceal eradication but may improve after shunt surgery[21,23].

Endoscopic outcome of esophageal varices

The majority of EHPVO patients present as variceal bleed. Unlike cirrhosis, adequately tackling the variceal bleed by endoscopic therapy ensures < 5% mortality. The rate of variceal growth in EHPVO varies among different individuals[9,10]. The 1-year, 3-year, and 5-year probability of development of esophageal varices is 2%, 22%, and 22%

respectively and growth from small to large size is 13%, 40%, and 54% respectively [24]. Endoscopic therapy of the esophageal varices consists of endoscopic variceal ligation (EVL) and endoscopic sclerotherapy (EST). Both are preferred endoscopic therapies for acute variceal bleeding (Figure 1). The eradication rate of esophageal varices with EST is 88%-100%. However, complications like esophageal ulcers (8%-30%) and strictures (6%-20%) are commonly seen with EST[25-27]. Though EVL has the advantages of rapid eradication of varices requiring fewer sessions and lesser incidence of complications, the studies of EVL are limited in children. EST is preferred for smaller children as there is difficulty in inserting the banding cylinder during EVL. Children lesser than 2 years have a physiologically narrow cricopharynx. Smaller band cylinders are compatible with thinner endoscopes but may not generate adequate pressure suction on the esophageal varices for banding. In developing countries, EST is possibly more cost-effective compared to EVL. In a randomized controlled trial of EST *vs* EVL in children by Zargar *et al*[28], the efficacy of controlling bleeding and rate of variceal eradication was similar in both groups (100% in both and 96% *vs* 91.7% respectively), but overall EVL was better as it required lesser number of sessions (3.9 *vs* 6.1), had lower re-bleeding (4% *vs* 26%) and complication rates (4% *vs* 25%)[28]. A study from the authors' center has shown that sequential EVL followed by EST (Group I, n = 101) is superior to EST alone (Group II, n = 60) in a 3 wkly endoscopy regimen till eradication. Group I required significantly fewer sessions (5.2 ± 1.8 *vs* 6.8 ± 2.8, P < 0.005), less sclerosant (13 ± 8.2 mL *vs* 30 ± 20 mL, P < 0.001) and had fewer complications (7% *vs* 28%, P < 0.001) as compared with group II[29]. Many pediatric hepatology centers in Asia consider a 3-weekly protocol of sequential downgrading of large esophageal varices by EVL followed by EST injection into the smaller varices till eradication. While EVL rapidly reduces the size of varices, EST effectively blocks the paraoesophageal perforators which ultimately lowers the risk of recurrence. This is advantageous as the cumulative dose of sclerosants and risk of complications are much lower in sequential therapy as compared to the EST alone[30]. Long-term sequelae of esophageal dysmotility is a concern with cumulative sclerotherapy.

Management and outcome of gastric varices

Gastric varices bleed less frequently but more profusely as compared to esophageal varices[31]. In a study with 274 children with EHPVO, 70% had primary gastric varices at presentation, of which 97% had gastroesophageal varices (GOV) and 3% had isolated gastric varices (IGV)[32]. After esophageal variceal eradication with EST, gastric varices may disappear or persist or develop afresh (secondary gastric varices). Disappearance is seen more often along the lesser curvature of the stomach (GOV1) than the greater curvature (GOV2). In a study from the author's center, GOV1 decreased from 45% to 30% and GOV2 increased from 8% to 13% during esophageal variceal eradication. Secondary gastric varices develop in 28%. Of these, 87% are constituted by isolated gastric varices in the fundus (IGV1) and the rest in the body and antrum (IGV2)[33]. The reduction of GOV1 is attributable to the fact that GOV1 arises from deep submucosal veins from the left gastric vein into which there has been a flow of sclerosant from the esophageal varices. GOV2 varices are formed by the collaterals from the left gastric and short gastric veins. IGV1 is formed exclusively by the short gastric veins. Short gastric veins do not receive any sclerosant as they do not communicate with the esophageal varices. As the esophageal varices and GOV1 shrink during endoscopic therapy, the blood is diverted through IGV1 and GOV2 to accommodate the persistent portal pressure and blood volume in the portal system. Following eradication of esophageal varices, IGV1 incidence increases significantly from 1% to 14% (P < 0.001), and the incidence of bleeding from gastric varices increases from 0% to 20%[32]. Acute gastric variceal bleeding is managed by 1-2 mL of glue (N-acetyl-2-butyl-cyanoacrylate) injection[3] (Figure 1). Repeated sessions of glue injection have the risk of glue cast fundal ulcers, obliteration of splenic vein for future portosystemic shunt surgery (PSS), and difficulties in the mobilization of the spleen during surgery. Hence, whenever large fundal varices are noticed, it is better to perform PSS if the anatomy is feasible. Antral varices (IGV2) rarely bleed even after eradication of esophageal varices and hence prophylactic endotherapy is not usually required[31].

Management and outcome of portal hypertensive gastropathy

Frequency, extent, and severity of portal hypertensive gastropathy (PHG) increase after esophageal variceal obliteration by endoscopic therapy. This results from increasing gastric mucosal venous congestion that occurs along with the decreasing collateral blood flow through the varices. In a study from our center, pre-EST PHG was documented in 40% of cases, all were mild. After eradication of esophageal

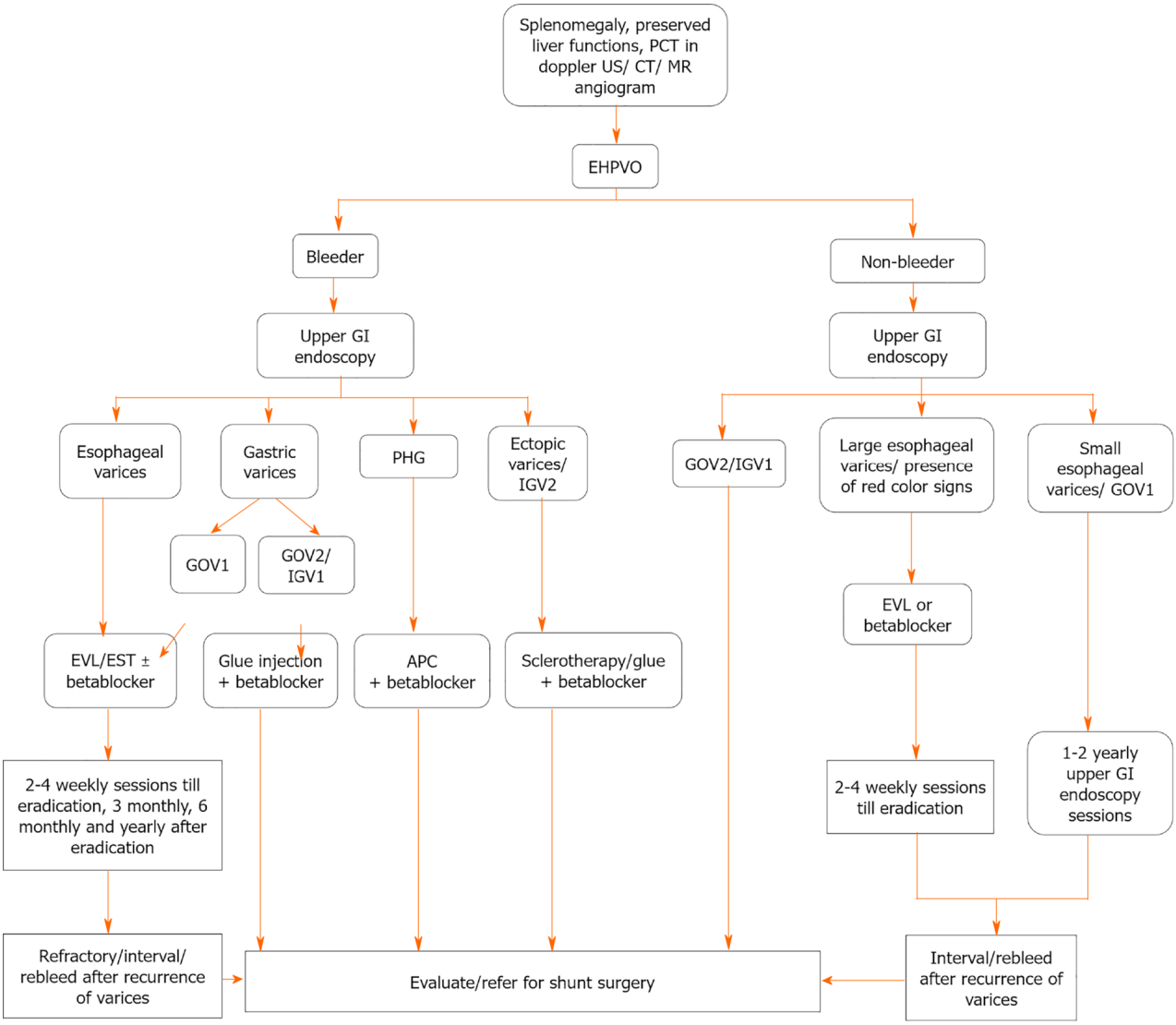

Figure 1 Algorithm for management of esophageal varices and gastric varices in extra-hepatic portal vein obstruction. EHPVO: Extra-hepatic portal vein obstruction; PHG: Portal hypertensive gastropathy; GOV: Gastroesophageal varices; IGV: Isolated gastric varices; EVL: Endoscopic variceal ligation; EST: Endoscopic sclerotherapy; APC: argon plasma coagulation.

varices, PHG increased to 80%, half were mild and the rest were severe[33]. In another study, the prevalence of mild and severe PHG increased from 25% to 52% and 3.2% to 16% respectively with statistical significance following esophageal variceal eradication. Bleeding from PHG is uncommon in EHPVO children[32]. Repeated sessions of argon plasma coagulation is a promising modality of management for symptomatic gastric antral vascular ectasia.

Natural history and outcome of portal cavernoma cholangiopathy

Portal cavernoma cholangiopathy (PCC) denotes the cholangiographic abnormalities involving both intra-hepatic and extra-hepatic bile ducts including gall bladder wall abnormalities in patients with portal hypertension. It is seen as biliary radical dilatation, filling defects, indentations, angulations, filling defects or a tumor mass (pseudocholangiocarcinoma sign)[34]. They occur due to compression of peri and para choledochal varices. Intracholedochal varices appear as filling defects within the lumen seen on endosonography and choledochoscopy. PCC is most commonly seen in EHPVO (80%-100%) as compared to cirrhosis (0%-33%)[1]. The prevalence of PCC is almost 100% in adults, however, the data is limited in children[15,35,36]. A prospective study conducted in the authors' center in 72 EHPVO children showed the prevalence of PCC as 92% of which 7% were symptomatic. In this study, the age at presentation and the duration of disease in asymptomatic PCC were 13.9 ± 2.3 and 6.9 ± 4.0 years respectively. This was significantly lower than the symptomatic group where age and duration were 16.1 ± 0.9 and 11.0 ± 1.4 years respectively. Age at presentation and

duration of disease had a significant linear correlation[37]. It has been observed symptoms of PCC are more commonly seen in adults as compared to children, implying that the duration of portal hypertension in EHPVO is responsible for progressive bile duct disease to cause symptoms[35]. In a study of adults with symptomatic PCC, the median age of presentation with symptoms of PCC was 41 years[38]. The mean interval between the first presentation with variceal bleed and jaundice was 7.4 years in another adult study[39]. In a study by Llop *et al*[40] in adults, it was shown that the 5-year and 10-year actuarial probability of developing symptomatic PCC after diagnosis of chronic portal vein thrombosis was 9% and 13%, respectively[40]. Zargar *et al*[41] followed 69 EHPVO children for 15 years and 4% developed biliary obstruction[41]. Symptoms arise due to obstruction of bile flow and result in cholestatic jaundice, pruritus, cholangitis, and gall stones. The implication of finding symptomatic PCC in children is grave. This would possibly mean tenacious strictures or stones that would entail multiple therapeutic endoscopies. A series of complications are anticipated. The endoscopic biliary interventions have technical limitations in younger children. Biliary drainage is associated with a risk of hemobilia from rupture of intracholedochal varices. Endoscopic intervention is easier for lower biliary strictures than higher strictures, more so in children. Refractory strictures may necessitate bilio-enteric anastomosis. Long-standing disease results in secondary biliary cirrhosis. In EHPVO, secondary biliary cirrhosis is an unfortunate consequence of a problem where a primary liver disease never existed in the first place. Considering the longevity of a child, QoL in the growing years, and gainful living, it is imperative to actively search for asymptomatic biliary changes with serial imaging. There are two hypotheses for biliary changes in EHPVO, extrinsic compression by portal collaterals and ischemic stricture due to bile duct injury or a combination of both[15,35,42]. In a study by Dhiman *et al*[42], endoscopic retrograde pancreato-cholangiography (ERCP) done in five cases post shunt surgery showed total disappearance of changes in two, partial response in one, and no improvement in two, indicating the relief of compression alone was not the reason for biliary changes[42]. The definitive diagnosis of PCC is by ERCP but due to its invasive nature, magnetic resonance cholangiopancreatography with gadolinium injection to delineate the cavernoma is preferred in children[43]. Symptomatic PCC should be managed but the requirement of management in asymptomatic PCC is doubtful. Also, steps for management of PCC are not clear. Should shunt surgery be offered in all symptomatic PCC followed by bilioenteric anastomosis (hepaticojejunostomy) or should endoscopic drainage be primarily performed before PSS[39,44]? Prior shunt surgery effectively decompresses the cavernoma in 6-12 mo and makes it easier for subsequent biliary drainage surgeries (Figure 2). Second stage hepaticojejunostomy is required in 28%-50% in adult studies following shunt surgery[39,44,45]. Issues with endoscopic biliary drainage are its invasive nature, need for technical expertise, and lack of smaller-sized endoscopes and biliary metallic stents in children. Meso-Rex shunt restores the blood flow to the liver to decompress the cavernoma adequately. In the study by Gauthier-Villars *et al*[46], 2/8 children with symptomatic PCC underwent Rex shunt, and liver biochemistry completely normalized post shunt surgery[46]. Meso-Rex shunt is not possible in most children due to unfavourable vascular anatomy where the left branch of the portal vein or SMV is blocked. Meso-Rex bypass is also ineffective if there is a large spleen at the time of presentation. In the study from the authors' center, 25 children with EHPVO underwent central end to side splenorenal shunt. Despite the patency of shunt 18 mo post-surgery, asymptomatic PCC did not improve in the majority. All the children who had progressive PCC after shunt surgery had concomitant SMV block. SMV block not only makes meso-Rex shunt non-feasible but also causes severe PCC[47]. The venous plexuses on the common bile duct drain into the portal vein and SMV territories. When the portal vein is occluded, the choked peribiliary collaterals compress upon the bile duct. In such a scenario, SMV is the only pathway for decompression. When the SMV is occluded too, the choking effect of the biliary venous plexuses is near total. Peribiliary collaterals enlarge further and compress the already narrowed common bile duct. A central end to side PSS does not effectively relieve the peribiliary portal hypertension since the connection is between the splenic and left renal vein. Future studies are required to address whether PSS is required in an asymptomatic PCC in children to prevent the burden of complicated PCC and the development of SMV block as they enter adulthood. The management of PCC poses great dilemmas in children. Issues such as choice of shunt surgery, adequate decompression of biliary varices, the appropriate time for bilioenteric anastomosis, and prophylactic biliary dilatation for strictures are well debated. Despite active screening for PCC in all children, we must understand that symptoms arise as a result of procrastination in treating asymptomatic PCC. Symptomatic PCC definitely

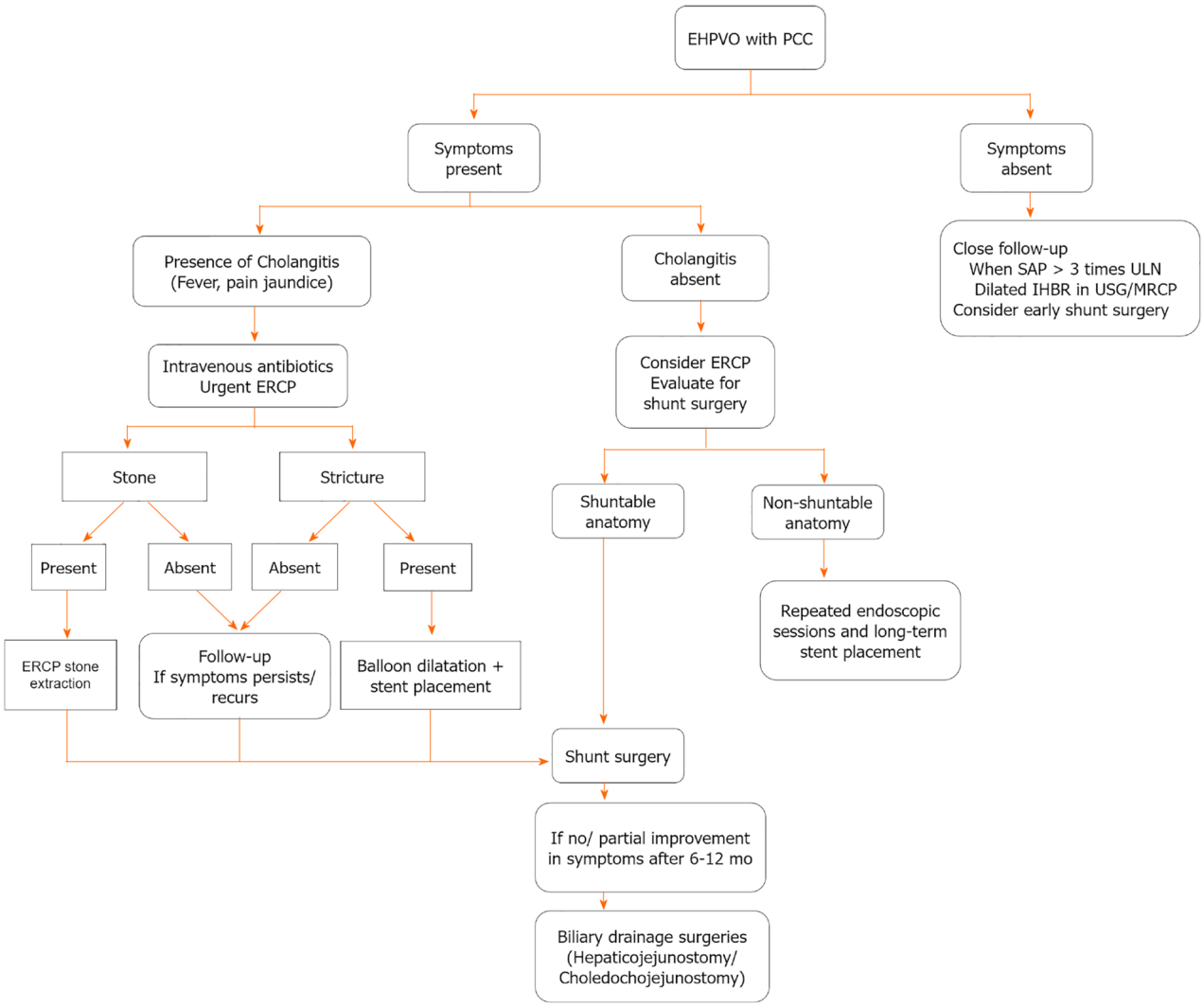

Figure 2 Algorithmic approach for management of portal cavernoma cholangiopathy in extra-hepatic portal vein obstruction. EHPVO: Extra-hepatic portal vein obstruction; PCC: Portal cavernoma cholangiopathy; ERCP: Endoscopic retrograde pancreato-cholangiography; MRCP: Magnetic resonance cholangiopancreatography; SAP: serum alkaline phosphatase; IHBR: intrahepatic biliary radicle; USG: ultrasonography; ULN: upper limit of normal.

requires biliary drainage but the requirement of biliary decompression in asymptomatic PCC is a dilemma not only in children but also in adults. After detecting PCC, the logical step forward has to be decompression of portal hypertension with PSS. In those with symptomatic PCC, endoscopic therapy may be required before the shunt surgery. Endoscopic therapy is reserved for selected cases of cholangitis and choledocholithiasis. Limitations such as lack of appropriate sized endoscopes and biliary metallic stents (not approved yet) are unique issues in children. The experience of meso-Rex bypass for relieving PCC is limited. Non-selective shunt surgeries may not have a wholesome outcome in PCC.

Natural history and management of portal colopathy

Portal colopathy is most commonly seen with EHPVO as compared to cirrhosis probably due to selective redistribution of portal pressure with time along the inferior mesenteric vein consequent to thrombosis at the junction of the splenic vein and SMV [48,49]. Similar to PCC, the prevalence of portal colopathy is lower in children compared to adults emphasizing the importance of the duration of portal hypertension. Unlike PCC, PSS effectively reverses colopathy. Portal colopathy is defined as the presence of colitis-like abnormalities (edema, erythema, ulcers), vascular lesions (cherry-red spots, ectasia, and spider angiomas) with or without the presence of colorectal varices (3-5 mm) by endoscopy and/or endosonography. Rectal endosonography is superior to sigmoidoscopy for identifying rectal varices[20,50]. Prevalence of rectal varices in adults is 63%-94%[20,49,51]. In a study from the authors' center, rectal varices were seen in 36% of 25 EHPVO children by sigmoidoscopy and 76% by rectal endosonography[50]. Rectal varices occur in 80%-90% of adults with

EHPVO but the overt bleeding frequency is low (3%-8%). In another study from our center, only 16.6% of EHPVO were symptomatic for colopathy/rectal varices. 94% showed rectal varices and 75% showed colitis-like changes on routine colonoscopy. Colopathy and colitis-like lesions were more common than vascular lesions (36/40 *vs* 23/40; $P = 0.001$). Colopathy changes were pancolonic in 52.5%, left-sided in 42.5%, and right-sided in 5% cases. 16% also had ileal changes. Children with colopathy had more often (90% *vs* 57%; $P = 0.01$) PHG, more endotherapy sessions (6[4-8] *vs* 2[1-4]; $P = 0.03$), and less often large esophageal varices (12.5% *vs* 43%; $P = 0.02$) than those without colopathy[52]. Mucosal changes like erythema, friability, and superficial ulcerations should not make the endoscopist suspect inflammatory bowel diseases, especially in the setting of portal hypertension as the shunt surgery effectively reverses the colitis like changes in these cases[53]. Bleeding rectal varices can be managed with sclerotherapy or band ligation[20]. PSS is preferred for large rectal varices and symptomatic colopathy. When PSS is anatomically not feasible, beta-blockers should be considered. Laser photocoagulation and Argon plasma coagulation are tried in adults in severe cases, but the studies in children are limited[54].

Rare complications in EHPVO

Minimal hepatic encephalopathy (MHE) in EHPVO without shunt surgery has been observed in 32% of cases using neuropsychological testing and 57% by critical flicker frequency techniques[55,56]. EHPVO is an example of type B hepatic encephalopathy where there is a portosystemic bypass in the absence of intrinsic liver disease. The other reasons attributed are chronic deprivation in hepatic blood flow leading to parenchymal extinction, increased brain glutamine, and increased proinflammatory cytokines[57]. Following shunt surgery, as the toxic substances bypass the liver into the systemic circulation, MHE is more prevalent in non-selective shunts as compared to selective shunts. The reversal of MHE following shunt surgery in EHPVO is not well established.

Ascites is an uncommon complication of EHPVO. In a study from the authors' center, 307 EHPVO children were analyzed, of which 26% developed ascites. 84% of ascites were following variceal bleeding. Younger age of onset, baseline malnutrition, hypoproteinemia are predictors of post-bleed ascites. The time interval between the first bout of bleed to the onset of ascites and hospital admission was 7 (3-20) and 12 (5-45) d respectively. 17% of patients had features of ascitic fluid infection requiring antibiotics. For the resolution of ascites, 32% required only salt restriction, 39% required the addition of diuretics, and 29% required single-time large-volume paracentesis. The overall resolution of ascites was seen in 46%, 76%, 88%, and 100% by days 7, 14, 30, and 60 respectively. In this study, 17 patients re-bled, of which 11 had a recurrence of post-bleeding ascites. None of the patients had any evidence of chronic liver disease on follow-up of 56 (9-112) mo[58]. The mechanism of de novo ascites is not well understood. Secondary causes and hepatic dysfunction are possible responsible factors. Rangari *et al*[14] analyzed 9 chronic EHPVO adults with ascites who had not bled in the last 3 mo. These patients had raised alanine transaminase, hypoalbuminemia, and deranged coagulation. Ascites in this study were attributable to increased age, longer duration of disease, and PCC. They postulated that the underlying liver dysfunction was caused due to a reduced parenchymal liver mass [14].

Hepato-pulmonary syndrome (HPS), though common in advanced cirrhosis, is rarely seen in EHPVO also. The prevalence of HPS in EHPVO is 2%-10%[59,60]. The incidence of HPS in EHPVO shows that apart from hepatic dysfunction, portal hypertension *per se* is responsible for HPS. Hepatic dysfunction is not unseen in EHPVO. It occurs more in the older age due to parenchymal extinction and is more seen with prolonged portal hypertension. PCC is commonly associated with hepatic dysfunction[14]. Portal hypertensive enteropathy is not unusual in children, seen in both cirrhotics and non- cirrhotics. In the study from the authors' center, children with EHPVO showed features of enteropathy as evident by duodenal morphometric features (60%). The features were lower villous to crypt ratio, dilated capillaries, increased thickness of muscularis mucosae) and increased small intestinal permeability (lactulose excretion test) as compared to healthy controls[61]. Portal hypertensive enteropathy is one of the most important causes of growth failure in children.

Outcome of shunt surgery

Endotherapy significantly improved mortality due to variceal bleeding as compared to the pre-endoscopic era. Endotherapy (EVL/EST) causes eradication of esophageal varices in 90%-95% EHPVO cases[62]. As endotherapy obliterates portosystemic collaterals in the esophageal region, the persistently elevated portal pressure causes

rebleed in 7%-41% of cases following endotherapy[33,41,62,63]. There is also a significant risk of developing other complications related to high portal pressures such as ectopic varices, gastropathy, colopathy, cholangiopathy, growth failure, and hypersplenism. A randomized trial comparing endotherapy and shunt surgery showed the risk of rebleeding is significantly higher in the endotherapy group[64]. The study by Krishna *et al*[23] showed the QoL remained poor even after variceal eradication on endotherapy due to various reasons like growth retardation, cholangiopathy, ectopic varices, massive spleen related pain, early satiety, and infarction[23].

Shunt surgery is indicated in EHPVO whenever feasible. However, there are various approaches in the surgical management of EHPVO (Figure 3). Baveno VI guidelines recommend that meso-Rex Bypass should be offered for primary, pre-primary, and secondary prophylaxis for all cases of EHPVO[3]. However various factors preclude meso-Rex bypass in all children with EHPVO such as anatomic non-feasibility, the need for technical expertise. Another feasible intervention is PSS, a procedure ideally performed after tackling the first episode of variceal bleed endoscopically. However, in developing nations, the bulk of the disease outweighs the number of centers that have expertise in conventional and physiological shunt surgeries. PSS is a popular shunt surgery for EHPVO as it not alone prevents rebleeding but also improves other complications like colopathy, cholangiopathy, growth, QoL, *etc.* PSS consists of selective (distal splenorenal shunt) and non-selective shunt (proximal or central-end to side splenorenal shunt with splenomegaly, side to side splenorenal shunt, and mesocaval shunt)[65,66]. Each of the above-mentioned PSS has its own merits and demerits and hence, the choice of surgical procedure is tailored as *per* the indication for surgery and the anatomy of the splenoportal axis (the patency and diameter of the veins)[67,68]. Broadly, if massive splenomegaly affects QoL adversely, then splenectomy with a central end-to-side splenorenal shunt is indicated. Splenectomy is required when issues related to massive splenomegaly and significant hypersplenism predominate. However, a spleen-preserving shunt is preferred if splenomegaly is not of concern. Side-to-side splenorenal shunts permit a large diameter vascular anastomosis if the splenic vein is of a small diameter (< 5 mm) calibre[68]. The mortality following PSS is 0%-1.9%. Shunt thrombosis occurs in 2.5%-13% following PSS[6,67,69-71]. On a few occasions, other surgical interventions may be required for selected indications when PSS is not feasible due to non-shuntable anatomy. Hepaticojejunostomy is required in symptomatic portal biliopathy, especially related to ischemic strictures. Emergency devascularization procedure is required when endotherapy fails to control acute variceal bleed, interval bleed, or recurrence of bleed following eradication. In the author's center, 110 children underwent surgical intervention for delayed sequelae post-variceal eradication. PSS was performed in 83% whereas esophagogastric devascularization was performed in 17%. 91% showed shunt patency after a median follow-up duration of 28 mo following shunt surgery. Growth parameters, colopathy, issues related to splenomegaly improved in all[72].

Meso-Rex bypass requires placement of autologous vein graft between SMV and left branch of the portal vein and it is an ideal curative procedure conceptually. However, there are various limitations of the meso-Rex bypass. Complete patency of intrahepatic portal veins, including the recess of Rex, is required for performing this procedure. In a pediatric EHPVO study, 62% had favourable anatomy before surgery, and eventually, only 37% culminated into a successful meso-Rex bypass[73]. Wedge hepatic venous portography is the gold standard for imaging of intrahepatic portal veins. 15% of all successful meso-Rex bypass need interventional radiological procedures like thrombectomy, shunt dilatation, or stenting to maintain shunt patency [74]. The shunt blockage following meso-Rex bypass is 4%-19%[74-77]. Meso-Rex bypass is not the procedure of choice when there is gross splenomegaly and hypersplenism.

Issues in developing countries

In the author's understanding, the issues in developing nations are uniquely different from those in developing countries. Due to poor referral systems, the patients are referred to tertiary care centers in an advanced state where one or more of the above complications would have ensued. Meso-Rex bypass is favourable in the early stages where the left branch of portal vein and confluence are patent. In advanced disease, the anatomy is no longer favourable as the entire portal vein and its branches are affected by stasis and progressive local thrombosis. 64% of EHPVO children also have additional thrombosis of SMV or splenic vein which limits the choice of PSS[37]. This possibly occurs at onset or due to local progression of thrombosis at the trijunction confluence. Proximal splenorenal shunt lowers portal pressure but does not ameliorate

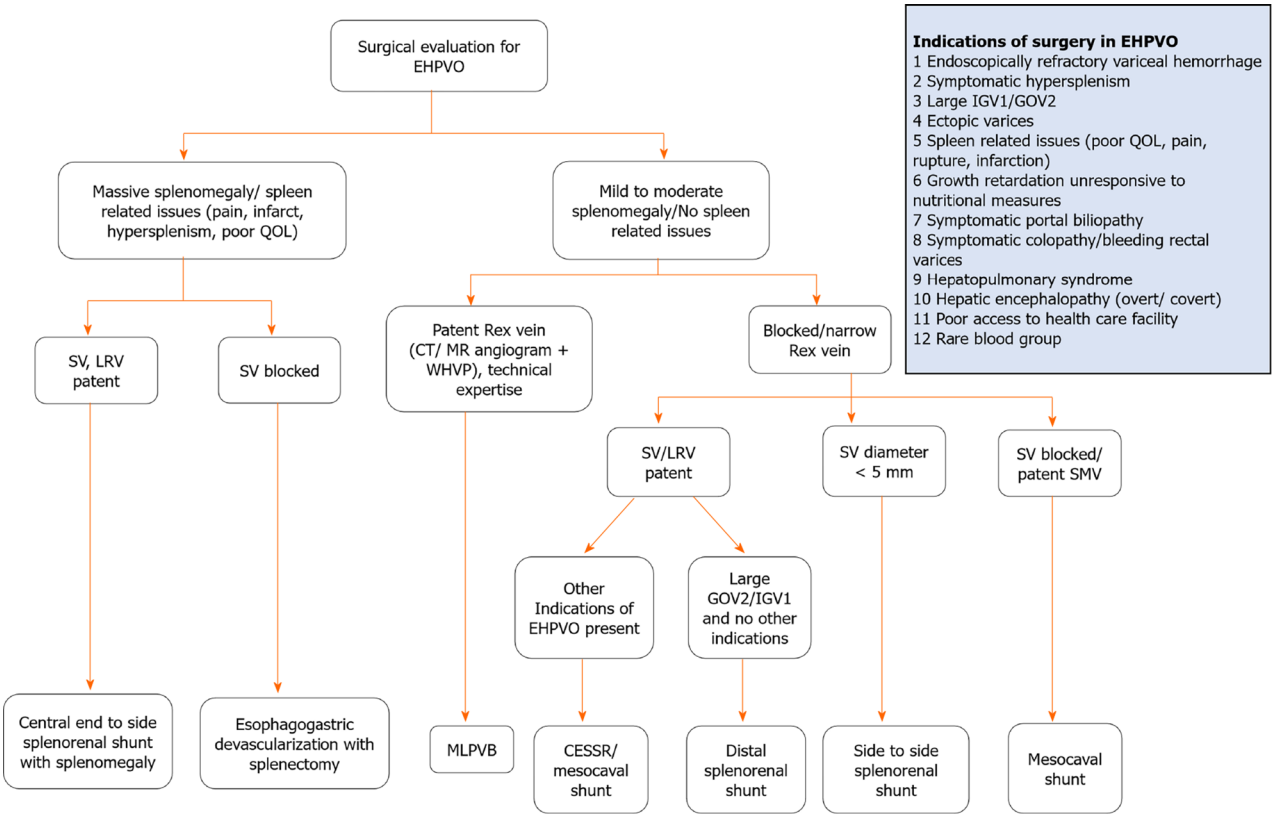

Figure 3 Indications of surgery in extra-hepatic portal vein obstruction and algorithmic approach for surgical management in extra-hepatic portal vein obstruction in developing countries. EHPVO: Extra-hepatic portal vein obstruction; GOV: Gastroesophageal varices; IGV: Isolated gastric varices; EVL: Endoscopic variceal ligation; SV: splenic vein; SMV: superior mesenteric vein; EST: Endoscopic sclerotherapy; CT: Computed tomography, LRV: left renal vein; MR: magnetic resonance, CESSR: central end-to-side splenorenal shunt, QOL: quality of life; WVHP: wedge hepatic venous pressure; MLPVB: mesenterico left portal vein bypass (meso-Rex).

the PCC. Distal splenorenal shunt and meso-Rex bypass do not ameliorate issues of a large spleen. Those with entire splenoportal axis thrombosis are subjected to esophagogastric devascularization which diverts the blood away from the life-threatening variceal territory but fails to lower the portal pressure. Hence the long-term choice of definitive therapy is that of a compromised one. Keeping in mind the logistic issues in developing countries, it is the authors' opinion that repeated endoscopic sessions should be performed till variceal eradication and an opportune time must be sought for a PSS if the disease is in an advanced state or if a meso-Rex bypass is not feasible. PSS has low post-operative mortality and good long-term shunt patency. Despite the compromise, PSS may be the only available option for amelioration of the disease.

NCPF

NCPF is also called idiopathic portal hypertension, hepatoportal sclerosis or obliterative venopathy. This is a disorder of no specified etiology characterized by massive splenomegaly, preserved liver function, and patent portal vein[1].

Pathophysiological implications

Etiopathogenesis of NCPF is not well understood and there are various theories for the same. Infections (*Escherichia coli*), prothrombotic states, immunological disorders, toxins (arsenic), and genetic factors are possible causative factors[75-78]. Human immunodeficiency virus and hepatic schistosomiasis also are responsible for liver fibrosis similar to NCPF[79-83]. Various theories explain the pathogenesis of NCPF though, none of the theories have been effectively proven. The unifying hypothesis suggested by Sarin and Kumar[84], suggests a major thrombotic event in a younger age is responsible for EHPVO but, a micro thrombotic event later in life is responsible for the obliteration of small and medium branches of portal veins resulting in NCPF

[84]. Schouten *et al*[85] proposed a dual theory of splenic vein dilatation (due to high levels of nitric oxide synthase in splenic endothelial cells) and intrahepatic portal vein obliteration as the main pathogenesis in the development of NCPF[85]. Sato and Nakanuma[86], suggested endothelial-mesenchymal transformation theory, according to which endothelial cells in portal vein branches acquire features of myofibroblast due to stress and ischemia thereby causing deposition of collagen in vessel walls causing obliteration[86]. The histological hallmark of NCPF is obliterative portal venopathy. Other prominent features include aberrant vessels in the portal tract (portal angiomatosis), portal tract fibrosis and inflammation, and absence of significant hepatocellular injury. Incomplete nodules and scattered regenerative nodules are seen on a few occasions[87].

Clinical features

The incidence of idiopathic portal hypertension has reduced in Japan in the past two decades. Though no national registries are available, the incidence in India also seems to have decreased along with EHPVO[87]. The change in the scenario could be due to the reduction in the incidence of umbilical sepsis, reduced diarrheal episodes in infancy due to better sanitation and vaccination programs[88,89]. Studies from India show that NCPF accounts for 3.3%-4.6% of all pediatric portal hypertension[90,91]. NCPF is commonly seen in the third to fourth decade in adults. Various pediatric series suggest that NCPF is not an uncommon entity in children[86]. Variceal bleeding is the most common presentation in adults (72%) with a relatively small proportion presenting as a lump in the left upper quadrant (12%)[92,93]. The scenario is different in children. In the author's experience, the median age at presentation of NCPF was 14.5 (6-18) years where 49% and 47% presented as variceal bleed and unbled isolated splenomegaly respectively[90]. Another pediatric series from India showed that only 16% presented as variceal bleed and the remaining 84% presented with isolated splenomegaly. Predominant presentation of variceal bleeding in adults is possibly due to a progressive increase in disease severity as age progresses[91]. However, the overall natural history in adults is not different from pediatric series. 87% of NCPF in the authors' study had hypersplenism with median spleen size 10.5 (1-17.5) cm on examination. Transient ascites and hypoalbuminemia were seen in 20% and 11% patients respectively, mostly after variceal bleeding. A small proportion of patients develop end-stage liver disease requiring liver transplantation[90].

Endoscopic outcome

The analysis of the NCPF cohort in the authors' experience showed the predominant presence of esophageal varices (96%) and portal hypertensive gastropathy (89%) followed by primary gastric varices (56%) at presentation. The majority of the children showed eradication of esophageal varices and GOV1 after 5 (2-12) sessions. 36% showed recurrence of esophageal varices in about 1 year of follow-up and 12% developed secondary gastric varices (GOV2 and IGV1). Most of the PHG was mild in severity and PHG was significantly higher in bleeders as compared to non-bleeders probably due to higher portal pressures[90] (Figure 4). Prevalence of esophageal varices in adult NCPF is similar in children (85%-95%) but the gastric varices at presentation were more common in adults compared to children[91]. In a study by Chawla *et al*[94], endoscopic sessions in 72 adult NCPF patients showed eradication after a mean of 5.7 sessions of EST, and recurrence of varices occur in 9.2% over a follow-up period of 21 mo[94]. Sarin *et al*[95] compared adults with cirrhosis with NCPF, and EHPVO. Cirrhotics had a similar recurrence of variceal bleeding as compared to NCPF. Unlike cirrhosis, none of the EHPVO or NCPF died at follow-up suggesting that despite the progression of portal hypertension in NCPF, the liver parenchyma is preserved like in EHPVO[95].

Natural history and surgical outcome

Pediatric data on long-term follow-up studies are lacking (Table 1). Overall survival of NCPF is favourable. Poor outcomes like death, decompensation, and requirement of surgery were seen in 24% of patients[90]. Adult series by Siramolpiwat *et al*[96] reported native liver survival of 72% at 5 years[96]. Similarly, the Spanish cohort of adults reported 86% native liver survival at 5 years[97]. In a French follow-up study, 46% of patients develop portal vein thrombosis during a follow-up period of 7.6 years [98]. Thus, the development of portal vein thrombosis is a major factor that also contributes to the progression of portal hypertension in NCPF. There is a paucity of published data on surgical management of NCPF both in children and adults. As most of the patients have predominant spleen-related issues, a non-selective PSS like central

Table 1 Clinical characteristics and outcome of non-cirrhotic portal fibrosis in pediatric studies

Parameters	Prasad *et al*[90] (*n* = 45)	Sood *et al*[91] (*n* = 19)	Poddar *et al*[109] (*n* = 11)	Franchi-Abella *et al*[110] (*n* = 48)
Mean or median (range) age at presentation	14.5 (6-18) yr	13.8 (5.9-17.6) yr	11 (5-14) yr	8.75 (1 mo-16 yr)
At presentation				
Variceal bleed	49%	15.70%	54.60%	18.80%
Lump upper abdomen	47%	84.20%	45.40%	43.80%
Ascites	20%	-	18%	-
Spleen size (mean) cm	10.5	12 (4.75–17.25)	8	-
Variceal recurrence	39%	-	18%	-
Poor outcome				
Decompensation	4%	0	0	12.50%
Hepatopulmonary syndrome	2%	5%	-	4.20%
Follow-up duration (mean)	48 (3-120) mo	18 (2-51) mo	57.5 (12-78) mo	15 (1-26) yr
Survival without transplant	93%	100%	100%	88%

end to side splenorenal shunt with splenectomy would be a favourable compromise. Long-term complications of shunt surgery include hepatic encephalopathy, glomerulonephritis, hepatopulmonary syndrome, and ascites[99]. In the authors' experience, 10% require a central end to side splenorenal shunt with splenectomy[90].

CHF

CHF is a liver ciliopathy disorder of irregularly shaped proliferating bile ducts and periportal fibrosis. CHF is one of the fibropolycystic diseases, that include Caroli disease/syndrome, autosomal dominant polycystic kidney disease (ADPKD), an autosomal recessive polycystic kidney disease (ARPKD)[100].

Pathophysiological implications

CHF and related disorders occur as a result of ductal plate malformation (DPM). The ductal plate is the embryonic precursor of intrahepatic bile ducts and it surrounds the portal vein. Remodelling of ductal plate starts at 12 wk of gestation and completes at 20 wk. Defect in the remodelling causes persistence of immature embryonic duct structures called DPM. The persistence of immature ductal elements activates hepatic stellate cells by transforming growth factor-beta secreted by Kupffer cells. The activated stellate cells stimulate the formation of fibrous tissue in the portal tract which is ultimately responsible for recurrent cholangitis and portal hypertension. As embryologically, bile duct development and hepatic vasculature have been closely related, DPM is commonly associated with 'pollard willow' malformation of the portal vein, which predisposes the portal vein to undergo thrombosis and cavernomas transformation[101]. Osteopontin gene mutation and microRNA (miR15α) have also been postulated in the pathogenesis of CHF[102,103].

Clinical features

The age of presentation widely varies with CHF diagnosed as early as infancy to late adulthood. A large systematic review of CHF patients showed the mean age of presentation as 11 years[104]. Four forms of CHF have been identified based on the clinical features, most common being portal hypertension followed by cholangitic, mixed, and latent forms. The associations of CHF also widely vary with renal diseases (ARPKD, ADPKD, Jeune syndrome, juvenile nephronophthisis, dysplastic kidney) and Caroli's disease/syndrome commonly seen. However, a few cases of CHF present without any association. Most patients present with features of portal hypertension. Physical examination usually shows firm to hard hepatomegaly with predominant left lobe enlargement, splenomegaly and occasionally nephromegaly. Laboratory workup reveals elevated alkaline phosphatase, gamma-glutamyl transpeptidase, and

Table 2 Clinical characteristics and outcome of congenital hepatic fibrosis in pediatric studies

Parameters	Rawat *et al*[106] (*n* = 40)	Poddar *et al*[105] (*n* = 15)	Parkash *et al*[111] (*n* = 25)	Luoto *et al*[112] (*n* = 27)
Mean or median age	1.3 yr	8 yr (10 mo-14 yr)	8.5 ± 2.7 yr	2.7 (0-13) yr
Associations				
Caroli's syndrome	52.50%	9%	8%	11%
Renal	92.50%	81.80%	24%	100%
CHF	47.50%	54.50%	92%	37%
Presentations				
Variceal bleeding	27%	54.50%	60%	15%
Cholangitis	25%	9%	0%	7.40%
Recurrent cholangitis	7.50%	9%	0%	3.70%
Decompensation	5%	18.20%	0%	19%
Endotherapy	27%	100%	60%	30%
Shunt surgery	0%	9%	20%	3.70%
Transplant				
Renal transplant	0%	-	-	41%
Liver transplant	5%	-	-	3.70%
Combined liver kidney transplant	45%	-	-	37%
Survival				
Overall survival	90%	100% [41 (1-80) mo]	100%	70% [10.6 (0.6-40) yr]
Survival post-transplant (follow-up duration)	80% [5 (1.2-9)] yr	-	-	73.30%
Survival non-transplant (follow-up duration)	100% [15 (4.5-19)] yr	-	-	100%

CHF: Congenital hepatic fibrosis.

cytopenias. Abnormal renal functions are present in those with significant renal disease[100,105]. There are various syndromes associated with CHF like Caroli's syndrome (intrahepatic bile duct cysts with CHF), Joubert syndrome (cerebellar vermis, retinitis pigmentosa, nystagmus, ataxia), Senior-Loken syndrome (cerebellar ataxia, skeletal abnormalities, nephronophthisis, retinal dystrophy, sensorineural hearing loss), COACH syndrome (cerebellar vermis hypo/aplasia, oligophrenia, ataxia, coloboma, polydactyly), Meckel syndrome (microcephaly, renal cystic disease, hypoplastic or ambiguous genitalia, polydactyly, congenital heart defect, cleft palate, ocular defects) and Bardet- Biedl syndrome (rod-cone dystrophy, postaxial polydactyly, congenital heart defect, cleft palate, mental retardation, hypogonadism). Table 2 describes a few series of pediatric CHF. In a systematic review of 1230 patients, 64% had associated ARPKD, 26% had Caroli's syndrome and 9.5% had isolated CHF. 71% had presented with features of portal hypertension (hepatosplenomegaly, variceal bleeding) however, only a small proportion presented with ascites, hepatopulmonary syndrome, and encephalopathy (< 5%). Features of portal hypertension are commonly seen with ARPKD. Cholangitis is seen in 12% which is commonly seen in Caroli's syndrome[104]. A study from the west (median age at presentation-1.3 years) showed 35% had a neonatal presentation and 78% had associated Caroli's syndrome. Features of portal hypertension are seen in 86% and cholangitis in 25%[106]. Another study from India also showed features of portal hypertension as predominant presentation [105]. In the author's experience (unpublished data) of 33 children, almost 69% presented with features of portal hypertension, and 11% presented with cholangitis. Only 10% developed ascites during follow-up.

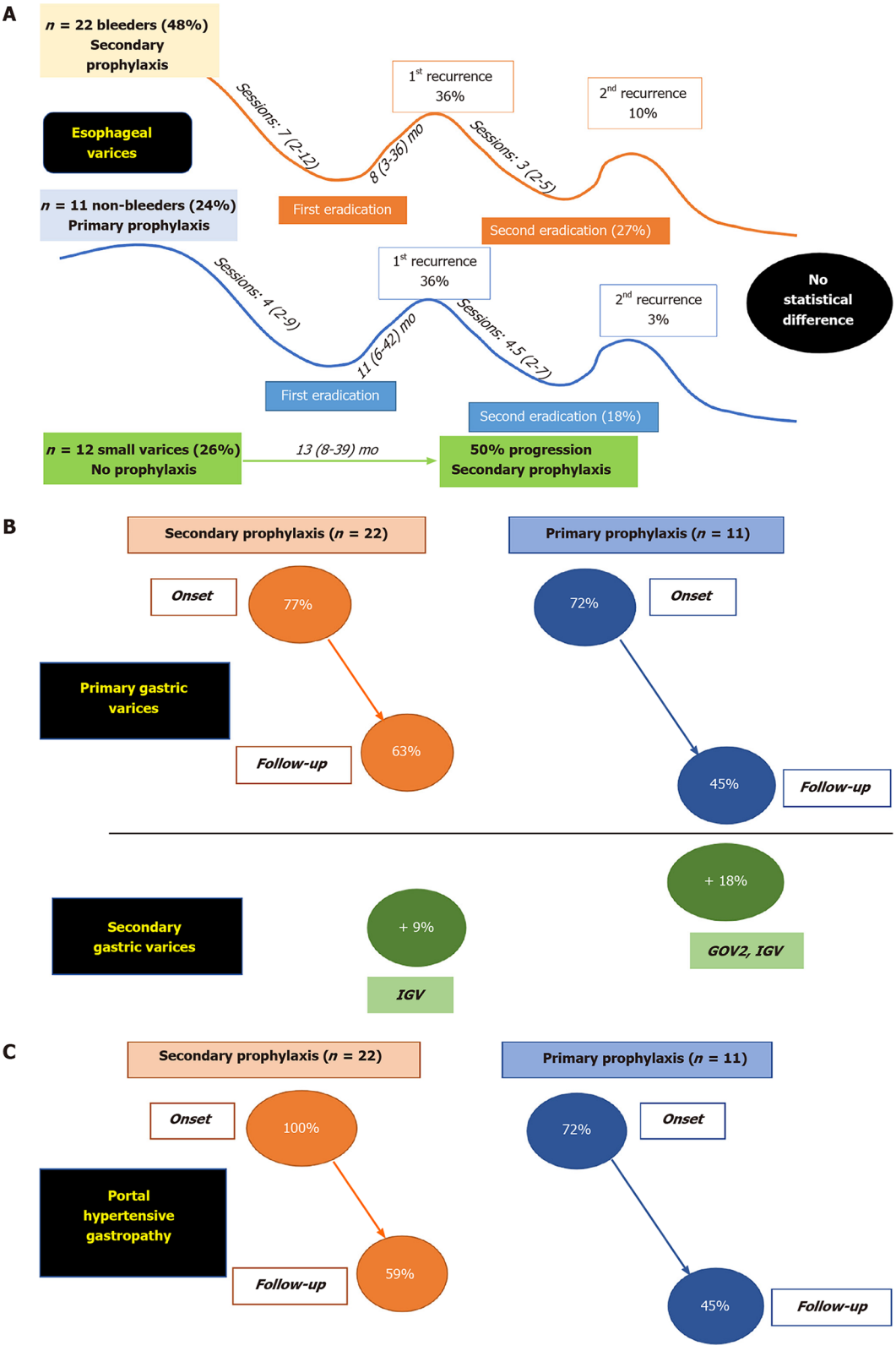

Figure 4 Natural history and follow-up outcome of esophageal varices, gastric varices and portal hypertensive gastropathy in pediatric non-cirrhotic portal fibrosis in author's experience. A: 22 children presented with variceal bleeding (bleeders), required Endoscopic Variceal Ligation and/or sclerotherapy. 11 children presented with large varices without bleeding (non-bleeders). The incidence of recurrence of varices following eradication is not statistically different between bleeders and non-bleeders. 50% small varices progressed to bleed in the follow-up (median time 13 mo); B: 77% bleeders and 72% non-bleeders had gastric varices (primary) at initial endoscopy. 9% bleeders and 18% non-bleeders develop gastric varices (secondary) in follow-up; C: 100% bleeders and 72% non-bleeders had portal hypertensive gastropathy at initial endoscopy, reduced to 59% and 45% respectively, in follow-up.

Natural history and outcome

An algorithm for the diagnostic approach and management of CHF is given in Figure 5. Cholangiocarcinoma is seen in 2.5%-16% of Caroli's syndrome but it is less

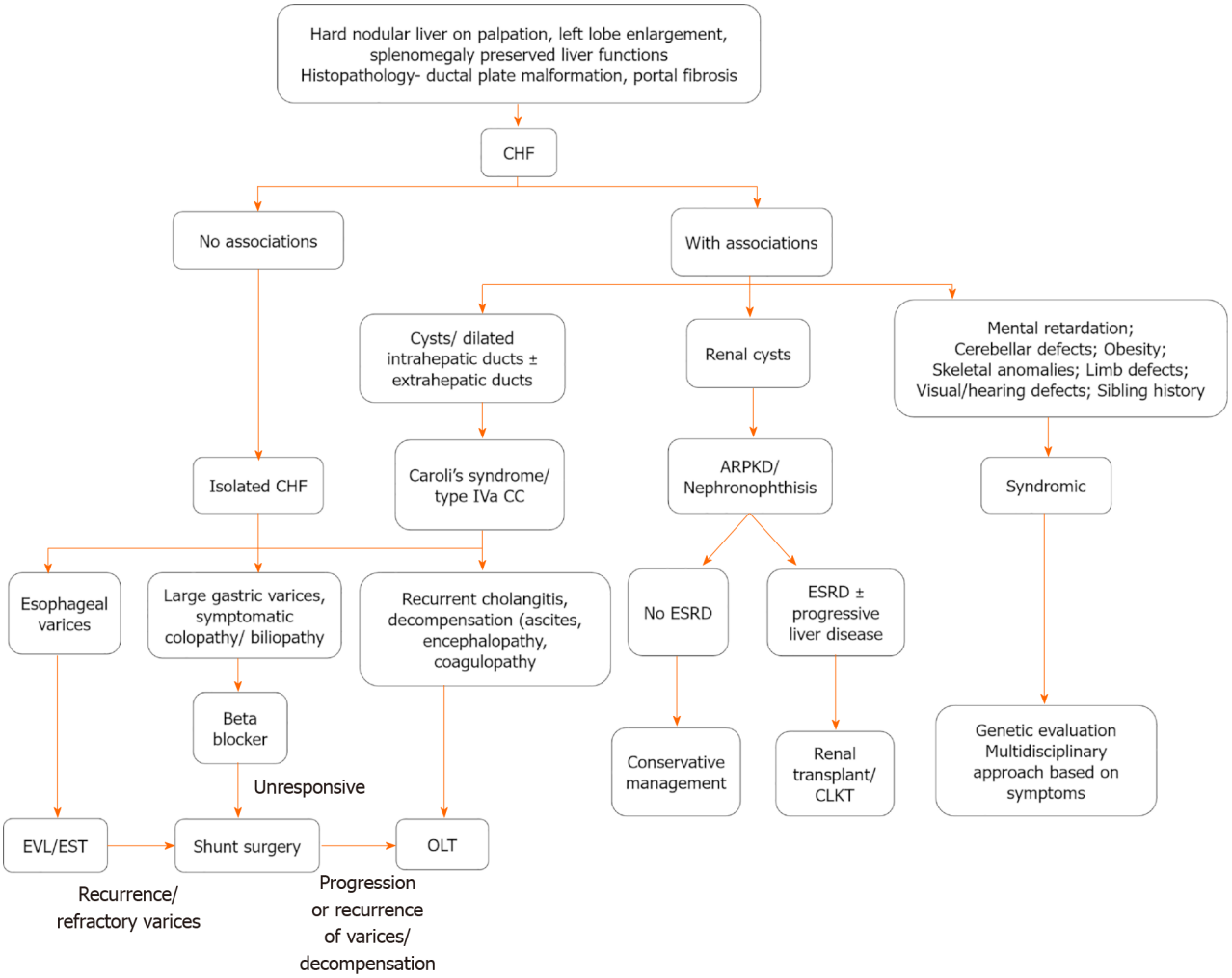

Figure 5 Algorithmic approach to diagnosis and management of congenital hepatic fibrosis. CHF: Congenital hepatic fibrosis; CC: Choledochal cyst; ESRD: End-stage renal disease; EVL: Endoscopic variceal ligation; EST: Endoscopic sclerotherapy; CLKT: Combined liver-kidney transplantation; OLT: Orthotopic liver transplantation, ARPKD: autosomal recessive polycystic kidney disease.

common with isolated CHF[100,107,108]. In the systematic review, 1.5% developed cholangiocarcinoma during median duration of follow-up 7.5 (0-38) years in adults, predominantly in patients with Caroli's syndrome. The incidence of cholangiocarcinoma is extremely uncommon in children. 23% required transplantation (liver, kidney, and combined liver and kidney). Most of the isolated renal transplantation had ARPKD and the majority of the isolated liver transplantation had Caroli's syndrome. 6% died during follow-up most commonly due to sepsis (post-transplant cholangitis) and complications related to cholangiocarcinoma. 2.7% of patients required shunt surgery of which approximately three-quarters showed improvement. A small proportion had shunt block and post-shunt encephalopathy[104]. In another pediatric study, all children with neonatal presentation required renal transplant before the second decade due to underlying ARPKD. In comparison only 23% of those presenting later require required liver/kidney transplantation[106].

CONCLUSION

In developing countries, NCPH is fraught with challenges of advanced presentation and associated complications related to portal hypertension. Though the management of variceal bleeding is taken care of by endoscopic measures, definitive therapy is often compromised. In a small subset of patients, the disease progresses to end-stage liver disease.

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MINIREVIEWS

Acute-on-chronic liver failure in children

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Abstract

Although various complex definitions of acute-on-chronic liver failure (ACLF) have been suggested in relation to adult patients, there is currently no universal definition of the syndrome in pediatric patients. In simplified terms, ACLF is characterized by the acute deterioration of the liver functions due to the effects of a precipitating factor on the basis of a chronic liver disease. Acute events and underlying liver diseases are very different in children from those seen in adults. Moreover, acute events and underlying chronic liver diseases vary among geographical regions, although it seems that the most common such diseases and acute events are autoimmune hepatitis, Wilson's disease, and their flares. ACLF is associated with a poor prognosis. While no scoring systems have been developed to predict the prognosis for children with ACLF, modified versions of the Asian Pacific Association for the Study of the liver's acute-on-chronic liver failure scoring system and the Chronic Liver Failure-Sequential Organ Failure Assessment criteria can be used in children until specific and validated scoring systems are available. Aside from liver transplantation, there is no proven treatment for ACLF. Thus, the early recognition of ACLF prior to the development of extrahepatic organ failure is important.

Key Words: Liver failure; Prognosis; Prevalence; Clinics; Histopathology; Scoring systems; Treatment

Core Tip: Acute-on-chronic liver failure (ACLF) remains poorly defined in pediatric patients. ACLF is associated with acute deterioration in patients with chronic liver disease or cirrhosis due to an underlying precipitating event. In the limited number of pediatric studies conducted to date, the underlying chronic diseases and acute precipitating events have been found to vary among geographical regions, while high rates of short-term mortality have also been reported. This review focuses on ACLF in

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INTRODUCTION

Acute-on-chronic liver failure (ACLF) remains poorly defined in pediatric patients. Although a few prior pediatric studies have relied on definitions of the syndrome formulated in relation to adult patients[1-9], no study has yet sought to develop a definition of the syndrome in pediatric patients. The simplest definition of ACLF equates it with the development of acute deterioration in patients with chronic liver disease or cirrhosis as a result of an underlying precipitating event[10]. ACLF differs from both acute liver failure (ALF) and acute decompensated cirrhosis. More specifically, ALF is defined as a form of coagulopathy that cannot be corrected with vitamin K when biochemical data indicate the presence of acute liver injury without prior evidence of chronic liver disease[11]. Furthermore, decompensated cirrhosis is defined as the loss of the liver's normal synthetic capacity over time accompanied by the development of jaundice and complications of portal hypertension, including ascites, variceal bleeding, and hepatic encephalopathy (HE)[12].

Many studies have been conducted among adults with ACLF, although such studies have utilized different criteria and etiologies, and they have been conducted in different geographical regions. European, American, and Asian hepatology authorities have devised different definitions of ACLF in light of their specific populations. Despite the use of different definitions and etiologies, the morbidity and mortality rates associated with ACLF have consistently been found to be high in adults[13-15]. In the limited number of pediatric studies conducted to date, the underlying chronic diseases and acute precipitating events in cases of ACLF have been found to vary among geographical regions, while high rates of short-term mortality have also been reported[1-7]. The present review will focus on ACLF in children.

DEFINING ACLF

Different definitions of ACLF have been suggested in relation to adult patients. For instance, as part of prospective observational studies, the European Association for Liver Studies (EASL)[13], the North American Consortium for End-Stage Liver Disease Studies (NASCELD)[14], and the Asian Pacific Association for the Study of the Liver (APASL)[15] have each suggested different definitions of ACLF in adults, which can sometimes lead to confusion (Table 1). According to both the EASL and the NASCELD, ACLF involves the development of acute hepatic decompensation accompanied by extrahepatic organ failure, which stems from an acute precipitating factor in patients admitted to hospital with cirrhosis. Moreover, the two authorities stress that ACLF is associated with high mortality. With reference to the definition of ACLF suggested by the EASL, in the conducted in the United Kingdom using European (CANONIC) study of cirrhotic patients, acute hepatic decompensation was defined as the development of ascites, HE, gastrointestinal hemorrhage, bacterial infection, or any combination of these disorders. In addition, as a requirement for a diagnosis of ACLF, the NASCELD defines organ failure as shock, HE grade III or IV, renal failure that requires dialysis, and/or respiratory failure that requires mechanical ventilation. Patients with a prior history of decompensated cirrhosis are included within both the EASL and the NASCELD definitions of ACLF. In its definition of ACLF, the APASL includes not only those with cirrhosis, but also those with chronic liver disease. The EASL specifies the time frame for developing ACLF as 4–12 wk, whereas the NASCELD does not specify a time frame[13,14]. The APASL does not include extrahepatic organ failure in its definition of ACLF, although it is recognized as a complication of ACLF. Moreover, patients with decompensated and acutely decompensated cirrhosis are excluded from the APASL definition of ACLF. In fact,

Table 1 Commonly accepted acute-on-chronic liver failure definitions

	APASL	EASL	NASCELD
Definition	An acute hepatic insult manifesting as jaundice (serum bilirubin ≥ 5 mg/dL) and coagulopathy (INR ≥ 1.5) complicated within 4 wk by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis, and is associated with a high 28-d mortality	An acute deterioration of pre-existing chronic liver disease usually related to a precipitating event and associated with increased mortality at 3 mo due to multisystem organ failure	A syndrome characterized by acute deterioration of cirrhosis with two or more extrahepatic organ failure
Included patients	Acute liver deterioration in patients with previously diagnosed or undiagnosed chronic liver disease including cirrhosis. Acute hepatic triggering factors	Cirrhosis (compensated or decompensated)	Cirrhosis (compensated or decompensated)
		Renal failure is mandatory	Two extrahepatic organ failure
		Patients with an acute decompensation of cirrhosis	Presentation not necessarily to be liver failure
		Patients with prior decompensation of cirrhosis	Can be repeated episodes of ACLF
Excluded patients	Patients with bacterial infections	HCC	HIV infection
	Patients with cirrhosis who develop acute deterioration of their clinical status are considered to have acute decompensation but not ACLF	HIV infection	Disseminated malignancies
	Prior decompensation.Non-hepatic acute insults (such as sepsis)	Receiving immunosuppressive treatments	
Pediatric definition	For children less than 3 years, modified HE assessment scale can be used	None	None
	Clinical and/or radiological ascites can be used for defining ACLF in children		

ACLF: Acute-on-chronic liver failure; APASL: The Asian Pacific Association for the Study of the Liver; EASL: The European Association for Liver Studies; HCC: Hepatocellular cancer, HIV: Human immunodeficiency virus; INR: International normalized ratio; NASCELD: The North American Consortium for End-Stage Liver Disease Studies.

decompensation preceding jaundice and repeated episodes are said to indicate acute decompensation, not ACLF. Another important difference that sets the APASL definition of ACLF apart from the other definitions is the requirement for the diagnosis of jaundice to be followed by the diagnosis of clinical ascites or HE. More specifically, the APASL definition of ACLF states the following:

ACLF is an acute hepatic insult manifesting as jaundice (serum bilirubin ≥ 5 mg/dL (85 micromol/L) and coagulopathy [international normalized ratio (INR) ≥ 1.5 or prothrombin activity < 40%] complicated within 4 wk by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis, and is associated with a high 28-d mortality[15].

As mentioned above, there is currently no universal definition of ACLF in pediatric patients. Only the APASL has stated, in its latest guidelines, that, with some minor modifications, its definition of ACLF in adults can be used for children. Due to the difficultly associated with identifying clinical ascites and HE in children, those necessary modifications include recognizing ascites as "clinical and/or radiological ascites" and "diagnosing HE in children younger than 3 years using modified HE assessment scale"[15]. However, there are still several major problems with the APASL definition. First, some instances of ALF in children may not be accompanied by a significant increase in the bilirubin level, such as ALF stemming from metabolic liver disease. Second, the cut-off INR for the diagnosis of ACLF is problematic. Indeed, when defining ALF in children, the INR must be ≥ 1.5 with HE or ≥ 2 regardless of the HE status[11]. The APASL has referred to these two issues, although it has not made any recommendations. In light of this, in a retrospective study conducted in children, we defined ACLF as follows:

The presence of an acute hepatic insult in previously diagnosed or undiagnosed chronic liver disease causing jaundice (total serum bilirubin ≥ 5 mg/dL) and coagulopathy (INR ≥ 2.0) and clinical and/or radiological ascites and/or HE within 4 wk[16].

Finally, in its consensus report, the World Gastroenterology Organization defined ACLF as follows:

ACLF is a syndrome characterised by acute hepatic decompensation resulting in liver failure (jaundice and prolongation of the INR] and one or more extrahepatic organ failures that is associated with increased mortality within a period of 28 d and up to 3 mo from onset[10].

PREVALENCE

Despite the use of different diagnostic criteria, the prevalence of ACLF has been found to range from 22.6% to 40% in adult patients with cirrhosis[17-19]. Moreover, according to the APASL and EASL criteria, the incidence rate has been determined to be 5.7 and 20.1 cases per 1000 person-years, respectively[20].

We searched the literature published in English and found nine studies concerning ACLF in children[1-9]. Of those nine, six studies were conducted in India. Given that prior studies have relied on different adult definitions and etiologies, and as they have mainly been conducted in a single Asian country, it is difficult to determine the true prevalence of pediatric ACLF. Indeed, the previously reported prevalences are not generalizable. In two centers in India in which the APASL definition of ACLF was used, its prevalence was reported to range from 11.2% to 22.1%[3-5].

CLINICAL FEATURES OF ACLF

Underlying chronic liver disease

The primary causes of chronic liver disease and cirrhosis in adults are alcohol abuse, hepatitis B (HBV) and C, and non-alcoholic fatty liver disease. While viral hepatitis is the most common cause in Eastern countries, alcohol abuse is the most common cause in Western countries[10,13,14]. In the few studies previously conducted in children, the most common underlying chronic liver diseases were found to be Wilson's disease (WD), autoimmune hepatitis (AIH), and indeterminate chronic liver diseases[4,6]. AIH can present as ACLF, as the exacerbation of a pre-existing chronic liver disease or liver injury caused by an overlapping infectious or toxic agent may lead to ACLF in cases of AIH. There are no definitive data regarding whether or not patients diagnosed with ACLF have a previous history of liver disease. In our prior study, 58.6% of ACLF patients were diagnosed with liver disease for the first time[16].

Precipitating acute events

A precipitating event can trigger the decompensation of liver disease and lead to multiple organ failure. Acute events are known to vary by region in adults. Bacterial infection, sepsis, and alcoholism are the most common acute events in the West, while the reactivation of HBV infection or superinfection, hepatotoxic drugs, and complementary and alternative medicines are the most common acute events in the East[13-15]. The most common acute events in pediatric ACLF were reported in one center in India to be WD (46.5%) and AIH (34.9%) flares[8]. In the other two centers in India, the most common acute events were reported to be hepatitis A virus (HAV) and hepatitis E virus infections[1,2]. In our prior study, the most common acute events were AIH (48.28%) and WD (27.58%) flares. Moreover, the other identified acute events were drug-induced liver injury, Epstein-Barr virus, cytomegalovirus, and HAV infection [16].

PATHOPHYSIOLOGY

Current knowledge regarding the pathophysiology of ACLF is insufficient. It has been stated that the main trigger of ACLF in adults is increased severe systemic inflammation. Systemic inflammation can cause ACLF through several mechanisms, including: (1) Immune-mediated tissue damage; (2) Mitochondrial dysfunction caused by oxidative stress; and (3) The development of renal hypoperfusion and multiple organ failure due to the effective arteriolar volume decrement caused by vasoactive substances[21,22]. The main causes of systemic inflammation have been reported to be bacterial infection and sepsis originating from the gastrointestinal tract, gastrointestinal bleeding, and severe alcoholic hepatitis[21,23]. It has been suggested

that gastrointestinal hemorrhage causes systemic inflammation through causing ischemia-reperfusion injury secondary to liver ischemia and intestinal bacterial translocation[24]. Excessive alcohol consumption is known to stimulate systemic inflammation by causing both intestinal dysbiosis and bacterial translocation in severe alcoholic hepatitis[23]. The differences in the triggering factors, underlying diseases, and comorbidities seen between children and adults suggest that factors other than those mechanisms also play a role in the pathophysiology of pediatric ACLF.

LIVER HISTOPATHOLOGY

A diagnosis of chronic liver disease or cirrhosis is typically made on the basis of a physical examination as well as specific laboratory, endoscopic, and/or radiological investigations[12]. A liver biopsy or histopathological examination of the explant liver provides information about necrosis, chronicity, and/or cirrhosis. However, it may not be possible to perform a liver biopsy due to coagulopathy. In such a case, a transjugular liver biopsy or non-invasive modality can be used[11,12,15]. While the histology of ACLF has not yet been thoroughly investigated, it can be predicted that the syndrome has the histopathological features of both ALF and chronic liver disease. Massive necrosis without chronicity is seen in the case of fulminant hepatitis or ALF [11]. Any degree of fibrosis, ductular reaction, or parenchymal collapse in the liver is a sign of ACLF[25]. This issue has not previously been studied in detail in children. In our prior study, massive confluent necrosis and fibrosis with mild to moderate inflammation (neutrophil and eosinophil), as well as evidence of regeneration, were observed in the hepatectomy materials of children who underwent LT. In those who did not undergo LT, the presence of underlying disease (*i.e.*, lymphoplasmacytic cell infiltration in AIH and micro- and macrovesicular steatosis in WD), rare or patchy hepatocellular necrosis, and advanced-stage fibrosis with bridging were all observed[16].

DIAGNOSIS AND SCORING SYSTEMS

ACLF is associated with a high short-term mortality rate. Data concerning the severity of the syndrome contributes to the selection of an appropriate treatment for it. The validity of a number of scoring models in ACLF has been extensively tested in adults. For instance, the model for end-stage liver disease (MELD), MELD-sodium (MELD-Na), and Child-Pugh-Turcotte scores, which are used in relation to organ allocation, have been found to exhibit low sensitivity because they do not evaluate extrahepatic organ failure, which is important in terms of the prognosis of ACLF[13,14]. Both the Chronic Liver Failure-Sequential Organ Failure Assessment (CLIF-SOFA) (Table 2) and the APASL-ACLF Research Consortium (AARC) (Table 3) scoring systems, which include parameters for evaluating kidney, brain, respiratory, and circulatory functions, have been found to be more reliable with regard to identifying the prognosis of ACLF [13,15]. The APASL has suggested that the AARC system is more sensitive than the CLIF-SOFA when it comes to determining prognoses. ACLF is a dynamic process, which means that the associated scoring systems should be evaluated dynamically. Scoring systems used at the 48th hour, after 3–7 d, or after 8–15 d predict the prognosis of ACLF better than a score calculated at the time of admission. An AARC score of < 10, or a score falling below 10 during the first week of admission, indicates a higher likelihood of survival in adults[13-15].

Although there is currently no validated scoring system for pediatric patients with ACLF, a few studies have made use of scoring systems (or their modified versions) designed for use with adults[2,4,8]. The modifications in this regard include the adjustment of the HE assessment, blood pressure, and serum creatinine levels according to the childhood age group[3] (Tables 4 and 5). In one pediatric study[3], the CLIF-SOFA and AARC scores were found to be superior in terms of predicting a poor outcome when compared with the Pediatric End-Stage Liver Disease, Child-Pugh and Pediatric Risk of Mortality-III scores. In the study, AARC and CLIF-SOFA scores of 11 were found to predict a poor prognosis with maximum sensitivity and specificity [area under the receiver operating characteristic curve (AUROC) > 0.9]. In another pediatric study[2] that tested the validity of the CLIF-SOFA system, the maximum sensitivity (100%) and specificity (76%) (AUROC = 0.95) were achieved at a 6.5 cut-off level with regard to predicting mortality. Moreover, in another pediatric study, children with a CLIF-SOFA score ≥ 10 at the time of admission were found to require an urgent referral to an LT center[4]. In our prior study, the AARC and CLIF-SOFA scores were

Table 2 Chronic liver failure–sequential organ failure assessment score

Organ/systems	0	1	2	3	4
Liver (bilirubin, mg/dL)	< 1.2	≥ 1.2 to < 2.0	≥ 2.0 to < 6.0	≥ 6.0 to < 12.0	≥ 12.0
Kidney (creatinine, mg/dL)	< 1.2	≥ 1.2 to < 2.0	≥ 2.0 to < 3.5	≥ 3.5 to < 5.0(or RRT)	≥ 5.0(or RRT)
Cerebral (HE grade)	No HE	I	II	III	IV
Coagulation (INR)	< 1.1	≥ 1.1 to < 1.25	≥ 1.25 to < 1.5	≥ 1.5 to < 2.5	≥ 2.5 or platelet < 20 × 10^9 /L
Circulation (mean arterial pressure, mm Hg)	≥ 70	< 70	Dopamine ≤ 5 or dobutamine or terlipressin(μg/kg/min)	Dopamine > 5 or E ≤ 0.1 or NE ≤ 0.1(μg/kg/min)	Dopamine > 15 or E > 0.1 or NE > 0.1(μg/kg/min)
Lungs					
PaO/FiO_2	> 400	> 300 to ≤ 400	> 200 to ≤ 300	> 100 to ≤ 200	≤ 100
or SpO_2/FiO_2	> 512	> 357 to ≤ 512	> 214 to ≤3 57	< 89 to ≤ 214	≤ 89

BP: Blood pressure; E: Epinephrine; FiO2: Fraction of inspired oxygen; HE: Hepatic encephalopathy; INR: International normalized ratio; NE: Norepinephrine; PaO2: Partial pressure of arterial oxygen; RRT: Renal replacement therapy; SpO2: Pulse oximetric saturation.

Table 3 Asian Pacific Association for the Study of the Liver Acute-on-Chronic Liver Failure score

Points	Total bilirubin (mg/dL)	HE grade	INR	Lactate (mmol/L)	Creatinine (mg/dL)
1	< 15	0	< 1.8	< 1.5	< 0.7
2	15-25	I-II	1.8-2.5	1.5-2.5	0.7-1.5
3	> 25	III-IV	> 2.5	> 2.5	> 1.5

HE: Hepatic encephalopathy; INR: International normalized ratio.

Table 4 Modified chronic liver failure–sequential organ failure assessment score

Organ/systems	0	1	2	3	4
Liver (bilirubin, mg/dL)	< 1.2	≥ 1.2 to < 2.0	≥ 2.0 to < 6.0	≥ 6.0 to < 12.0	≥ 12.0
Kidney (creatinine, rise from baseline)	< 1.5 ×	1.5 to ≤ 2.0 ×	> 2.0 to ≤ 3 ×	> 3 ×	Need for RRT
Cerebral (HE grade)	0	I	II	III	IV
Coagulation (INR)	< 1.1	≥ 1.1 to ≤ 1.25	> 1.25 to < 1.5	≥ 1.5 to ≤ 2.5	> 2.5
Circulation (systolic BP)	Normal for age	< 5^{th} centile for age	NE < 0.5 μg/kg/min	NE > 0.5 μg/kg/min	NE > 0.5 μg/kg/min and 2^{nd} inotrope
Lungs					
PaO/FiO_2	> 400	> 300 to ≤ 400	> 200 to ≤ 300	> 100 to ≤ 200	≤ 100

BP: Blood pressure; HE: Hepatic encephalopathy; INR: International normalized ratio; NE: Norepinephrine; PaO2: Partial pressure of arterial oxygen; FiO2: Fraction of inspired oxygen; RRT: Renal replacement therapy.

found to have high LT-predictive specificity and sensitivity. The CLIF–SOFA system focuses on extrahepatic organ failure, but there were no patients with multiorgan failure in our study. Furthermore, we found that the total bilirubin level ranges were high in the AARC system. Based on these findings, we concluded that the CLIF-SOFA and AARC scoring systems need to be modified for use in children[16].

A previous study found acute kidney injury to occur in 22.6% of children with ACLF and to be associated with a poor prognosis[8]. In a study CANONIC criteria,

Table 5 Modified Asian Pacific Association for the Study of the Liver Acute-on-Chronic Liver Failure score

Points	Total bilirubin (mg/dL)	HE grade	INR	Lactate (mmol/L)	Creatinine (rise from baseline)
1	< 15	0	< 1.8	< 1.5	< 1.5 ×
2	15-25	I-II	1.8-2.5	1.5-2.5	1.5 to ≤ 3 ×
3	> 25	III-IV	> 2.5	> 2.5	> 3 × or need RRT

HE: Hepatic encephalopathy; INR: International normalized ratio; RRT: Renal replacement therapy.

20% of 99 patients with biliary atresia were determined to have developed ACLF. Sepsis and gastrointestinal bleeding were identified as the most common precipitants of ACLF. Moreover, the ACLF mortality rate was found to be 20%[9]. In a study conducted among pediatric ACLF patients in the United States, most of the included patients were found to have biliary atresia, while the mortality rate was calculated to be 22% in patients who required hospitalization. In addition, the creatinine and aspartate transaminase levels, the INR, and a positive blood culture on admission were all shown to be associated with the development of ACLF. In this study, the triggers of the underlying decompensation were identified as bleeding, ascites, and an altered mental status in a significant portion of patients[6]. Cholangitis is known to be the most common cause of hepatic decompensation in patients with biliary atresia. Due to it not being a primary parenchymal disease, experts from the APASL study group excluded biliary atresia from among the diseases said to cause ACLF. Additionally, the APASL does not consider extrahepatic causes to be trigger factors in relation to ACLF [15].

BIOMARKERS

The treatment strategies for ACLF are mainly supportive. Biomarkers have previously been the subject of research concerned with predicting the prognosis of ACLF. These biomarkers aim to predict organ dysfunction at an early stage. Oxidative stress factors (*e.g.*, S100A12 and sRAGE), markers of cell death such as the caspase pathway proteins (which reflect the death of hepatocytes), and immune functions have been investigated in adults patients with ACLF. Unfortunately, the validity of such markers remains unknown[26]. Due to the role of infections in the etiopathogenesis of adult ACLF, the use of certain biomarkers, such as galactomannan or beta-d-glucan for invasive fungal infections and C-reactive protein and procalcitonin for bacterial infections, has been recommended by the APASL in relation to early diagnosis[15]. Renal complications are common in cases of ACLF. While hepatorenal syndrome improves following LT, acute tubular necrosis and structural acute kidney injury, which may cause permanent renal damage, require both LT and kidney transplantation. Thus, the use of new biomarkers of acute tubular necrosis (*e.g.*, N-GAL, Kim-1, IL-18, and 1-FABP) in ACLF may prove beneficial in terms of identifying an appropriate treatment approach[15, 27]. Finally, non-invasive tools and biomarkers developed to measure liver fibrosis may provide useful information when it comes to predicting the prognosis of ACLF.

PROGNOSIS

Overall, patients with ACLF have a poor prognosis. The APASL emphasizes that ACLF should be recognized during the "golden therapeutic window" prior to the development of extrahepatic organ failure, which is associated with mortality[15]. Studies conducted in adults have reported ACLF mortality rates ranging from 33% to 50%[15,21]. Pediatric cases of ACLF can be predicted to have better prognoses than adult cases for three main reasons: (1) There are specific treatments for the two most common causes of pediatric ACLF (WD and AIH); (2) Children are likely to have greater liver reserves; and (3) Children exhibit fewer comorbidities[15]. In two studies involving pediatric ACLF cases in two non-transplant centers, the 28-d and three-month mortality rates were reported to be 19.4% and 59%, respectively[1,2]. In another study, the 28-d mortality and LT rates was reported to be 25% and 8.3%, respectively [3]. In a study conducted in the same center, the three-month mortality and LT rates were reported to be 30.4% and 8.9%, respectively[4]. In a study that used the Pediatric

Acute Liver Failure (PALF) study group's ALF criteria (rather than ACLF criteria), which only included children with the etiologies of AIH, WD, Budd-Chiari syndrome, inborn errors of metabolism affecting the liver, and HBV reactivation, some 59% of patients survived without LT[28]. In a prior study we conducted among 29 pediatric ACLF patients, 24.14% of patients required LT and no patients died[16]. Interestingly, the presence of acute kidney injury increases the likelihood of death or LT by 7.7 times when compared with those who do not develop acute kidney injury[8]. When comparing the mortality rate of ACLF with that of ALF, more than 50% of children with non-acetaminophen-induced ALF died or underwent LT in the PALF study[29].

TREATMENT OF ACLF

There is no proven treatment for ACLF other than LT. The early recognition of ACLF and its precipitating events during the "golden therapeutic window period" prior to the development of extrahepatic organ failure is important in relation to the success of treatment[15]. ACLF treatments mainly include supportive treatments for hepatic and extrahepatic organ failure (if present). Extracorporeal liver support systems (*e.g.*, the molecular adsorbent recirculating system and plasmapheresis) have long been used as a bridge to LT in both adults and children with liver failure. The purpose behind using such modalities is to improve the clinical situation (especially neurologically) and biochemical parameters. However, the efficacy of extracorporeal liver support systems in adult and childhood liver failure remains unclear[11,15,30]. Optimizing the extracorporeal liver support modalities in children may improve outcomes. A few adult studies have assessed the treatment of ACLF using granulocyte colony–stimulating factor (GCSF)[31,32]. It has been suggested that GCSF may reduce mortality through promoting hepatic regeneration by mobilizing the bone-marrow-derived CD34+ cells and reducing sepsis. In a pediatric study conducted in India, 5 mcg/kg/d GCSF therapy for five days was found to be ineffective in terms of improving survival outcomes[5].

LT

Although ACLF is associated with high short-term mortality, a significant number of patients recover due to the use of medical and extracorporeal liver support systems[1-4]. The final treatment option is LT in patients who do not otherwise recover. There is no conclusive evidence concerning the efficacy of LT in children, although LT in adults with advanced ACLF has been found to result in good outcomes[13-15]. However, deciding on the timing of LT can be difficult. There are no data available concerning who would benefit from early LT, although the procedure should be performed prior to the development of multiple organ failure and advanced-stage encephalopathy. It may prove useful to use the scoring systems mentioned above when assessing the need for LT.

CONCLUSION

A definition of ACLF in children has not yet been developed. The etiology of ACLF varies among geographical regions. Moreover, organ dysfunctions are seen less frequently in pediatric ACLF patients than in adult patients. However, the mortality rate associated with ACLF remains high. Although there is no proven scoring system for predicting the prognosis of ACLF, if the AARC system score is > 8–10, a poor prognosis is indicated.

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MINIREVIEWS

Coronavirus disease 2019 in liver transplant patients: Clinical and therapeutic aspects

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has profoundly impacted liver transplant (LT) activity across the world, with notable decreases in the number of donations and procedures in most Western countries, in particular throughout the first wave. The cumulative incidence of COVID-19 in LT recipients (with estimates ranging from 0.34% to 1.56%) appears to be at least comparable to that observed for the general population. Clinical and radiological features at presentation are also similar to non-transplant patients. The risk of death among LT recipients requiring hospital admission is high (from 12% to 19%), although some authors have suggested that overall mortality may be actually lower compared to the general non-transplant population. It is likely that these poor outcomes may be mainly influenced by the older age and higher comorbidity burden of LT recipients, rather than by the transplant status itself. In fact, it has been hypothesized that post-transplant immunosuppression would exert a protective role, with special focus on tacrolimus-containing regimens. There is scarce evidence to guide the optimal management of post-transplant COVID-19 and the use of antiviral or immunomodulatory therapies, although both clinical practice and guidelines support the dose reduction or withdrawal of anti-proliferative agents such as mofetil mycophenolate. Preliminary reports suggest that the antibody response to messenger RNA vaccines is significantly impaired as compared to non-immunocompromised individuals, in line with other transplant populations. Finally, it is foreseeable that the future will be conditioned by the emerging variants of severe acute respiratory syndrome coronavirus 2 with

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increased transmissibility among LT recipients.

Key Words: COVID-19; Liver transplantation; Clinical features; Therapy; Immunosuppression; SARS-CoV-2

Core Tip: Coronavirus disease 2019 incidence and clinical and radiological features are similar in liver transplant recipients and the general population. Reported mortality in hospitalized patients is 12%-19%. Risk factors are older age and comorbidity. Tacrolimus could be protective, but anti-proliferative agents such as mycophenolate mofetil should be avoided.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) produced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China in December 2019[1,2]. The initial outbreak rapidly spread all over the world, being declared a pandemic by the World Health Organization by March 11, 2020, with 118000 cases declared in 114 countries and 4291 deaths at that time[3]. The pandemic has now affected more than 172 million people and has reached a death toll exceeding 3.7 million[4,5].

Liver transplant (LT) recipients are considered susceptible to infectious complications due to their long-term immunosuppression (IS)[6]. At the time COVID-19 was first described, the potential impact of this emerging condition on this patient population was unpredictable. Previous experiences with related coronaviruses, such as SARS-CoV or Middle East respiratory syndrome coronavirus (MERS-CoV), did not clearly show an increased incidence or case-fatality rate among immunocompromised patients[7,8]. A systematic review and meta-analysis that summarized the literature available between January and April 2020 identified hypertension, diabetes, cardiovascular disease, chronic obstructive pulmonary disease, malignancy, cerebrovascular disease, and human immunodeficiency virus infection as risk factors for severe COVID-19 in the Chinese population. Of note, chronic liver disease was not identified in this preliminary study[9]. Nevertheless, a systematic review focused on solid organ transplantation (SOT), which pooled 60 studies from January to October 2020 and 2772 unique patients, including 505 LT recipients, revealed high rates of both hospitalization (81.0%) and all-cause mortality (18.6%)[10].

In the present review, we summarize the current experience regarding COVID-19 in LT recipients, with particular focus on clinical and therapeutic aspects. Early experiences from different locations all over the world led to the scientific societies to develop guidelines for the management of these patients. This pandemic has exerted a deep impact on the transplant activity. There remain concerns about the medium- and long-term outcomes of infected recipients as well as on the optimal management of IS.

EARLY EXPERIENCES

On April 19, 2020 it was reported from Wuhan a 50-year-old male patient that had undergone LT in 2017 and developed SARS-CoV-2 pneumonia with mild respiratory failure by the end of January. Tacrolimus was restarted 4 wk later, with normal liver function. The authors suggested that reduction or temporary withdrawal of IS may be beneficial for the reconstitution of the immune response[11]. Huang *et al*[12] subsequently reported a second 59-year-old LT recipient that died on 45 d of

admission due to multiorgan failure in the setting of suspected chronic rejection and septic shock.

During March 2020, three long-term (> 10 years) LT recipients that were receiving low-dose IS and rapidly developed acute respiratory distress syndrome (ARDS) requiring mechanical ventilation died at the Istituto *Nazionale dei Tumori di Milano* between 3 and 12 d after the onset of symptoms. Three other recipients that developed COVID-19 less than 2 years from transplantation had an uneventful disease. This led the authors to suggest that post-transplant IS might be protective, whereas metabolic-related comorbidities would be associated with an increased risk of severe infection [13].

Six LT recipients from our institution had been admitted by March 23, 2020. Two of them died due to ARDS associated to renal failure and refractory shock, respectively. Both patients were receiving mycophenolate mofetil (MMF) at admission, associated to everolimus in the first case. Two further LT recipients were treated as outpatients. Two patients were temporarily converted to tacrolimus, MMF was halted in one patient, and no modifications were made in the remaining three[14].

Some of the earliest cases of post-transplant COVID-19 from the United States were reported on March 22, 2020. These 4 cases included a 67-year-old man that had undergone LT 19 years before. The patient was initially admitted to the intensive care unit (ICU), cyclosporine therapy was continued without adjustment, and he was discharged home after 6 d[15]. A report from New York City described the initial experience at two centers during the first weeks of the outbreak, including 13 LT recipients, four of them with severe disease. Sixteen out of 90 SOT recipients died, resulting in an overall case-fatality rate of 18%, 24% for hospitalized patients and 52% for those admitted to the ICU[16].

Shortly after the outbreak of the pandemic, first experiences with recent transplant recipients started to be reported. For instance, a 69-year-old patient admitted for LT on January 28, 2020 in Iran became febrile on post-transplant day 4, being diagnosed with hospital-acquired pneumonia. He developed respiratory failure and loss of consciousness on day 9. A brain computerized tomography (CT) scan revealed a hypodensity in the right parietal lobe suggestive of middle cerebral artery ischemic stroke. The patient died on day 23 after transplantation, with SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) being reported positive on the next day[17]. Qin *et al*[18] reported a 37-year-old male patient that underwent LT on January 21, 2020. He started with persistent fever on post-transplant day 9, and a thoracic CT scan revealed minor changes. A second scan performed 9 d later showed multiple ground glass opacification in the left lobes. Tacrolimus and steroids were maintained though titrated to lower doses, and supplemental oxygen therapy through high-flow nasal cannula maintained oxygen saturation ranging from 95% to 99%. The patient was successfully discharged 51 d after transplantation[18].

COVID-19 AND THE LIVER

Although COVID-19 is primarily a respiratory disease, SARS-CoV-2 may also infect the digestive system through its viral receptor angiotensin-converting enzyme 2 (ACE2). The ACE2 cell surface receptor is more strongly expressed in cholangiocytes, at a similar level in fact than type 2 alveolar cells in the lungs, than hepatocytes (59.7% *vs* 2.6%, respectively)[19]. Increased transaminases is a common laboratory finding in COVID-19, and liver injury has been associated to drug-induced liver toxicity, systemic hyperinflammatory response, or hypoxia-ischemia reperfusion injury[20], rather than direct viral cytopathic effect[21]. Coagulopathy and liver endotheliopathy have been suggested to be at least partially driven by interleukin (IL)-6 trans-signaling, which would lead to the expression of procoagulant (such as factor VIII or von Willebrand factor) and proinflammatory factors as well as increased platelet attachment in liver sinusoidal endothelial cells. Interestingly, these effects were blocked by soluble gp130, which acts as an IL-6 trans-signaling inhibitor, and the janus kinase inhibitor ruxolitinib, providing support for these therapeutic approaches[22]. Histopathologic features suggestive of some level of cytopathic injury, however, have been also observed in liver biopsies[23].

Cai *et al*[24] reported in a large cohort that individuals with abnormal liver tests were at a higher risk of progression to severe COVID-19. Abnormal liver function was observed in 76.3% of patients, with 21.5% of them developing liver injury. The detrimental effect on liver function was mainly related to therapies used during hospitalization, which should be closely monitored and evaluated.

In a retrospective study from Wuhan, 1282 out of 2073 patients (61.8%) had abnormal liver function test during hospitalization, and 14.3% experienced some degree of liver injury. Increased aspartate aminotransferase (AST) and direct bilirubin levels at admission were independent predictors of all-cause mortality, whereas the presence of hepatitis B virus infection did not increase the risk of poor outcome[25].

In a retrospective cohort comprising 234 patients hospitalized in two referral hospitals in France, the rate of abnormal liver function tests at admission was as high as 66.6% and was associated with in-hospital aggravation [odds ratio (OR): 4.1: 95% confidence interval (CI): 1.5-10.8; $P = 0.004$] and mortality (OR: 3.3; 95%CI: 1.04–10.5; $P = 0.04$). A minority of patients (3.8%) had underlying liver disease, and there were no significant differences in the prevalence of alcohol consumption or metabolic syndrome between patients with or without abnormal liver tests on admission, suggesting that this finding may be COVID-19-related and not due to pre-existing liver disease[26].

In a retrospective cohort from New York that included 2273 patients, acute liver injury was common and categorized as mild [alanine transaminase (ALT) levels < 2 times the upper limit of normal (ULN)] in 45% of the cases, moderate (ALT levels two to five times the ULN) in 21%, and severe (ALT levels > 5 times the ULN) in 6.4%. In the multivariate analysis adjusted for age, body mass index, comorbidities, and requirement of invasive mechanical ventilation (IMV) and renal replacement therapy, peak ALT levels were significantly associated with death or discharge to hospice (OR: 1.14; $P = 0.044$)[27].

Underlying cirrhosis has been identified as a risk factor for increased severity of COVID-19, with mortality rates ranging from 12% to 43%[28]. Indeed, SARS-CoV-2 may produce acute-on-chronic liver failure (ACLF) among cirrhotic patients[29]. The mortality in 20 patients with ACLF reported from India reached 30%, as compared to 5% among cirrhotic patients without ACLF[30]. Metabolic dysfunction-associated fatty liver disease has been also associated with the severity of SARS-CoV-2 infection in patients below 60 years (OR: 4.07; 95%CI: 1.20–13.79; $P = 0.02$)[31].

In the earlier post-mortem examinations, Xu *et al*[32] found moderate microvesicular steatosis and mild inflammatory infiltrates in the hepatic lobule and portal tract. Mild sinusoidal dilatation, focal macrovesicular steatosis, and mild lobular lymphocytic infiltration has been also reported[33]. Fiel *et al*[23] described the biopsies of two patients that successfully recovered from COVID-19, showing a mixed inflammatory infiltrate with prominent bile duct damage, endothelitis, and numerous apoptotic bodies. *In situ* hybridization and electron microscopy suggested the intrahepatic presence of SARS-CoV-2, thus supporting the possibility of a direct cell injury.

Macrovesicular steatosis was the most common finding (75%) in 40 liver biopsies from patients that died due to a complicated COVID-19 course. Mild lobular necroinflammation and portal inflammation were present in 20 cases each (50%), whereas viral RNA was detected by RT-PCR on liver tissue in 55% of patients tested[34].

Both the diagnosis and treatment of cancer have been negatively affected by the COVID-19 pandemic and the resulting pressure on the health care services worldwide. Patients with hepatocellular carcinoma (HCC) represent a vulnerable population with a significant treatment delay. In a multicenter, retrospective study performed in Paris, Amaddeo *et al*[35] found a significant decrease in the number of patients with HCC presented to the multidisciplinary tumor committee. The proportion of patients that experienced a treatment delay longer than 1 mo increased between 2019 and 2020 from 9.5% to 21.5%.

IMPACT OF COVID-19 ON LT ACTIVITY

The effect of the pandemic has been heterogeneous in terms of donation and transplant activity. Nevertheless, a notable reduction has been reported from most institutions across Europe and North America during the peak of COVID-19 incidence, mainly related to the burden of patients admitted to the ICU and the associated effects on candidate referral and perioperative care[36]. Such a decrease in LT activity was particularly profound in March and April 2020, during the first wave that affected many Western countries. De Simone described the reorganization of LT units carried out in so many centers worldwide during the first wave: Cancellation of routine patient follow-up, outpatient care limited to recent LT recipients, pre-transplant referral limited to priority patients after telephone triage, follow-up by means of phone calls on the waiting list, and implementation of health care worker (HCW) safety

policies[36]. This rapid reorganization allowed for maintaining the activity of a high-volume center in Pisa during the Italian national lockdown (February 18 to May 4, 2020), despite the marked drop observed between March 16 and April 5. This was achieved due to the increase in ICU bed capacity, systematic screening for SARS-CoV-2, creation of COVID-19-dedicated ICUs, recruitment of additional medical and nurse staff, rescheduling of elective surgery to priority cases, and continuation of LT activities in COVID-19-free areas[37].

A preliminary analysis of the impact on Italian LT programs was done by means of a survey issued on March 16, 2020 and completed by 22 centers[38]. There were two major geographical areas with different incidence of SARS-CoV-2 infection, north-central Italy and south-central Italy. Between February 15 and March 15, all transplant programs reduced their outpatient activity by 68% in terms of pre-transplant evaluation and 100% in the post-transplant face-to-face follow-up. A reduction in transplant activity was also seen in northern-central Italy during the first 2 wk of March, but not in the southern-central area. Recovered donors dropped by 46% during the first peak (the 4-wk period after February 23) as compared to the preceding 8-wk period[39].

In Spain, according to data provided by the Spanish National Transplant Organization [Organización Nacional de Trasplantes (ONT)], the mean number of donors declined since the national state of alarm was declared on March 13 from 7.2 to 1.2 per day, and the mean number of transplants from 16.1 to 2.1 per day[40]. There was a saturation of the health care system and ICU capacities (although most hospitals had increased the number of ICU beds), and many HCWs became infected (15.5% of the infected population at that time) or forced to quarantine. The number of potential donors declined due to the decrease in neurocritical patients or due to a positive result in SARS-CoV-2 screening. In addition, logistical problems arose as a consequence of the restricted mobility and declining organ offers following a risk assessment that included the clinical situation of the recipients, and even human resources were reduced due to cases of COVID-19 among HCWs. Finally, in the pandemic scenario, some candidates refused transplantation after informed consent[40].

The impact of the first wave on the LT activity in France resulted in an overall 28% decrease in the number of donations when comparing the first 4 mo of 2019 with the corresponding period of 2020, whereas the number of LT effectively performed dropped by 22%. The north-eastern region of the country (with the highest incidence rate of COVID-19) experienced reductions in multiorgan procurement and LT activity of 33% and 26%, respectively[41].

A national state of emergency was declared in the United States by March 13, 2020. A retrospective analysis of data collected from January 5 to September 5, 2020 by the Organ Procurement and Transplantation Network revealed a decrease of 37% in the number of LT procedures performed between March 8 and April 5[42]. Since mid-March, many waitlist patients were placed in temporarily inactive status due to COVID-19 concerns. This practice affected over 2000 waitlist registrations during the week of March 22. LiveOnNY, the organ procurement organization for the greater New York metropolitan area, suffered a drop to 10 donors in April 2020 from 26 in March, although this figure recovered to 18 donors in May[43].

A multinational study performed in India, the United Kingdom, and the United States compared the weekly organ donation and LT numbers over a 3-mo period (February 17 to May 17, 2020) and the LT activity in six centers with varying local COVID-19 caseload[44]. Peak reduction ranged from 25% in the United States to more than 80% in the United Kingdom and India.

On the contrary, the impact of COVID-19 on LT activity has been reported to be almost negligible in other countries. Lee concluded that establishing safe processes and procedures can be beneficial in reducing the negative effects of the national lockdown and saving patients' lives, as he analyzed LT procedures performed in South Korea[45]. He compared the MERS outbreak, the COVID-19 pandemic, and the average number of LT performed throughout the prior 5 years. There was a significant decrease of 11% in the LT activity during the MERS outbreak, although the number of procedures was maintained from January to March 2020. In addition, none of the 401 patients undergoing LT during the COVID-19 outbreak were confirmed to be infected with SARS-CoV-2. Some Italian centers located in medium- or high-incidence areas were also able to maintain a stable LT activity by means of appropriate screening and isolation practices, dedicated COVID-19-free routes, and reorganization of ICU resources[36,46,47].

A great variability in the adaptation of LT practices in response to the COVID-19 pandemic has been observed within the same country and even the same region[48]. On the other hand, the detrimental impact on LT activity seems to have been not

restricted to those areas facing the highest COVID-19 burden. According to Agopian *et al*[48] such differences across centers likely reflect variations in the allocation and prioritization of hospital resources, local capacities to timely screen for SARS-CoV-2 infection among SOT candidates and recipients, and concerns with respect to donors (*e.g.*, accuracy of testing), recipients (*e.g.*, role of baseline IS), and transplant team members (*e.g.*, risk of hospital-acquired COVID-19).

The effect on the LT waiting list in the United States has been studied by Strauss *et al*[49] using data from the Scientific Registry of Transplant Recipients. From March 15 to April 30, new listings were 11% lower than expected, and deceased donor LTs (DDLTs) decreased by 9%. In May, new listings were 21% lower and living donor LTs were 42% lower, whereas DDLTs increased by 13%. In states with the highest incidence of COVID-19, the number of deaths in the waiting list increased by 59%. By August, waitlist outcomes were occurring at expected rates except for DDLT. According to the authors, these results reflect the adaptability of the transplant community in addressing the COVID-19 pandemic and applying new knowledge to patient care.

Putzer *et al*[50] found a 29% decrease in the number of LT procedures performed in the Eurotransplant area between mid-March and mid-June 2020, with regards to the corresponding periods from 2015 to 2019. Of note, the activity in Germany continued at the same pace during the initial phase of the crisis, likely thanks to the higher number of ICU beds in that country. However, the number of LTs increased slowly compared to the first month of observation.

INCIDENCE OF COVID-19 IN LT RECIPIENTS

According to the survey performed by the European Liver and Intestine Transplantation Association (ELITA) and the European Liver Transplant Registry (ELTR), the crude incidence of SARS-CoV-2 infection among LT candidates and recipients during the first wave in Europe has been overall estimated in 1.05% (range: 0.5%-20%) and 0.34% (range: 0.1%-4.8%), respectively[51]. One hundred nine out of 149 (73.2%) ELTR centers located in 28 European countries responded to the survey. Eighty-eight centers reported the diagnosis of COVID-19 in 57 LT candidates and 272 recipients. The highest numbers of infected recipients were reported from Spain (77), Italy (66), and France (59). Crude case fatality rates in candidates and recipients were 18% and 15%, respectively. The authors concluded that both LT candidates and recipients are at high risk of COVID-19 and highlighted the need for an early and proactive screening for SARS-CoV-2 infection in these populations.

Cumulative incidence of COVID-19 has been highly variable across European countries. The King´s College group only reported 5 cases out of about 4500 LT recipients (0.1%) followed-up in their institutional cohort during the first wave[52]. In fact, LT recipients appeared to have a lower incidence of COVID-19, with less severe symptoms, as compared to the general population or other SOT populations, likely due to the better individual adherence to self-isolation recommendations or the optimal level of IS, which would favorably modulate the response against SARS-CoV-2.

A nationwide study promoted by the Spanish Liver Transplantation Society (SETH) recruited 111 LT patients from February 28 to April, 7 2020 and revealed a higher incidence of COVID-19 compared to the general population, almost doubling the expected number of cases[53]. A preliminary experience from our institution showed a cumulative incidence from March 15 to May 5 of 1.6% (19 out of 1200) among LT recipients compared to 0.95% in the general population of Madrid, although potential underreporting due to limited diagnostic capacities at that time could not be ruled out [54].

A detailed study carried out in the United Kingdom comprised SOT recipients diagnosed with SARS-CoV-2 infection in England up to May 20, 2020 and showed a cumulative incidence of 1.3% and 0.7% (64 out of 8734) for the specific group of LT recipients[55].

As the pandemic evolved during 2020, different institutions and groups have provided updated epidemiological data. On the basis of data collected by the Italian Information Transplant System until June 22, Trapani *et al*[56] found a cumulative incidence of 1.02% among SOT recipients as compared to 0.4% in the non-transplant population ($P < 0.05$). This figure was lower (0.63%) for LT recipients. Authors from the Shiraz University of Medical Sciences in Iran, one of the largest transplant centers in the world, published their results by mid-July[57]. They found 85 cases of COVID-

19 among abdominal transplant recipients (66 in LT recipients). As of July 2020, 0.32% of the population of the country was infected, with a mortality rate of 5.1%. Among 6969 SOT recipients followed-up at their center, 85 (1.21%) had been diagnosed with COVID-19, and 17 (20%) had died. Their conclusion was that LT and kidney transplant recipients face a poorer outcome due to COVID-19.

Not surprisingly, cumulative incidence has steadily increased over the last months, reflecting variations in the epidemiology of COVID-19 in the general population. In our institution, we have registered 67 cases of SARS-CoV-2 infection by the end of January 2021, accounting for more than 5% of followed-up LT recipients (data not published).

RISK FACTORS FOR SEVERE COVID-19 IN LT RECIPIENTS

In an early retrospective, multicenter cohort study, Zhou *et al*[58] reported detailed clinical course and risk factors for mortality in 191 non-transplant patients with COVID-19 from Wuhan that had been discharged or died by January 31, 2020. Hypertension (30%), diabetes (19%) and coronary heart disease (8%) were the most common comorbidities in the general population. The authors found that older age, higher Sequential Organ Failure Assessment score and D-dimer levels above 1 μg/mL on admission were associated with in-hospital death at multivariable regression.

Mainly reflecting the risk factors identified in the general population, older age, the presence of chronic comorbidities (congestive heart failure, chronic obstructive pulmonary disease, or obesity), lymphopenia (absolute lymphocyte count < 0.5 x 10^9 cells/L), and abnormal chest imaging at admission were independently associated with mortality (20.5%) in a cohort study comprising 482 SOT recipients (73 LT recipients) from more than 50 United States centers[59].

Preliminary data from the ELITA/ELTR registry on 103 LT recipients diagnosed with COVID-19 between March 1 and April 24, 2020 revealed the following co-morbidities: Overweight (56%), hypertension (51%), diabetes (41%), chronic renal impairment (serum creatinine level > 2 mg/dL) (15%), smoking history (13%), and coronary artery disease (7%). After a median follow-up of 18 d, overall all-cause mortality rate was 16%, but it reached 22% among patients ≥ 60 years and 44% in those requiring IMV[60]. Although the difference did not achieve statistical significance, mortality was found to be lower among patients that had undergone LT within the previous 2 years as compared to those with longer intervals since transplantation (5% *vs* 18%). Of note, all deaths occurred among patients aged 60 years or older.

In the SETH study the most common comorbidity was hypertension (57.7%), whereas risk factors for severe COVID-19 among hospitalized patients included Charlson comorbidity index, male gender, dyspnea at diagnosis, and baseline immunosuppression containing MMF, particularly at doses higher than 1000 mg/d [53].

The assessment of SARS-CoV-2-attributable mortality after LT must take into account the impact of baseline conditions. A multicenter study from the COVID-Hep and SECURE-Cirrhosis international registries performed between March 25 and June 26, 2020 compared the outcomes of 151 adult LT recipients and 627 patients with SARS-CoV-2 infection who had not undergone transplantation. Older age, serum creatinine levels, and non-liver cancer were associated with mortality. In a propensity score-matched analysis (adjusted for age, sex, major comorbidities, and ethnicity), LT did not significantly increase the risk of death in patients (absolute risk difference: 14%; 95%CI: -7.7–10.4)[61]. Similar findings have been also reported for kidney transplant recipients[62].

COVID-19 PRESENTATION IN THE SETTING OF LT

There is a male predominance across different series of LT recipients with COVID-19, from 68%[61] to 78.8%[57]. Median age in adult patients ranges from 60[59] to 65 years [53,60]. Low-grade fever was the most frequent symptom in the earlier reports from Wuhan, followed by cough, fatigue, myalgia, and digestive symptoms (diarrhea, nausea, or vomiting)[58]. Among LT recipients with COVID-19, the presence of fever is also reported in 62.7%[57] to 79%[62] of cases. Cough (with rates ranging from 40.9% [57] to 70.3%[52]), myalgia (37%[60] to 45.5%[57]), fatigue (40.9%[57] to 56%[62]), dyspnea (30.3%[57] to 46%[63]), gastrointestinal symptoms (22.6%[60] to 39.4%[57]), and smell and taste disorders (7%[63]) are also common at presentation.

Becchetti *et al*[63] observed a higher prevalence of fever and dyspnea in long-term LT recipients (more than 10 years from the procedure), whereas the presence of fever and cough was significantly less likely among very short-term recipients (≤ 1 year) [63]. Asymptomatic patients are scarce. In the SETH series they accounted for 6.3% of cases only, whereas most of the patients admitted to the hospital (66%) required some type of respiratory support[52].

Chest X-ray or computed tomography scan showed typical features of COVID-19 in 62% of patients in the series by Belli *et al*[60] and 78.4% in the SETH cohort (unilateral in 19.8% and bilateral in 58.6%)[52]. Becchetti *et al*[63] reported typical radiological features (bilateral, peripheral, consolidation, or ground glass opacities) in 43% of computed tomography scans and 40% of X-ray examinations performed[63].

Only 8% of the patients reported by Becchetti *et al*[63] had a significant increase in transaminases (AST and/or ALT > 2 times the ULN), whereas this figure reached 14.7% in the series by Colmenero *et al*[53]. Mean lymphocyte and platelet counts were decreased in patients with severe disease. Lymphopenia was present in 68.8% of the patients reported by Malekhosseini *et al*[57] and 76% of those reported by Becchetti *et al*[63]. The nadir of absolute lymphocyte count during hospital stay was 0.31 x 10^9 cells/L among severe cases (versus 0.5 × 10^9 cells/L in the non-severe forms of infection; P = 0.013). Other markers as D-dimers of ferritin levels were significantly higher in severe cases[53], although data were not available for most patients[63].

OUTCOME IN LT RECIPIENTS WITH COVID-19

The percentage of mild cases managed as outpatients varied in different series from 13.5%[53] to 42.4%[57]. Most of the published cohorts reported rates of hospitalization in the range of 66% to 82%[60,61,63], with a mean hospital stay of 9-10 d[57,63]. Notable variation was observed in the proportion of ICU admission (from 10%[63] to 31.6%[57] of hospitalized patients), which likely reflect regional differences in the availability of critical care resources. Regarding respiratory support, invasive or non-invasive mechanical ventilation was used in 10%[63] to 20%[61] of recipients, including extracorporeal membrane oxygenation in 10.6% of the patients in one series [57].

Reported mortality rates ranged between 12%[61] and 19%[61], close to those observed in large series in the general population (15-21%)[1,64]. Colmenero *et al*[53] showed that, after adjusting for age and gender, the number of observed deaths among LT patients was slightly lower than expected in the general population, resulting in a standardized mortality ratio of 95.55 (95%CI: 94.25–96.85).

Four out of 5 patients that contracted COVID-19 within the first month after transplantation in Shiraz died[57]. The authors attributed this dismal outcome to the higher amount of IS given during the very early post-transplant period. On the other hand, there are several reports on successful recovery in patients diagnosed with SARS-CoV-2 infection very shortly after LT[65-69].

Bhoori *et al*[13] were the first to suggest that long-term LT survivors on minimal IS therapy would face a greater risk of death following COVID-19 infection, thus proposing that a higher IS level could play a protective role. A systematic review pooling outcomes of 223 LT recipients from case-series and cohorts published up to June 15, 2020, however, revealed no significant differences in mortality rates between recent (< 2 years) and remote (≥ 2 years) LT recipients (16.7% *vs* 21.9%, respectively; P = 0.5)[70].

THERAPEUTIC APPROACHES IN LT RECIPIENTS WITH COVID-19

Antiviral therapies

Most LT recipients included in the series reported during the first pandemic wave were treated with repurposed drugs with *in vitro* activity against SARS-CoV-2, despite the lack of supporting clinical evidence at that time. For instance, the use of hydroxychloroquine (HCQ) (66%), azythromycin (33%), and lopinavir/ritonavir (LPV/r) (17%) was common among LT recipients recruited in the ELITA/ELTR registry between March 1 and April 24, 2020[60]. These rates were even higher in the SETH registry, with as many as 88% and 40% of patients receiving HCQ and LPV/r, respectively[53]. Of note, no differences in the use of these agents were observed according to the severity of COVID-19. In addition, the multicenter registry collected by the ONT in Spain showed that the proportion of recipients treated with protease inhibitors

(mainly LPV/r), HCQ, and azithromycin was similar across different SOT populations, suggesting that the therapeutic approach in LT recipients did not substantially differ from that used in patients usually exposed to a higher level of IS, such as heart or lung transplant recipients[71]. As expected, the management of drug-to-drug interactions between LPV/r, a potent cytochrome P450 3A4 inhibitor, and calcineurin or mammalian target of rapamycin (mTOR) inhibitors was particularly challenging[16,72]. In our experience, two LT recipients under everolimus were converted to low-dose prolonged-release tacrolimus (0.5 mg/wk) in order to facilitate the adjustment of IS during hospitalization[54].

No outcome benefit has been demonstrated from the use of LPV/r, HCQ, or subcutaneous interferon-β in the setting of randomized controlled trials (RCTs) conducted over the past months[73-75]. The RNA-dependent RNA polymerase inhibitor remdesivir is the only antiviral agent currently approved for the treatment of COVID-19, in view of the shorter time to clinical recovery obtained with this agent as compared to placebo[76]. The clinical experience with remdesivir in LT recipients, nevertheless, is scarce, with only a few treated patients in large multicenter cohorts[53,71]. Since remdesivir and its main active metabolite GS-441524 are mainly excreted by the kidney, no major drug-to-drug interactions with tacrolimus, MMF, or mTOR inhibitors are to be expected, whereas limited experience with cirrhotic patients has revealed no new safety signals[28]. Abnormal liver function test was not reported as a common adverse event in the ACTT-1 trial, although exclusion criteria included the presence of ALT or AST levels > 5 times the ULN[76].

Immunomodulatory therapies

The clinical course of severe forms of COVID-19 is characterized by the presence of an excessive inflammatory response triggered by SARS-CoV-2 and orchestrated by the host immune system, which contributes to the development of tissue damage, multiorgan failure, and ARDS[77]. Such a pathogenic mechanism has led to the widespread use of various immunomodulatory strategies aimed at blocking this "cytokine storm", including corticosteroids[78], anti-IL-6 (such as tocilizumab or sarilumab)[79] and anti-IL-1β (canakinumab or anakinra)[80] agents, or janus kinase inhibitors (baricitinib)[81]. With the exception of low-to-intermediate-dose systemic corticosteroids (*i.e.* dexamethasone 6 mg daily for 10 d), which have been shown to decrease 28-d mortality in patients requiring respiratory support[82], there remains controversy regarding the clinical benefit to be expected from these agents in the general population with COVID-19, with conflicting results from observational studies and RCTs.

The available evidence supporting the use of immunomodulatory therapies in SOT recipients is even more limited[83]. Nevertheless, multicenter registries revealed that anti-IL-6 agents were commonly administered during the first pandemic wave (with overall rates ranging from 13%[59] to 21%[71]). In the specific group of LT recipients, 5% and 1% of patients included in the ONT registry as of July 2020 had received tocilizumab and anakinra, respectively[71]. The off label use of tocilizumab in other cohorts ranged from 6.2% in the ELITA/ELTR registry[84] to 15.6% in the SETH registry[53]. As previously stated, no RCTs have assessed to date the role of therapeutic IL-6 blockade in the setting of post-transplant COVID-19 with cytokine release syndrome. A small retrospective study compared 29 SOT recipients treated with tocilizumab for severe COVID-19 (including one single LT recipient) with a matched control group of recipients who did not receive this agent. No significant differences were observed in terms of in-hospital mortality (41% *vs* 28%, respectively; $P = 0.27$), hospital discharge (52% *vs* 72%; $P = 0.26$), or secondary infections (34% *vs* 24%; $P = 0.55$), although the higher rates of IMV and renal replacement therapy observed in the tocilizumab group suggest some degree of confounding by indication not completely controlled by the matching process[85].

Management of immunosuppression

As commented above, some preliminary reports showing a worse outcome among long-term LT recipients on minimal immunosuppressive regimen (as compared to recently transplanted, fully immunosuppressed patients)[15] led to propose during the first weeks of the pandemic that post-transplant IS might be actually protective in severe COVID-19[86]. Clinical experience accumulated over the past months, however, does not seem to confirm this hypothesis. Indeed, the SETH registry demonstrated the deleterious impact of baseline MMF-containing regimens (particularly when given at doses higher than 1000 mg/d). This negative effect was not observed for calcineurin or mTOR inhibitors. Complete MMF withdrawal during hospitalization showed a trend towards a reduced risk of progression to severe COVID-19 (41.7% *vs* 69.2%; $P = 0.16$)

[53].

The most common adjustment of baseline IS among more than 600 SOT recipients enrolled within the ONT registry was the withdrawal of the anti-metabolite drug (MMF or azathioprine), whereas calcineurin inhibitors were generally managed with dose reduction[71]. It is likely that the impact of baseline IS on the outcome of SARS-CoV-2 infection differ according to individual drugs. Belli *et al*[84] have recently shown that the use of tacrolimus was independently associated with a reduced mortality risk in the ELITA/ELTR registry (hazard ratio: 0.55; 95%CI: 0.31–0.99). The authors propose that tacrolimus could exert a direct antiviral effect through the immunophilin FK506-binding proteins[87].

In accordance with the survival benefit demonstrated for dexamethasone in the RECOVERY trial[82], baseline corticosteroid dose was usually maintained or increased in most LT recipients hospitalized due to COVID-19. In addition, corticosteroids boluses were given in 12.5% of patients in the SETH registry (4.9% and 25.7% of those with non-severe or severe COVID-19, respectively)[53].

SARS-COV-2 VACCINATION IN LT RECIPIENTS

Whereas messenger RNA SARS-CoV-2 vaccines provide excellent rates of seroconversion and clinical effectiveness in the general population[88,89], immunogenicity in the setting of SOT appears to be severely compromised. Most available reports, however, are focused on kidney[90-92] or lung transplant recipients[93]. In addition, only a few studies have assessed the development of SARS-CoV-2-specific T-cell-mediated immunity in addition to antibody responses[94,95]. Rabinowich *et al*[96] tested for SARS-CoV-2 immunoglobulin G antibodies against the SARS-CoV-2 spike glycoprotein 10-20 d after the administration of the second BNT162b2 vaccine dose in 80 LT recipients. Detectable humoral response was demonstrated in 47.5% of patients only (as compared to 100% of HCWs used as control group). In addition, the mean antibody titer was significantly lower in LT recipients (95.41 AU/mL *vs* 200.5 AU/mL, respectively). Older age, lower estimated glomerular filtration rate, and treatment with MMF or high dose steroids were associated with the lack of vaccine response, with no apparent impact of the time since transplantation. The vaccine was well tolerated, and there were no episodes of suspected or confirmed graft rejection during the follow-up [96]. This disappointing immunogenicity is, however, in line with the rates reported for other SOT populations. The deleterious effect of the anti-metabolite drug has been also shown for kidney and lung transplant recipients[90,93].

GUIDELINES FOR THE MANAGEMENT OF LT DURING THE COVID-19 PANDEMIC

On November 9, 2020, the American Association for the Study of Liver Diseases (AASLD) issued updated guidelines for LT providers in the current pandemic scenario [97]. Regarding the management of the waiting list, the document recommends to continue to prioritize the initial evaluation of patients with HCC or those with severe disease and high Model for End-stage Liver Disease (MELD) scores who are more likely to benefit from immediate LT listing. Some listed patients should be still seen in person according on the local incidence of SARS-CoV-2 infection and individual patient factors (such as their Model for End-stage Liver Disease score). Telemedicine alternatives may be considered for the remaining candidates. In addition, the AASLD guidelines recommend to develop hospital-specific policies for organ acceptance, taking into account the availability of ICU beds and other hospital resources. Potential donors and recipients must be screened for SARS-CoV-2 exposure and clinical symptoms compatible with COVID-19 (regardless of test results or availability). In addition, all donors and recipients should be screened for SARS-CoV-2, by means of nasopharyngeal swab, bronchoalveolar lavage, or both, taking into account the risk of false negative results, disease prevalence, and testing turnaround time in your area. Alternatives to RT-PCR-based testing such as chest X-ray may also be also considered. Ideally, LT in SARS-CoV-2-positive candidates should be delayed for at least 14-21 d after symptom resolution and one or two negative SARS-CoV-2 diagnostic tests. Of note, the decision to ultimately proceed with LT in a candidate recovering from COVID-19 must be individualized based on several factors (such as the urgency of transplantation, the presence of respiratory symptoms, and the risk of exposing HCWs

to SARS-CoV-2).

Regarding the approach to LT recipients diagnosed with COVID-19 in the AASLD guidelines, it should be considered lowering the overall level of IS (particularly anti-metabolite doses) based on general principles for managing post-transplant infections and in order to decrease the risk of secondary infection. The risk of COVID-19-associated kidney injury should be also taken into account and calcineurin inhibitor levels must be closely monitored. Likely due to the lack of supporting evidence, no clear recommendations are provided regarding the optimal regimen and timing for antiviral and immunomodulatory therapies.

In addition, the AASLD expert panel advises against making anticipatory adjustments in current immunosuppressive regimens in LT recipients with no diagnosis of SARS-CoV-2 infection. Prevention measures (*e.g.*, hand washing, cleaning frequently touched surfaces, staying away from large crowds, *etc*.) should be emphasized in this at-risk population[97].

Finally, although specific guidelines on the optimal vaccination strategy are scarce and based on low-level evidence, the Italian Association for the Study of the Liver recommends that LT candidates should be prioritized due to the high risk of mortality in the waiting list. Vaccination of the partners and caregivers of cirrhotic patients and LT recipients should be also encouraged[98].

CONCLUSION

Although with geographical differences across countries, COVID-19 has exerted a negative impact on LT transplant activity (both in the number of donors and procedures) during the first months of the pandemic, with decreases ranging from 28% to 46%[38,40,42,43]. The cumulative incidence of SARS-CoV-2 infection in LT recipients has been estimated between 0.34%[50] to 1.56%[52]. These figures appear to be comparable to that observed for the general population, although some studies suggest that the incidence of COVID-19 after LT would be lower as compared to other types of SOT[54]. The clinical and radiological characteristics of COVID-19 at presentation are overall similar to non-transplant patients, including predictive factors of poor outcomes. All-cause mortality among hospitalized recipients is high (from 12%[61] to 19%[59]), and great heterogeneity in the rates of ICU admission is observed across different series (10%[61] to 31.6%[55]). It has been also proposed that the risk of death may be actually lower compared to the non-transplant population[51]. The outcome of post-transplant COVID-19 seems to depend mainly on the age of the recipient and the number of chronic comorbidities, rather than by the transplant status itself[59]. Some studies have suggested that post-transplant IS —in particular tacrolimus-containing regimens— may play a protective role by abrogating the deleterious effect of the cytokine release syndrome occurring during the course of SARS-CoV-2 infection or through a direct antiviral activity[83]. To date, there is scarce evidence to guide the use of antiviral or immunomodulatory therapies for COVID-19 after LT, including the potential effectiveness and safety of remdesivir or anti-IL-6 agents[82]. Both clinical experience and guidelines recommend the dose reduction of IS or withdrawal of MMF and other anti-proliferative agents[51,87]. Although specific studies are still scarce, messenger RNA vaccines seem to be safe in LT recipients in terms of serious adverse events or risk of alloimmunity, although the magnitude of SARS-CoV-2-specific immunoglobulin G antibody response is severely decreased as compared to non-immunocompromised individuals[97].

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MINIREVIEWS

Pediatric vascular tumors of the liver: Review from the pathologist's point of view

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Abstract

Differential diagnosis of pediatric vascular liver tumors can be challenging due to inconsistent nomenclature, histologic overlap and the rarity of some entities. Here we give an up-to-date overview of the most important entities. We discuss the clinic, histology and pathophysiology of hepatic congenital and infantile heman - gioma, hepatic epithelioid hemangioendothelioma and hepatic angio-sarcoma.

Key Words: Hepatic congenital hemangioma; Hepatic infantile hemangioma; Hepatic epithelioid hemangioendothelioma; Hepatic angiosarcoma; Hepatic vascular tumors of infancy; Hepatic hemangiomas

Core Tip: Overview of the most important pediatric hepatic vascular tumors from the point of view of the pathologist, including hepatic hemangiomas, hepatic epithelioid hemangioendothelioma and hepatic angiosarcoma.

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INTRODUCTION

Through the years the classification of vascular anomalies in the liver has evolved due to better biological understanding with substantial contribution of molecular genetics

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and immunohistochemical correlates. However, terminology can be difficult due to the existence of multiple (general and organ specific) classifications and inconsistent nomenclature through the years. In 1997, vascular tumors were differentiated from vascular malformations for the first time[1]. In brief, the main difference between the above entities is that vascular tumors are considered as cellular vascular neoplastic proliferations and vascular malformations as errors in the morphogenesis lined by mature endothelium[2,3]. In 2014, The *International Society for the Study of Vascular Anomalies* (ISSVA) divided vascular tumors further in benign, locally aggressive or borderline and malignant entities[4]. Here, we give an overview of the most important pediatric hepatic vascular tumors, including hepatic hemangiomas, hepatic epithelioid hemangioendothelioma and hepatic angiosarcoma.

HEPATIC HEMANGIOMA

Hepatic hemangiomas belong to the group of benign vascular tumors[4]. The term "*hemangioma*" has been used through the years for a variety of vascular malformations of the liver. In 2018, the ISSVA reserved this term for vascular lesions that match the definition of congenital or infantile hemangiomas[5]. These benign endothelial neoplasms can occur in the liver and belong to the histologic group of "*hepatic hemangioendothelioma, type 1*" (Figure 1). However, the term '*hemangioendothelioma*' has to be used with caution, due to the terminology overlap with epithelioid hemangioendothelioma (which is considered as a malignant vascular entity) and should be avoided in absence of histologic evaluation[5,6]. Further, histologic confirmation of hemangiomas is often not required, since the diagnosis can easily be made with physical examination, imaging and review of patient's history. Still, a biopsy can be performed when the history or clinical/radiological features are atypical[5]. Hemangiomas are characterized by a proliferation, plateau and involution phase. They occur due to an imbalance in angiogenesis, resulting in an uncontrolled proliferation of vascular elements. Involution of the lesions is characterized by a decrease in angiogenic factors, endothelial cell apoptosis and high levels of angiogenic inhibitors, replacing the endothelial cells by loose stromal tissue [2,6].

Hepatic congenital hemangioma

Hepatic congenital hemangiomas (HCH) are benign high-flow vascular tumors that proliferate in utero and are fully grown at birth with no postnatal increase in size. They are less common than hepatic infantile hemangioma (HIH) and present mostly as a solitary lesion[5,7,8]. Diagnosis can be made on prenatal imaging showing a large mass with extensive central infarction, hemorrhage, calcifications and sometimes large abnormal vessels, suggestive for arteriovenous malformation[5,9]. They can be asymptomatic or can cause intratumoral bleeding, thrombocytopenia, hypofibrinogenemia (Kasabach-Merritt syndrome, occasionally associated with large hepatic hemangiomas) and high-output cardiac failure[5,10].

The most important clinical differential diagnoses of a liver mass in infants include hepatic infantile hemangioma (HIH), epithelioid hemangioendothelioma, hepatoblastoma, germ cell tumors, (metastatic) neuroblastoma, mesenchymal hamartoma, cysts and abscesses[10,11].

There are 3 clinical subtypes depending on the pattern of evolution: *rapidly involuting congenital hemangioma* (RICH), *partially involuting congenital hemangioma* (PICH) and *noninvoluting congenital hemangioma* (NICH)[4,5,10]. These subtypes share common histopathologic features and have to be seen as part of a single entity with differences in their clinical behavior[12,13].

Histologically (Figure 2), HCHs are usually well-demarcated vascular lesions which can show entrapment of hepatocytes and bile ducts in interface areas[9]. RICH is composed of lobules of variable sized, mostly small thin-walled vessels lined by plump endothelium without cytonuclear atypia[7,10]. There may be evidence of thrombosis and the central part (*i.e.*, the first area of involution) may contain necrotic and hemorrhagic areas, fibrosis and focal dystrophic calcifications. Extramedullary hematopoiesis can also be observed. At the periphery of the lesion abundant larger vessels occur, sometimes associated with aneurysmal changes[7]. In contrast to RICH, NICH shows lobules of small vessels with interlobular fibrosis but without signs of involution. Arteriovenous microfistulae with large irregular vessels in the center can occur[10]. PICH shows histologic overlap between RICH and NICH and cannot be distinguished histologically[12,13]. Endothelial cells show immunoreactivity for Wilms' Tumor 1 (WT-1), CD34, CD31, factor VIII and Erythroblast transformation-

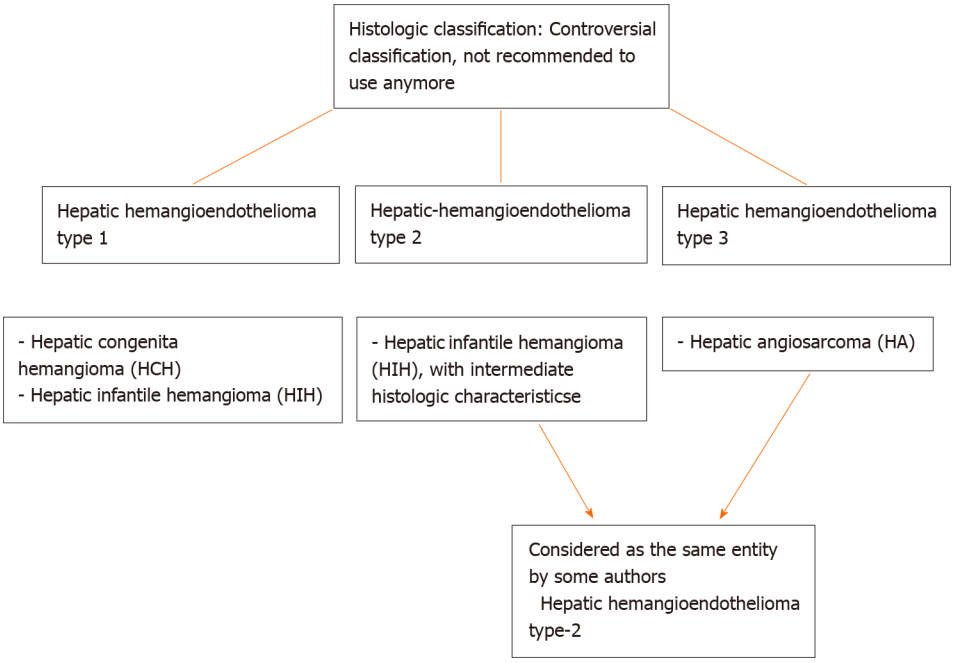

Figure 1 Histologic classification of hepatic hemangioendothelioma.

specific [ETS]-related gene (ERG)[13-15]. Triana *et al*[16] showed there was no expression of podoplanin (D2-40) in HCH. However, El Zein *et al* showed focal positivity for podoplanin in congenital hemangiomas of the skin, mainly in abnormal extralobular lymphatic vessels or in patients with concomitant thrombocytopenia (with decrease of intensity when platelet count normalized)[13]. The endothelial cells of HCH do not stain for glucose transporter-1 (GLUT-1), which is an important hallmark in the differentiation of HCH with HIH (Figure 3)[5,10].

Genetic studies revealed that almost all HCHs have mutually exclusive, missense mutations that alter glutamine at amino acid 209 (Gln209) in the alleles which code for guanine nucleotide-binding protein G(q)alpha (*GNAQ*) and guanine nucleotide-binding protein subunit alpha-11 (*GNA11*), regardless of subtype. This implies that also other genetic, epigenetic and/or environmental factors may influence the behaviour of these lesions[10,17]. A subset shows missense mutations in *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* (*PIK3CA*) (c.3140A > T; p.His1047Leu)[16].

Hepatic infantile hemangioma

HIH is the most common benign hepatic tumor in infancy, with female predominance [7]. It proliferates rapid after birth, reaching a maximal size at 6 to 12 mo, and then it gradually involutes until 3 to 9 years[5,6]. Most hemangiomas are asymptomatic and remain undetected or are incidental findings on postnatal imaging. Still, a subset can be symptomatic due to their size, location or hemodynamic effects[8]. The high flow within the tumor or presence of shunts can cause cardiac failure. Also, thrombocytopenia and anemia can be observed when intralesional thrombosis occurs[5,8,18,19]. Due to high expression of type 3 iodothyronine deiodinase in these vascular lesions, which inactivates thyroid hormone, acquired consumptive hypothyroidism occurs. All of these complications are detected after birth during the proliferation phase and can be missed initially on newborn screening[5]. Further, HIH can occur in association with Beckwith-Wiedemann syndrome[6].

The clinical differential diagnosis of HIH is broad and includes arteriovenous malformations, arterioportal fistula, mesenchymal hamartoma, hepatoblastoma, angiosarcoma and (metastatic) neuroblastoma[8].

HIH presents clinically/macroscopically as white-tan nodules with occasionally degenerative changes in the centre[9]. They can be divided into 3 categories based on degree of unaffected liver parenchyma: focal, multifocal or diffuse disease. Focal HIH shows overlap with RICH, as it does not express GLUT-1 and can be found on prenatal imaging[8,18,19]. Therefore, focal HIH is not considered as a true HIH[8]. Multifocal HIH presents as areas of hemangioma with intervening segments of normal hepatic parenchyma, whereas a diffuse pattern is defined as innumerable tumors with nearly

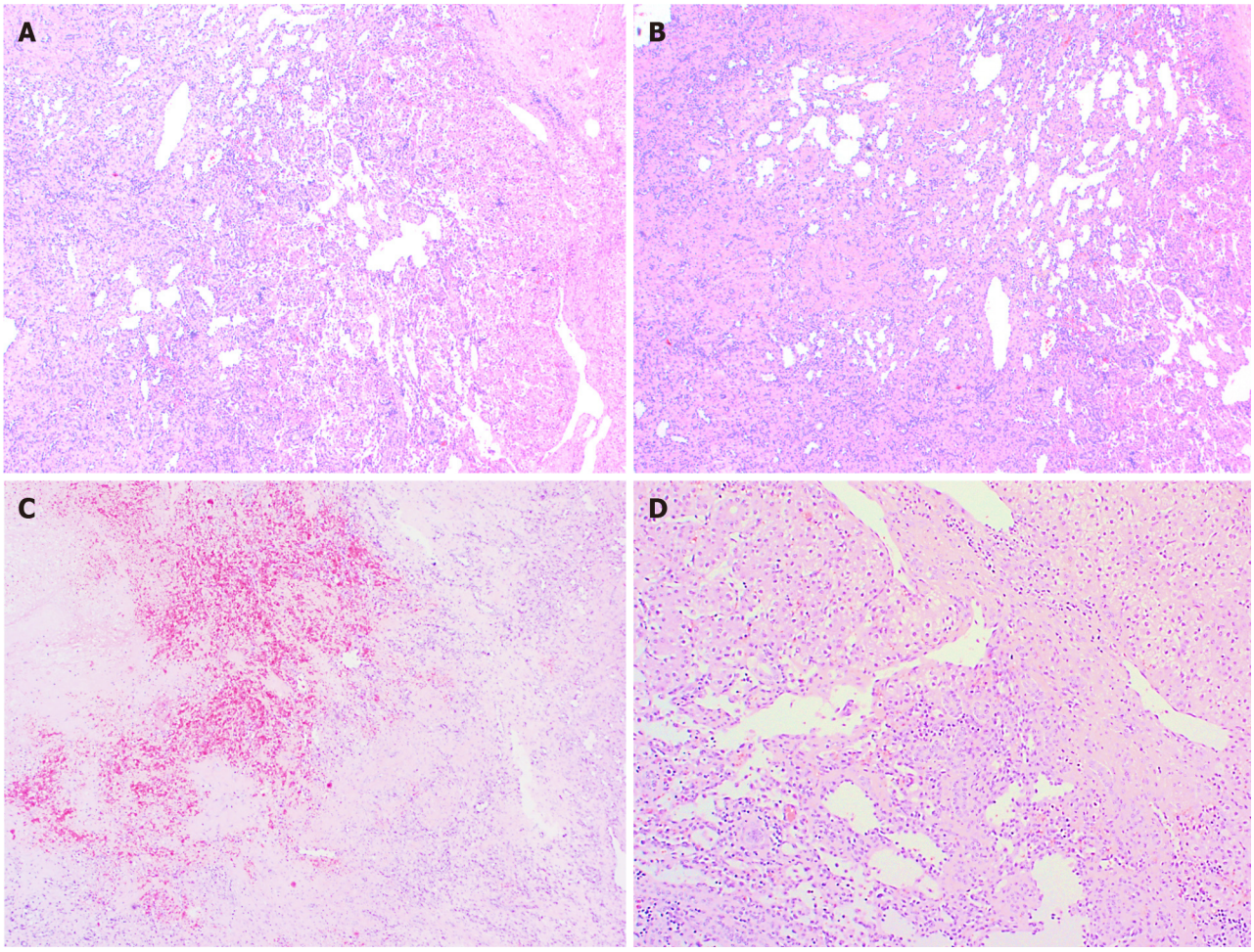

Figure 2 Hepatic congenital hemangioma. A: A relatively well-demarcated vascular lesion; B: Lobules of variable sized, mostly small thin-walled vascular spaces and more abundant larger vessels at the periphery; C: Necrotic and hemorrhagic areas in the central part (area of involution); D: Entrapment of hepatocytes and bile ducts in interface areas.

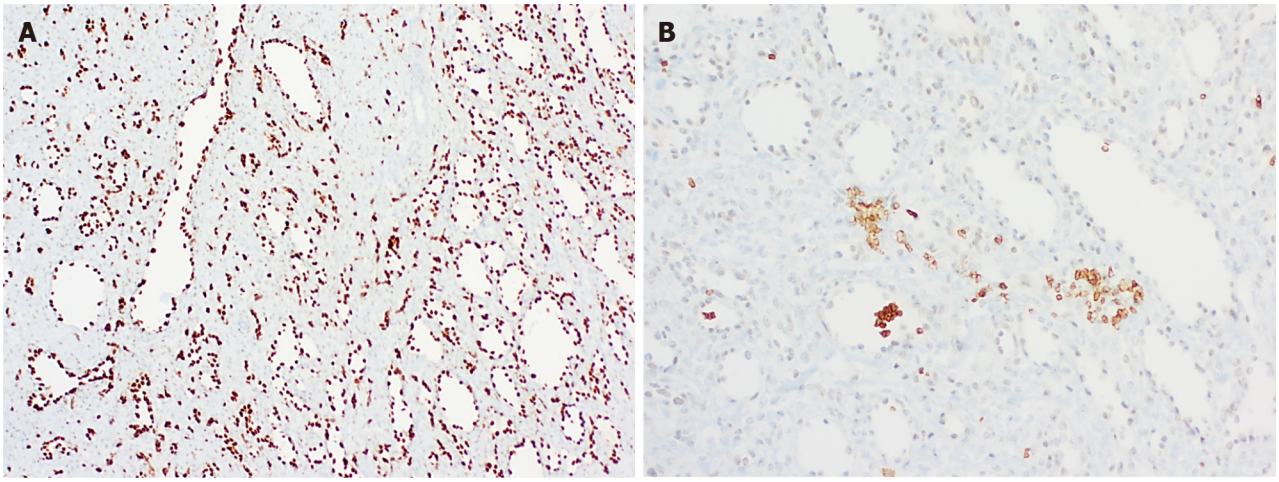

Figure 3 Hepatic congenital hemangioma. A: Erythroblast transformation-specific-related gene expression of endothelial cells; B: No GLUT1 expression of endothelial cells.

complete hepatic parenchymal replacement[5]. Diffuse HIH shows a higher risk of complications, *e.g.*, abdominal compartment syndrome, heart failure, profound hypothyroidism, and even mortality[5,8]. Associated cutaneous infantile hemangioma is often present in patients with multifocal or diffuse HIH and increases with prematurity. Screening for HIH is therefore advised when multiple cutaneous infantile

heman-giomas occur (mostly 5 or more), as the liver is the most common visceral site [8,18].

Histologically (Figure 4), HIH are well-demarcated, non-encapsulated vascular lesions composed of lobular, mostly small-sized vessels (capillary-like) with a pericytic cuff, highlighted by the immunohistochemical staining smooth muscle actin (SMA) (Figure 4)[6,9,11,20]. The periphery of these vascular lesions is cellular and mitotic active, with plump endothelial cells (suggesting active growth). Involution is particularly prominent in the center of the lesion and is characterized by reduced cellularity and enlarged vascular spaces lined by flat, mitotically inactive endothelium. The interstitium is fibrotic or fibromyxoid[9]. Bile ducts and hepatocytes are often entrapped within the advancing edge of the tumor. Areas of extra-medullary hematopoiesis may be present[11]. Central infarction, hemorrhage, calcification and abnormally enlarged vessels or arteriovenous malformation (AVM) can be observed[9]. Rarely, these vascular lesions show irregular anastomosing vascular spaces with prominent papillary formation lined by plump, pleomorphic endothelial cells with hyperchromatic nuclei [also known as *hepatic hemangioendothelioma type 2* with intermediate histologic characteristics, by some reports considered as a low-grade angiosarcoma (Figure 1)][5,9,11,21]. The interstitium of these lesions can contain nests of epithelioid endothelial cells, entrapped nests of liver cells, and bile duct epithelium [9]. When these atypical features are seen or when a lesion is persistent or present in an older child, follow-up is indicated, because of the potential of malignant transformation[6,9].

Multifocal and diffuse HIH show positive staining for GLUT-1, which correlates with a high cell-proliferation and distinguish them from other types of vascular liver tumors (Figure 5)[5,10,22]. The endothelial cells are also positive for ERG, CD31, CD34 and factor VIII but do not express the lymphatic marker podoplanin [D2-40 (Figure 5)] [5,15,21].

There are several hypotheses for the pathophysiology of HIH and its cutaneous counterpart. Clinical observations suggested hypoxia as a trigger for infantile hemangioma (IH). Hypoxia may be due to maternal events as well as the infant's own hypoxia-induced factors and is associated with GLUT-1, as GLUT-1 is a downstream target of hypoxia-inducible factor-1-alpha (HIF-1α), along with vascular endothelial growth factor A (VEGF-A) and insulin-like growth factor 2 (IGF-2). Also, the renin-angiotensin system (RAS) may play a role because high concentrations of angiotensin II (ATII), due to local expression of angiotensin-converting enzyme (ACE) in IH, stimulate cell proliferation. Further, IH expresses GLUT-1 and vascular antigens like Fc-gamma-receptor II, merosin, and Lewis Y antigen, which are also expressed in placental tissue. Another study found that IH endothelial cells share a similar immunophenotype (CD34 and CD133 positive) with embryonic veins, suggesting IH endothelial cells are arrested in an early stage of vascular differentiation[23]. Further, Takahashi *et al* observed an imbalance of vasculogenic factors in IH. During the proliferating phase, IH shows a high expression off type IV collagenase and vascular endothelial growth factor (VEGF) and when involuting there is an increase in tissue metalloproteinases, inhibiting new vessel formation[24]. Moreover, Walter *et al* showed allelic loss after methylation-based and transcription-based polymerase chain reaction clonality assays, suggesting a nonrandom X-inactivation pattern and, thus, a monoclonal origin of IH. In addition, they found 2 cases of IH with a missense mutation, one in the kinase domain of the *vascular endothelial growth factor receptor* (*VEGFR2*) gene and one in the kinase insert of the *VEGFR3* gene. These observations all suggest an alteration in the VEGF signaling pathway in IH[25].

EPITHELIOID HEMANGIOENDOTHELIOMA

Epithelioid hemangioendothelioma (EHE) is a rare malignant vascular tumor, which can occur anywhere in the body but typically arises in liver and lung[4,26]. It is mostly seen in adults, but can be diagnosed in children (estimated prevalence of 1/1000000, mean age 13,8 years)[27]. Hepatic EHEs show a more aggressive course than when arising in bone/soft tissue and are mostly multifocal. Hepatic EHE presents in most cases as a tumoral mass and has an unpredictable clinical course. It may be indolent, stable or aggressive[26,27]. Size > 3 cm and high mitotic index (> 3 mitoses/50 HPF) are poor prognostic factors in elderly[26].

EHEs appear macroscopically as solid, white lesions with some hemorrhagic changes[20]. Histologically (Figure 6), EHEs are relatively distinctive from the normal liver parenchyma and are composed of nests, cords, strands or single infiltrative epithelioid cells set in a myxohyaline stroma. The cells in HEH are epithelioid with

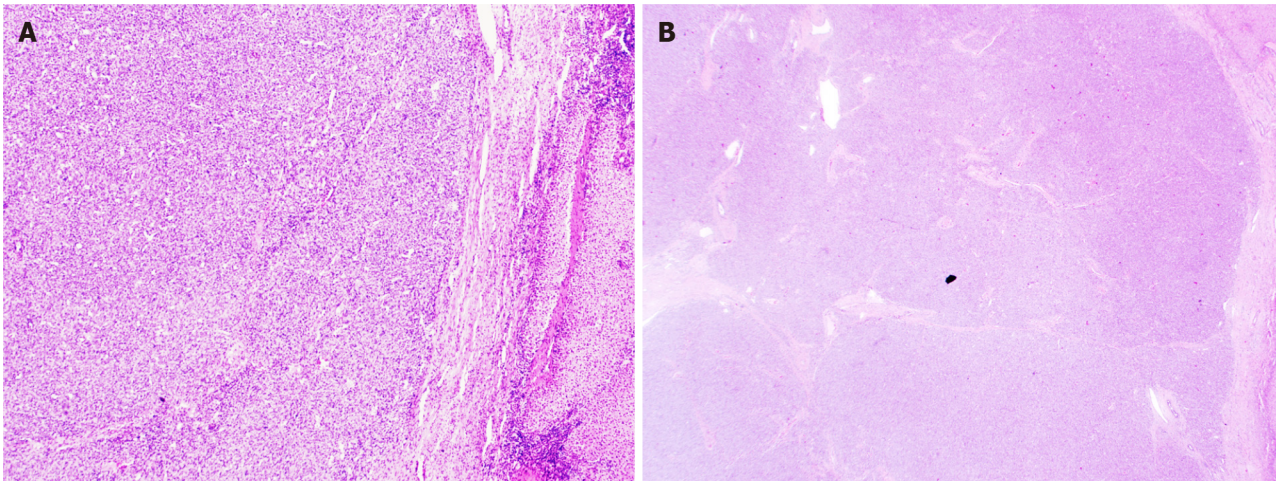

Figure 4 Hepatic infantile hemangioma. A: A well-demarcated vascular lesion; B: Lobular, small-sized vascular spaces.

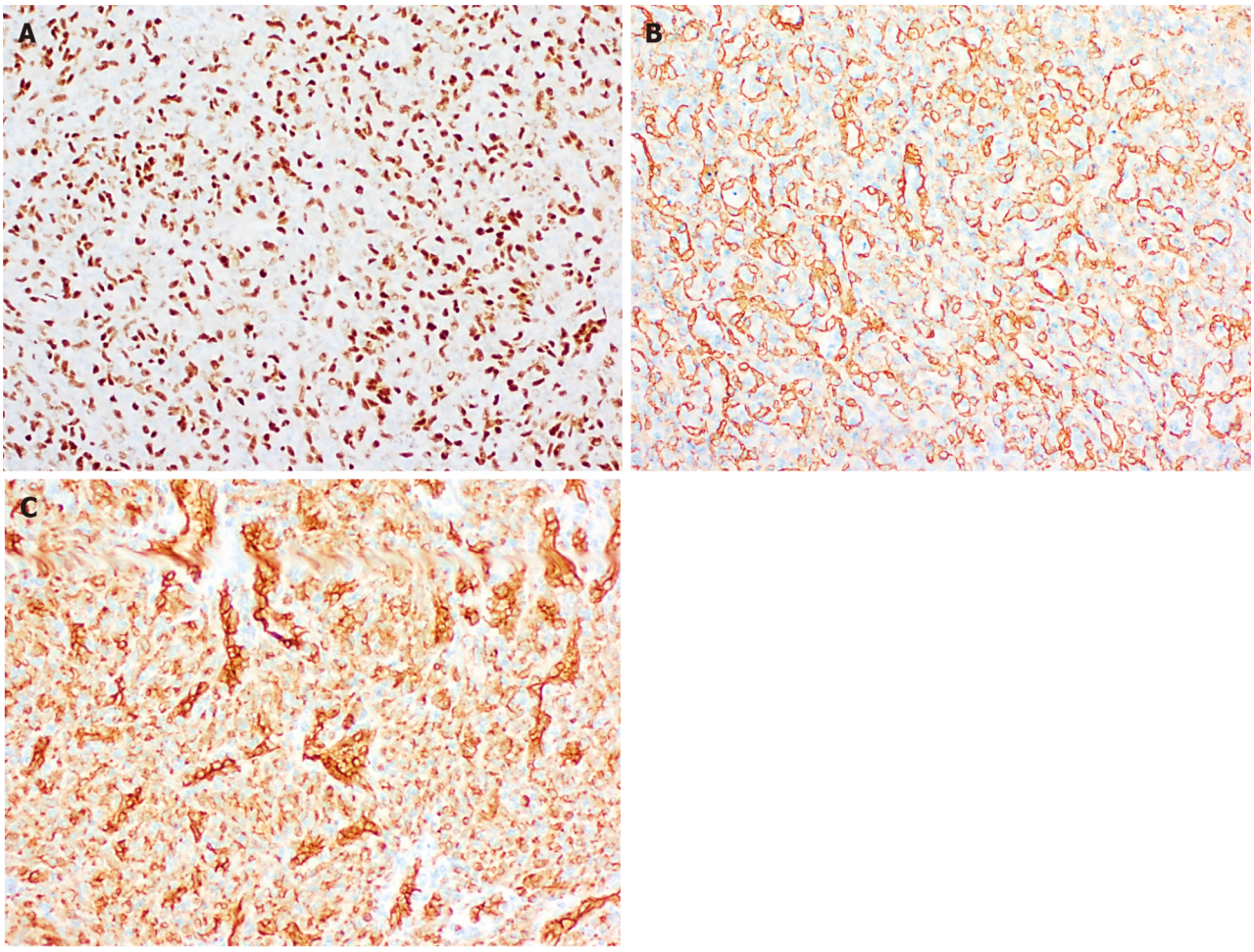

Figure 5 Hepatic infantile hemangioma. A: Erythroblast transformation-specific-related gene expression of endothelial cells; B: Smooth muscle actin expression of the pericytic cells; C: GLUT1 expression of endothelial cells.

eosinophilic cytoplasm and frequently show intracytoplasmic vacuoles (so-called "blister cells")[20,28]. Occasionally, there are tufts or papillary projections into the vessels. A subset of EHE shows histologic overlap with hepatic angiosarcoma (HA) containing necrosis or moderate to severe cytonuclear atypia (with large hyperchromatic cells), without the typical myxoid stromal component. In this setting, the distinction between EHE and HA can be difficult for a pathologist, especially in small liver biopsies. Usually EHE shows nuclear calmodulin-binding transcription activator1

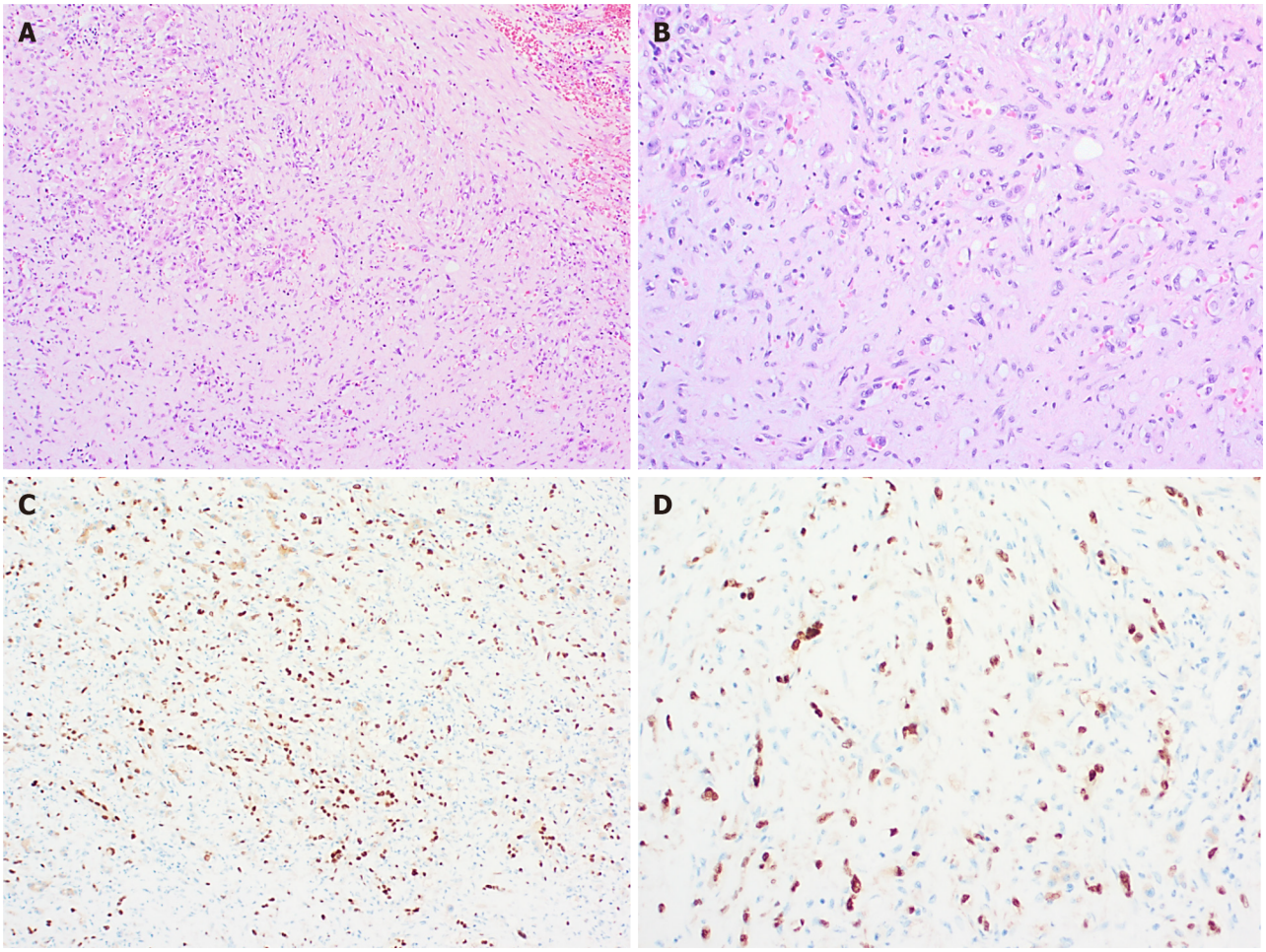

Figure 6 Hepatic epithelioid hemangioendothelioma. A: Nests, cords, strands and single infiltrative epithelioid cells with intracytoplasmic vacuoles; B: Nests, cords, strands and single infiltrative epithelioid cells with intracytoplasmic vacuoles; C: Nuclear calmodulin-binding transcription activator1 (CAMTA1) expression; D: Nuclear CAMTA 1 expression.

(CAMTA1) expression, which can be very helpful in the differential diagnosis with HA since this is a highly specific and sensitive marker for EHE with a CAMTA1 rearrangement (Figure 6)[28]. EHE also stains for ERG, CD31, CD34, factor VIII and podoplanin (D2-40)[15,20,28]. Nuclear positivity for transcription factor E3 (TFE3) is seen in most cases of EHE, irrespective of an underlying *TFE3* rearrangement[20,28]. A small subset of EHE expresses pan-cytokeratin or cytokeratin 8/18 (CK8/18)[20].

Most of the EHEs are characterized by chromosomal translocations involving 1p36.3 and 3q25 resulting in WW domain-containing transcription regulator1 (*WWTR1*, also known as *TAZ*) – *CAMTA1* fusion genes. A small subset shows Yes-associated protein 1(*YAP1*)-*TFE3* gene fusions[26,28]. *TAZ* and *YAP* are transcriptional coactivators and effectors, which are downregulated by the Hippo tumor suppressor pathway. *WWTR1*-*CAMTA1* fusion genes therefore induce oncogenic transformation due to constitutive nuclear localization and activation of *TAZ* independent of the Hippo pathway[26].

HEPATIC ANGIOSARCOMA

Hepatic angiosarcoma (HA) is a rare high-grade malignant vascular tumor that occurs mostly in elderly[5,29,30]. Seldom they occur in children and the majority of pediatric angiosarcoma cases arises in the heart/pericardium and mediastinum[29]. When occurring in the liver angiosarcoma presents as a rapid enlargement of the liver associated with jaundice, abdominal pain, vomiting, fever, tachypnea, dyspnea and anemia[30]. Consumptive coagulopathy, disseminated intravascular coagulation and congestive heart failure are known complications[31]. In children HA has a female predominance and occurs mostly around 40 mo. It represents 1%-2% of all pediatric liver tumors and has the potential to metastasize, even at the onset of the disease. Metastasis is commonly found in the lungs[30,32]. HA can occur in the background of

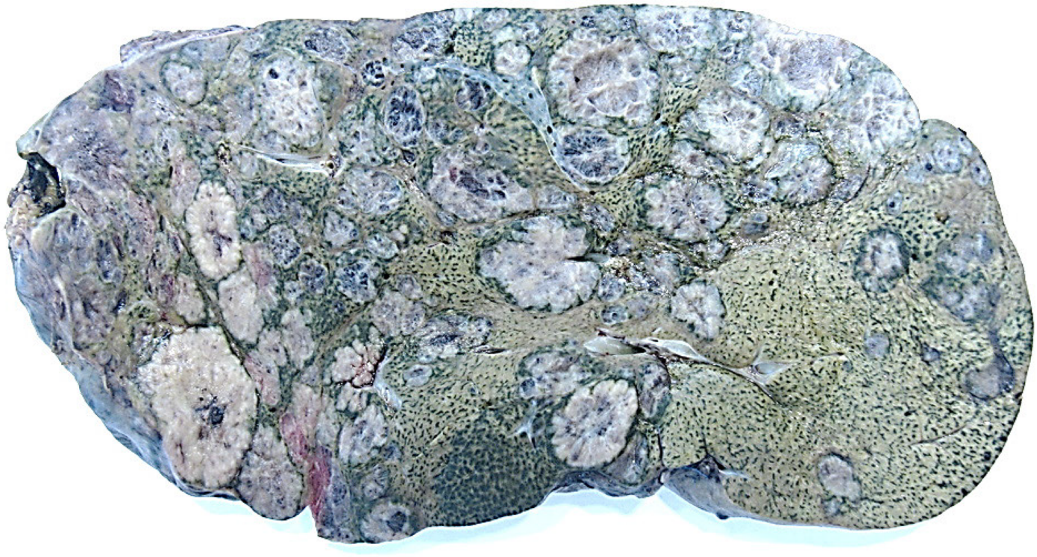

Figure 7 Hepatic angiosarcoma, macroscopical features.

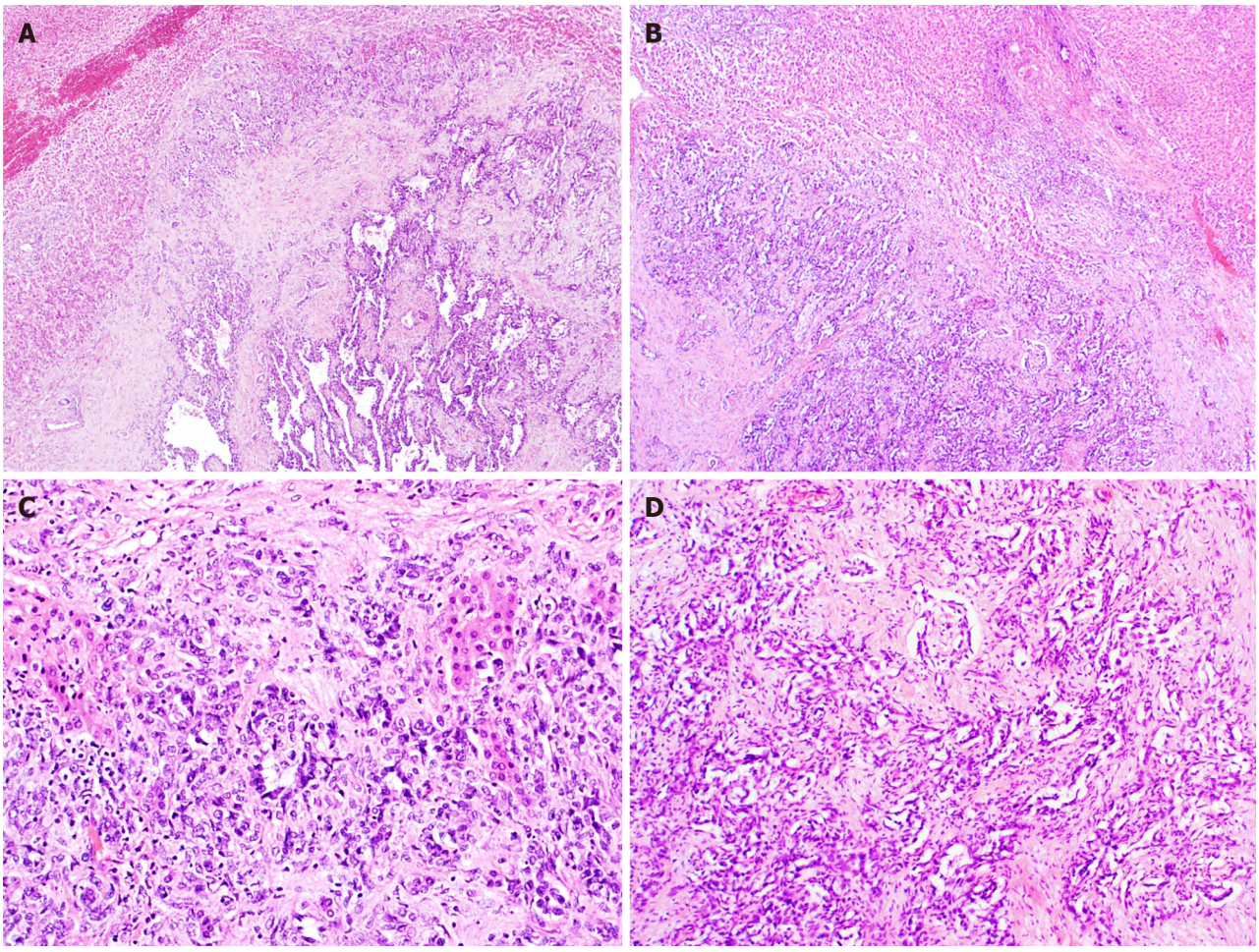

Figure 8 Hepatic angiosarcoma. A: Unencapsulated vascular lesion; B: Infiltrative growth pattern; C: Anastomosing vascular spaces lined by endothelial cells with marked cytological atypia and multilayering. D: Anastomosing vascular spaces lined by endothelial cells with marked cytological atypia and multilayering.

a HIH or can develop 4 to 5 years after primary diagnosis of HIH. Therefore, HIH in patients older than 1 year, should be followed carefully[30]. Also, in the past, several chemical carcinogens, including vinyl chloride monomer (VCM), thorotrast, radium and arsenic, have been associated with HA formation[33,34]. Pediatric HA has a poor

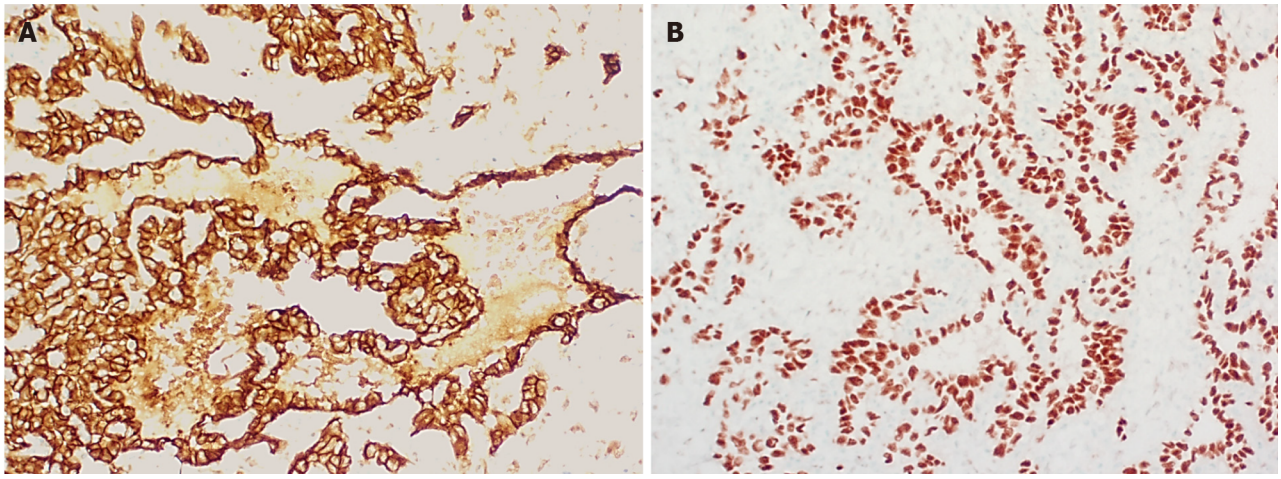

Figure 9 Hepatic angiosarcoma. A: CD31 expression of the endothelial cells; B: Erythroblast transformation-specific-related gene expression of the endothelial cells.

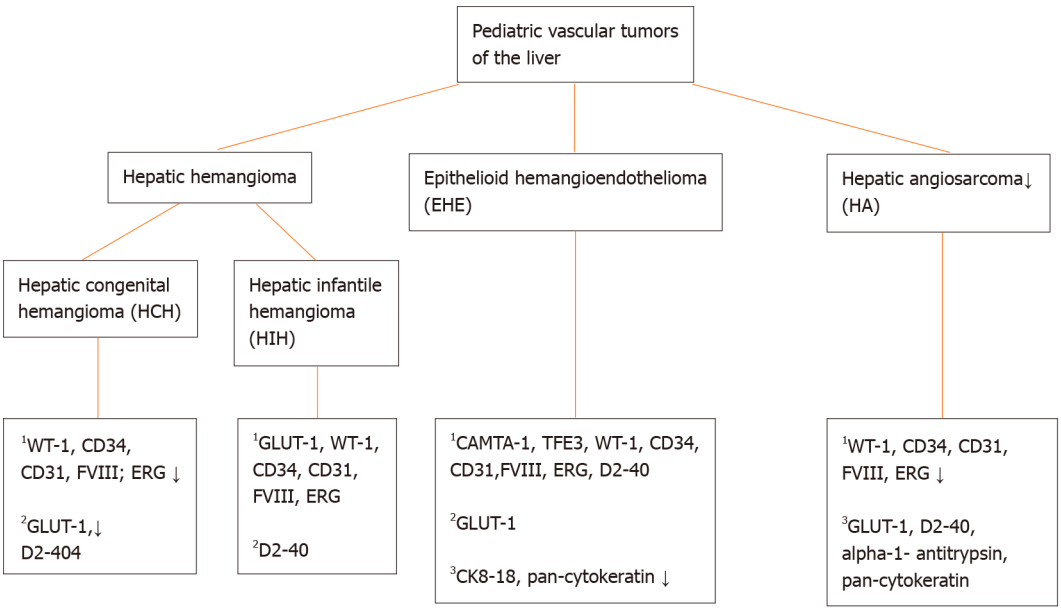

Figure 10 Overview pediatric vascular tumors of the liver and their immunohistochemistry. [1]Positive immunohistochemical staining [2]Negative immunohistochemical staining [3]Occasionally positive immunohistochemical staining. WT-1: Wilms' tumor 1; FVIII: factor VIII; ERG: Erythroblast transformation-specific-related gene; GLUT-1: glucose transporter-1; D2-40: podoplanin; CAMTA1: calmodulin-binding transcription activator1; TFE3: transcription factor E3; CK8-18: cytokeratin 8-18.

prognosis with an average survival of 16 mo and a 5-year overall survival of 20%-35% [30,32].

Diagnosis of a HA can be really challenging, as it is an extremely rare tumor and there are no specific radiographic characteristics that differentiate malignant vascular hepatic tumors from benign ones[33,35]. Histologic diagnosis can only be obtained by adequate and representative tissue biopsies, received by laparotomy[35].

Macroscopically, HA presents as a large solitary mass, or as multiple or diffuse nodules in the centre and periphery of the liver. Often sponge-like hemorrhagic areas alternate with solid gray-white nodules, surrounded by normal liver parenchyma (Figure 7)[31,36]. Commonly, both liver lobes are affected[35,36]. Histologically (Figures 8 and 9), HA shows an unencapsulated vascular tumoral lesion composed of anastomosing vascular spaces and sinusoids lined by endothelial cells with marked cytological atypia and multilayering[29,31,33,35]. The cells are plump, pleomorphic with hyperchromatic nuclei and show brisk mitotic activity[33]. Focally infiltrative whorls or glomeruloid foci of sarcomatoid cells or kaposiform spindle cells with intracytoplasmic PAS positive eosinophilic globules can be seen[30,32,33,35]. Tumor necrosis can be observed[29]. Histologically, HA is classified as *hepatic hemangioen-*

dothelioma, type 3 (Figure 10)[5]. HA shows immunoreactivity for ERG, CD31, CD34 and factor VIII[15,28,33]. A small percentage expresses pan-cytokeratin[33]. Ki-67 shows a proliferation of more than 10%[36]. HAs are occasionally positive for GLUT-1 and podoplanin (D2-40)[15,22,32]. The spindle cell component may show cytoplasmic immunopositivity for alpha-1-antitrypsin[30].

Uptil now, little is known about the genetics of HA, due to examination of small cohorts with a selected gene panel[34]. *KRAS* mutations have been described in sporadic and thorotrast-induced HA, and *TP53* mutations in VCM-related HA[37,38]. Also alterations in the *RAS-RAF-MAPK* pathway, *CDKN2A/p16* and *PTEN* gene have been found[34,39]. Recently a *ROS1-GOPC/FIG* (Fused In Glioblastome) fusion has been found in 1 case[34,37]. This fusion gene can act as a potential target for therapy. Further, upregulation of VEGF-receptor and consistent increased expression of VEGF are commonly seen[34].

CONCLUSION

Diagnosis of a pediatric hepatic vascular tumor can be challenging, not only for the clinici/radiologist, but for the pathologist as well. Throughout the years immunohistochemical markers[10] and molecular genetics have been proven very helpful in the differential diagnosis of vascular tumors. Here we gave an overview of the most important pediatric hepatic vascular tumors and their histology and pathophysiology. Still there is a lot to discover about these vascular lesions.

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MINIREVIEWS

Autoimmune hepatitis in genetic syndromes: A literature review

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Abstract

Genetic syndromes represent relevant and rare diseases. These conditions include a large amount of epidemiological, pathogenetic and clinical features. However, a systematic approach to genetic syndromes is often prevented by the rareness of these diseases. So, although clinical features are usually precisely defined, nowadays more uncommon associations between genetic syndromes and internal medicine related diseases have been insufficiently studied. Autoimmune hepatitis (AIH) is a chronic liver disease caused by loss of tolerance to hepatocyte-specific auto-antigens. Conversely, a better knowledge about specific genetic syndromes in which AIH is more frequent could be important in the clinical management of patients, both for an early diagnosis and for a prompt therapy. Furthermore, a systematic approach could explain if onset, clinical course, and response to treatment of AIH are typical for specific genetic syndromes. We took in consideration all the scientific articles reported in PubMed in the last 10 years, from 2010 to 2020. The purpose of this review is to explore the prevalence of AIH in genetic syndrome, but also to suggest new classification, that could be useful for pathogenetic hypothesis and clinical approach to genetic syndrome. From the 139 publications selected using keywords "autoimmune hepatitis" and "genetic syndrome", 30 papers (21.6%) respected the chosen inclusion criteria, reporting the association between AIH in patients with a genetic syndrome. We have collected in all 47 patients with AIH and genetic syndrome, and with median age of 12.6-year-old. We suggest that when a patient presents a clinical picture of cryptogenic chronic hepatitis, that is unexplained, it is useful to explore differential diagnosis of AIH associated with genetic syndrome. Given the clinical relevance of this topic, further reports are needed to demonstrate our hypothesis and collect new evidence in this field.

Key Words: Autoimmunity; Hepatitis; Gene; Syndrome; Liver; Disease; Immunity

quality classification
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Core Tip: Autoimmunity is a relevant health problem, burdened by delay in diagnosis and difficult therapeutic approach. Genetic syndromes often include autoimmune diseases in their typically complex clinical picture. This review explores the association between genetic syndromes and a specific autoimmune disease, autoimmune hepatitis in order to understand if there are pathogenetic mechanisms based on specific mutations, but also how much autoimmune hepatitis is frequent in genetic syndromes. This systematic approach showed an interesting correlation between these two important groups of diseases.

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INTRODUCTION

Rare genetic diseases are a topic of relevant importance for multi-organ complications and complex clinical pictures. These conditions include a large amount of epidemiological, pathogenetic and clinical features. The most of them have defined DNA mutations, typical phenotypes and characteristic clinical courses. Auto-inflammatory and autoimmune complications are described in several genetic syndromes. This occurs more often when immunoregulatory genes are involved in the pathogenesis of the disease.

The autoimmune hepatitis (AIH) is a complex immune-mediated and chronic liver disease, caused by loss of tolerance to hepatocyte-specific autoantigens.

It is an autoimmune disease of unknown etiology. There is no clear evidence for a hereditary etiology of this disease. Association studies of major histocompatibility complex and other genes demonstrate an influence of immunogenetics[1].

The AIH have annual incidence ranges from 0.67 cases to 2.0 cases per 100000 and annual prevalence ranges from 4.0 to 24.5 per 100000 people depending on the geographical location[2]. Familial cases of AIH are reported to occur in only 1% of AIH cases[3]. This observation suggests role of genetic predisposition. The pathophysiologic mechanisms of AIH are not fully understood. Both genetic predisposition and an imbalance between effector and regulatory immunity are key pathologic factors for disease development[1,2]. Due to an aggressive course of the disease, the diagnosis must be made early and therapy with steroids and immunosuppressant drugs started [1,4].

In 2015, we described a 6-year-old girl with Noonan syndrome (NS) and AIH type 1 [5]. Molecular analysis of *PTPN11* gene showed heterozygous mutation c.923A>G (Asn308Ser) in exon 8. This was the second case described in literature of association between NS and AIH type 1. We supposed that it was not a causality and we thought that autoimmunity represents a characteristic of NS, even if the etiopathogenesis is still unknown.

Then in 2018, we published with Le Coz *et al*[6] two cases with *ctla-4* haploinsufficiency, due to heterozygous microdeletions of chromosome 2q, complicated by autoimmune manifestations. One of these patients had AIH. It is known that about 15% to 20% of patients with the autoimmune polyglandular syndrome type 1 (APS1), also referred to as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a rare disease with prevalence of 1-9:1000000, suffer from an autoantibody-positive AIH, linked to mutations in the autoimmune regulator gene (AIRE)[1,7].

In this review we report literature data of association between AIH and genetic syndromes. Through a detailed and systematic analysis of the literature, we aim to evaluate AIH as a possible complication in patients affected by a genetic syndrome.

We do a systematic review through the choice of the best current works and which refer to the association between AIH and patients with genetic syndrome diagnosis.

The purpose of this work is to evaluate how many reports of genetic syndromes have AIH as a complication and to suppose pathogenetic mechanisms related to the causative mutation of the syndrome and the autoimmune or autoinflammatory processes that may have the liver as a target organ. The correlation between AIH and

genetic syndromes is still controversial and the cause and effect relationship is under investigation in order to understand if it is a simple coincidence/co-occurrence.

When a genetic syndrome has the possibility of developing AIH, the monitoring of this risk is a non-negligible aspect during the follow-up of these patients. AIH is a severe complication, which can have an unfavorable outcome, even with the death of the patient. Indeed, the untreated AIH has a very poor prognosis, with reported survival rates of 50% and 10% at 5 and 10-years respectively[4]. We also investigate the etiopathogenetic hypotheses related to the underlying genetic conditions. Besides, as more is becoming understood, it is also clear that in some cases, there is important overlap between genetic disease causation and the development of AIH.

Any classification is arbitrary and should be considered as a new proposal, as an evolving classification. Here, we try to distinguish the influence of genetic factors in causing AIH complication in a specific population, like patients with a genetic syndrome. We present the state of the art, by reporting all the well described cases, reported in literature.

The collection of clinical evidence could increase the knowledge in this field, improving the management of rare syndromes and AIH, as possible complication with high morbidity and mortality.

METHODOLOGY

We conducted a standard systematic literature review on PubMed, using the combination of keywords: "autoimmune hepatitis", "liver disease", "genetic syndrome".

The application of these search terms aimed to cover most of the publication regarding the description of the association of AIH and genetic syndromes.

We consider only those studies in which the above-mentioned terms are present, alone or variously combined together, in the main text, in the title, in the abstract and in MeSH terms. Since genetic syndromes are rare diseases, we have chosen both previous reviews and case reports. We took in consideration all the scientific articles reported in PubMed in the last 10 years, from 2010 to 2020. The search performed on February 17th, 2021 retrieved 8094, if we use combination of "liver disease" and "genetic syndrome" as keywords, while there are 139, if the combination used is "autoimmune hepatitis" and "genetic syndrome". The inclusion criteria include a clear clinical diagnosis of AIH and genetic syndrome. We checked in each article the congruence of the diagnosis of AIH with the recognized criteria and the confirmation of the diagnosis of specific genetic syndrome with a proper genetic test. Of 139 articles, 30 are accessible, compatible with our inclusion criteria and are included in the analysis. The exclusion criteria for the remaining 109 articles are in a language different from English, regarding familiar but not syndromic cases and a not specific diagnosis of AIH.

It has been paid attention to diagnostic criteria in diagnosis of AIH[1]. According to the Ab profile, AIH can be divided into three subtypes: AIH type 1 by the presence of antinuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA); AIH type 2 by anti-liver-kidney microsomal autoantibodies (LKM-1) directed against cytochrome P450 (CYP) 2D6; AIH type 3 by autoantibodies against a soluble liver antigen (SLA/LP)[1,2].

The established specific diagnostic criteria and scoring systems of AIH include analysis of autoantibodies (ANA, SMA, anti-LKM1, and anti SLA), immunoglobulin (Ig) G, viral markers (IgM anti-HAV, HBsAg, HBV DNA, and HCV RNA) and histological findings[1,2,8]. The diagnosis of syndromes condition is confirmed through genetic tests, using a cytogenetic, cytogenomic or molecular approach.

RESULTS

From the 139 publications selected using keywords "autoimmune hepatitis" and "genetic syndrome", 30 papers (21.6%) respected the chosen inclusion criteria, reporting the association between AIH in patients with a genetic syndrome.

From 2010 to 2020, the articles which have reported AIH as complication of a genetic syndrome have a median of 1.7% of all scientific production on liver disease in genetic syndromes, with a peak between 2014 and 2015 years of publication.

There are many case reports (24/30) and some reviews (2/30) and few original or research articles, cohort studies or clinical trials. Here, we considered the review which described case reports, because of the rarity of diseases.

Most of the syndromes found are forms of immunodeficiency or immunodysregulation, such as APS1, Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome (IPEX), Immunodeficiency-centromeric instability-facial anomalies syndrome, spondilocondrodisplasia (SPENCDI), X-linked agammaglobulinemia (XLA), Shwachman-Diamond syndrome (SDS) and severe combined immunodeficiency (SCID).

A new findings are the unbalanced genomic diseases, like Down syndrome, Smith-Magenis syndrome (SMS), 22q13.3 deletion syndrome and 2q deletion syndrome.

Interesting is the presence of 2 articles about Wilson disease (WD), that is a disease with primary hepatic involvement, describing 2 patients in which a form of autoimmune liver disease is hypothesized.

Moreover, we found some very different syndromes in association with AIH: NS, cutaneous amyloidosis, H syndrome, familial hemophagocytic lymphohistiocytosis (FHL) with STXBP2 mutations, progressive familiar intrahepatic cholestasis type 3 (PFIC3) and sclerosis tuberous syndrome (TSC).

We have collected in all 47 patients, with variable age of AIH onset. We observed median age of patients of 12.6-year-old and a high incidence (70.2%) of patients with age < 12-year-old. The ratio of males to females is 40.4% to 55.3% respectively, with female prevalence. The 30% of patients were died. We found also some publication that includes pathogenetic hypothesis, which are reported and commented in the discussion.

The articles and case reports are described in Tables 1-3.

DISCUSSION

AIH is a relatively rare progressive chronic liver disease that mainly affects women and is usually characterized by increased IgG levels, circulating autoantibodies and a favorable response to immunosuppressive treatment[1,2,4]. The etiology of AIH is still unknown and all the causes of chronic liver disease must be excluded in advance before diagnosing AIH. The literature data exhibit that AIH can show up in any age of both sexes and all ethnic groups, with peaks around puberty and between 4th and 6th decades. The onset of AIH may be insidious, acute or chronic, and one third of patients have already developed cirrhosis at the moment of diagnosis, suggesting a delay in diagnosis[8]. The presence of other autoimmune or immune-mediated diseases is frequent and an unusual form of AIH has been reported in 10%-18% of patients with APECED, also known as APS1[7-9]. AIH develops in genetically predisposed individuals, after exposure to triggering factors like microbes, viruses or drugs. When the autoimmune attack against the liver starts, it continues through "molecular mimicry" mechanisms, and is promoted by the diminished control of regulatory T-cells[8].

The evidence of an hepatic CD4 and CD8 T cell and B cell infiltration confirms the immune-mediated pathogenesis, related to defective regulatory mechanisms, antigen-specific immunization, pro-inflammatory CD4 T cell and their cytokines profile. The dysregulation of adaptive immune response has a pathogenetic role, due to the production of autoantibodies and the persistence in the liver of autoreactive CD4 T cells that maintain inflammation with a predominant secretion of tumor necrosis factor (TNF), interferon-γ (IFN-γ), interleukin (IL)-21. Furthermore, T-reg cell are not able to stop inflammation[10].

AIH is principally divided in type 1 (AIH-1) and type 2 (AIH-2), based on autoantibodies. The authors confirm that there are many differences between two types. AIH-2 is more frequent in children and young adults, has an acute or severe course and treatment failure, with relapse after stopping treatment and need for long-term treatment, compared to AIH-1[8,11,12]. A panel of experts, namely International AIH Group (IAIHG), reported the descriptive criteria of AIH, updated periodically [13]. Some AIH patients has clinical cholestatic presentation, that is known as primary biliary cholangitis or primary sclerosing cholangitis (PSC). In 2001, Gregorio *et al*[14] introduced the term "autoimmune sclerosing cholangitis" for the patients characterized by lesions of both AIH and sclerosing cholangitis. This presentation was named "overlap syndromes or variants of AIH" and its appearance was more frequent in children. The authors suggested an investigation of the biliary tree in all children with a diagnosis of AIH[8,15]. The IAIHG do not support the concept of "overlap

Table 1 Group-1: Disease gene is one of immunoregulatory genes

Genetic syndrome	Inheritance	Gene	Ref.	Number of AIH cases	Sex	Age at diagnosis	Nucleotide variant	Protein variant	Outcome
APECED/APS1	AD, AR	*AIRE*	Meloni *et al* [17], 2017	6	F; F; F; F; M	3 yr; 6 yr; 11 yr; 5 yr; 8 yr; 12 yr	c.[415C>T];[415C>T]	p.[(R139X)];[(R139X)]	Alive; Alive; Death; Death; Alive; Alive
			Huibregtse *et al*[7], 2014	1	F	10 yr	c.[20_115de196];[967_979del13]	p.[(?)];[(?)]	Alive
			Zaidi *et al* [18], 2017	2	M; M	3 yr; 5 yr	NR	NR	Alive; Death
IPEX	XLR	*FOXP3*	López *et al* [21], 2011	1	M	4 yr	c.[748-750delAAG];[0]	p.[(250Kdel)];[(0)]	Alive
			Baris *et al* [22], 2014	1	M	3 yr	c.[816+5G>A];[0]	p.[(?)];[(0)]	Death
			Magg *et al* [23], 2018	1	M	3 yr	c.[816+2T>A];[0]	p.[(?)];[(0)]	Death
			Duclaux-Loras *et al* [20], 2018	3	M; M; M	4 wk; 4 wk; 3 wk	c.[751_753delGAG]];[0]; c.[1157G>A];[0]; c.[227delT];[0]	p.[(E251del)];[(0)]; p.[(R386H)];[0]; p.[(L76Qfs*53)];[(0)]	Death; Death; Alive
ICF2	AR	*ZBTB24*	von Bernuth *et al*[25], 2014	1	F	3 yr	c.[1222T>G];[1222T>G]	p.[(C408G)];[(C408G)]	Alive (not responding to therapy)
ICF1	AR	*DNMT3B*	Sterlin *et al* [24], 2016	1	M	5 yr	c.[2324C>T];[2324C>T]	NR	Alive
SPENCDI	AR	*APC5*	Briggs *et al* [26], 2016	3	F; F; F	9 yr; 3 yr; 6 mo	c.[725A>G];[725A>G]; c.[389+1G>A];[389+1G>A]; c.[131C>T];[712T>C]	p.[(H242R)];[(H242R)]; p.[(?)];[(?)]; p.[(T44M)];[(C238R)]	Alive; Alive; Alive
SDS	AR	*SBDS*	Veropalumbo *et al*[28], 2015	2	NR; NR	9 mo; 12 mo	c.[258+2T];[183-1847A>CT]; c.[258+2T>C];[183-184TA>CT]	p.[(?)];[(?)]; p.[(?)];[(?)]	Alive; Alive
SCID	AR	*CD3γ*	Tokgoz *et al* [30], 2013	1	F	12 yr	c.[IVS2-1G>C];[IVS2-1G>C]	p.[(?)];[(?)]	Alive

AIH: Autoimmune hepatitis; AD: Autosomal dominant; AR: Autosomal recessive; XLR: X-linked recessive; F: Female; M: Male; NR: Not reported; SDS: Shwachman-Diamond syndrome; SCID: Severe combined immunodeficiency; SPENCDI: Spondilocondrodisplasia; ICF: Immunodeficiency, centromeric instability and facial dysmorphism; IPEX: Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome; APECED: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; APS1: Autoimmune polyglandular syndrome type 1.

syndromes" as new and distinct disorders[13].

We suspect that genetic syndromes with particular imbalance of immune response, could represent a genetic predisposition to develop autoimmune disease, especially AIH. Some genetic syndromes are known to have autoimmune complications, for examples APS, IPEX syndrome and Down syndrome. Also in rare genomic imbalance diseases could appear autoimmune complications.

We have found some case reports of patients with genetic syndrome complicated by AIH. The main found syndromes are APS/APECED, IPEX syndrome, unbalanced genomic syndromes, RASopathies.

We propose a classification system for genetic syndromes associated with AIH due to genetics and etiopathogenesis aspects. There are three possible groups: group-1, that includes genetic syndromes whose disease gene is one of immunoregulatory genes, directly involved in AIH pathogenesis; group-2, that includes those syndromes in which there is a polygenic involvement of immune-mediated risk and of AIH pathogenesis; group-3, that includes those in which there is a possible association related to the disease causative mutation, seems to be not directly involved in AIH pathogenesis. For the last group, we try to propose some possible pathogenesis mechanism in AIH development.

Table 2 Group-2: Polygenic involvement of immune-mediated risk (unbalanced genomic disease)

Genetic syndrome	Inheritance	Chromosomal region	Ref.	Number of AIH cases	Sex	Age at diagnosis	Deletion breakpoints [build GRCh37/hg19]	Outcome
Down syndrome	IC	-	Ravel *et al* [32], 2020	1	M	29 yr	-	Death
SMS	AD, IC	del17p11.2	Yang *et al* [36], 2014	1	F	24 yr	chr17: 16,660,721-20,417,975 *dn*	Alive
PHMDS	AD	del22q13.31-qter	Bartsch *et al* [37], 2010	1	F	3 yr	-	Alive
del2q	IC	del2q33.1-q34	Le Coz *et al* [6], 2018	1	F	12 yr	chr2:197,942,576–209,522,220 *dn*	Alive

AIH: Autoimmune hepatitis; IC: Isolated cases; AD: Autosomal dominant; F: Female; M: Male; SMS: Smith-Magenis syndrome.

Group-1 genetic syndromes includes

Autoimmune polyendocrinopathy syndromes: The term APS refers to a group of rare endocrine diseases characterized by autoimmune activity against more than one endocrine organ, with possible additional involvement of non-endocrine organs. Autoimmunity is typically directed against different target antigens in different tissues. The two more common autoimmune polyendocrine syndromes, APS type 1 and type 2, have a strong genetic background and have Addison's disease as a major feature. The group furthermore includes APS type 3 and type 4.

The APS type 1 is a rare recessive autosomal disease, also named APECED syndrome (OMIM 240300), and related to *AIRE* gene mutations. Because of a founder effect, APECED is particularly prevalent in Finland (1:25000) but is observed worldwide with variable prevalence[15]. Diagnosis is classically based on presence of at least two out of three "majors" criterions of Whitaker's triad (chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism and adrenal insufficiency or Addison disease). *AIRE* gene (21q22.3), coding for the *AIRE* transcription factor, is involved in immune tolerance mechanisms and contributes to the negative selection of autoreactive T lymphocytes in the thymus, lymph nodes and spleen. AIH and hepatitis as an APECED component may be distinguished on the basis of a different autoantibody profile. The anti-LM antibodies are specific of AIH, which develops in individuals with APECED.

The major target autoantigen of anti-LM antibodies has been documented as the CYP1A2[8,12,14]. In the considered period, we have found four papers reporting in all six patients with APECED syndrome and AIH, that is non-endocrine complication[7,16-18].

The girl described by Huibregtse *et al*[7] had homozygous 967-979del13bp mutation. Meloni *et al*[17] described a longitudinal cohort study in which AIH was seen in 27% of their APS1 Sardinian patients. There are five female patients with a median age of 6.5-year-old and one male of 12-year-old. The course of AIH varied from chronic moderate/severe hepatitis to fatal forms (in two Sardinian and one Indian children) [17,18].

They noted predominance in females, presence in all AIH patients of R139X homozygotes and *HLA-DRB1*0301-DQB1*0201* combination plus LKM autoantibodies (anti-CYP1A2), onset in infancy/childhood, a hitherto unreported predilection for hepatitis and that AIH can be the initial manifestation of APS1. Then they concluded that the role of HLA, in addition to the R139X *AIRE* variant, could influence the APS1 phenotype. Therapy for severe AIH consisted of oral prednisone, tapered off in about 6 mo, and azathioprine, that was continued for years.

In the review of Gatselis *et al*[8], published in 2015, the AIH associated with APECED is considered a component of this syndrome, that the authors described as a third type of AIH, because of the presence of characteristic autoantibodies, such as ANA, anti-LC, anti-LKM, anti-LM.

This review is not included in our listed papers, because of the lack of the established inclusion criteria, but it was interesting for improvement of information about this syndrome. In 2016, Sorkina *et al*[19] described an interesting 4-year-old patient with *AIRE* mutation and AIH, but their diagnosed criteria are not reported; for this reason we exclude the paper in this review. The authors concluded that regular screening for autoantibodies can help identify higher risk for development of AIH.

Table 3 Group-3: Association not directly related to the disease causative mutation

Genetic syndrome	Inheritance	Gene	Ref.	Number of AIH cases	Sex	Age at diagnosis	Nucleotide variant	Protein variant	Outcome
NS	AD	*PTPN11*	Quaio *et al* [38], 2012	1	M	19 yr	c.[836A>G];[=]	p.[(Y279C)];[(=)]	Alive
			Loddo *et al*[5], 2015	1	F	6 yr	c.[923A>G];[=]	p.[(N308S)];[(=)]	Alive
WD	AR	*ATP7B*	Ganesh *et al* [40], 2017	1	M	6 yr	c.[2906G>A];[2906G>A]	p.[(R969Q)];[(R969Q)]	Alive
			Santos *et al* [41], 2019	1	F	25 yr	N.R.	N.R.	Alive
H syndrome	AR	*SLC29A3*	Bloom *et al* [42], 2017	1	M	17 mo	c.[1087C>T];[1087C>T]	p.[(R363W)];[(R363W)]	Alive
FHL5	AR	*STXBP2*	Esmaeilzadeh *et al*[43], 2015	1	M	7 yr	c.[1247-1G>C];[1247-1G>C]	p.[(?)];[(?)]	Death
TSC	AD	*TSC1*	Di Marco *et al* [44], 2017	1	F	47 yr	c.[682C>T];[=]	p.[(R228*)];[(=)]	Alive
SCD	AR	*HBB*	Jitraruch *et al* [45], 2017	7	F; M; M; F; F; F; F	5 yr; 16 yr; 13 yr; 13 yr; 8 yr; 8 yr; 3 yr	c.[20A>T];[20A>T]	p.[(E7V)];[(E7V)]	Alive; Alive; Death; Alive; Alive; Alive; Death
			Zellos *et al* [46], 2010	1	F	25 yr	c.[20A>T];[20A>T]	p.[(E7V)];[(E7V)]	Death
			Hurtova *et al* [47], 2011	1	F	54 yr	c.[20A>T];[20A>T]	p.[(E7V)];[(E7V)]	Death
GD	AR	*GBA*	Ayto *et al*[48], 2010	1	F	51 yr	c.[1226A>G];[115+1G>A]	p.[(N409S)];[(?)]	Death
PLCA	AD	-	González-Moreno *et al* [50], 2015	1	M	36 yr	NR	NR	Alive
			Yan and Jin [49]	1	F	50 yr	NR	N.R.	Alive
PFIC3	AR	*ABCB4*	Oliveira *et al* [51], 2017	1	M	22 yr	c.[874A>T];[3680T>C]	p.[(K292*)];[(I1227T)]	Alive

AIH: Autoimmune hepatitis; AD: Autosomal dominant; AR: Autosomal recessive; F: Female; M: Male; NR: Not reported; NS: Noonan syndrome; WD: Wilson disease; FHL: Familial hemophagocytic lymphohistiocytosis; TSC: Tuberous syndrome; SCD: Sickle cell disease; GD: Gaucher disease; PLCA: Primary cutaneous amyloidosis; PFIC3: Progressive familiar intrahepatic cholestasis type 3.

IPEX syndrome: The IPEX syndrome (OMIM 304790) is a rare X-linked recessive life-threatening disorder characterized by autoimmunity and early death. The causative gene is *FOXP3*. We report four papers and six patients with IPEX syndrome and AIH [20-23]. These patients were hemizygote males of median age of 1.7-year-old. In 2018, Duclaux-Loras R *et al*[20] reported 14% of AIH in a cohort of French IPEX patients. Among these, three patients had AIH with early onset in the first months of life and two died at 8 and 7 mo. In IPEX syndrome the course of AIH is very severe.

Immunodeficiency, centromeric instability and facial dysmorphism syndromes: The immunodeficiency, centromeric instability and facial dysmorphism (ICF) syndrome (OMIM 242860) is a rare autosomal recessive immunodeficiency, that involves agammaglobulinemia or hypoglobulinemia with B cells, centromere-adjacent instability of chromosomes 1 and/or 16 (and sometimes 9) in mitogen-stimulated lymphocytes, with facial anomalies and psychomotor delay. Approximately 50 patients have been reported.

It is distinguished in ICF1 correlate to *DNMT3B* gene mutations and ICF2 due to *ZBTB24* gene, ICF3 caused by mutation in the *CDCA7* gene and ICF4 caused by

mutation in the *HELLS* gene. There are two papers which described two patients, one male and one female, with 5 and 3-year-old respectively, affected by ICF1 and ICF2 with AIH[24,25].

Spondyloenchondrodysplasia with immune dysregulation: SPENCDI (OMIM 607944) is a very rare autosomal recessive genetic skeletal dysplasia, that may have a heterogeneous clinical spectrum with neurological involvement or autoimmune manifestations. The prevalence is < 1.1000000 and onset is in childhood. In all, we found four patients who have AIH and SPENCDI. In the original article of Briggs *et al* [26], three female patients of 9-year-old, 3-year-old and 6-mo-old have been AIH and SPENCDI, confirmed by homozygous variants in APC5 gene.

In an abstract in Chinese language, for this not included in Table 1, the authors reported a case of a 12-year-old girl with type IIAIH, associated with systemic lupus erythematosus (SLE), treated with methylprednisolone and immunosuppressants, with improvement. Gene sequencing was performed, revealing a compound heterozygous mutations in ACP5 gene. The same paper showed a review of 25 articles (1 Chinese, 24 English) with 74 SPENCDI patients (92%) with autoimmune diseases. They concluded for a strong predisposition to these complications in SPENCDI[27].

SDS: SDS (OMIM 260400) is a rare autosomal recessive multisystemic syndrome characterized by chronic and usually mild neutropenia, pancreatic exocrine insufficiency, caused by mutations in the *SBDS* gene. It might be hepatomegaly and liver abnormalities. We found an article which described two patients with SDS and AIH [28].

Immunodeficiency: The primary immunodeficiency disorders are a rare heterogeneous group of inherited defects characterized by poor or absent function in one or more components of the immune system. The estimated prevalence of these disorders in the United States is approximately 1:1200 live births[29]. The clinical presentation involves increased susceptibility to infection, chronic diarrhea, failure to thrive, severe and recurrent infections with opportunistic pathogens.

In SCID there is a lack of functional T cells and immune function. We found an article reporting one of two siblings, 12 year-old girl, with SCID, due to homozygous splicing mutation (IVS2-1G>C) in the *CD3γ* gene and AIH[30]. About immunodeficiency syndromes, we want to cite one article, excluded for language, which describe a very rare case of a girl of 18-month-old with chronic granulomatous disease and AIH [31].

Group-2 includes

Down syndrome: Trisomy of chromosome 21 (OMIM 190685) is characterized by cognitive impairment, cardiac and gastrointestinal abnormalities and immunodeficiency.

Relevant is also the incidence of autoimmune diseases. Our research found a review in which only two cases with Down syndrome were associated to autoimmune chronic active hepatitis and autoimmune PSC[32]. Because the case reported have been excluded for publication over the years, we evaluated the aforementioned review, which is the only publication in the period considered, that referred to cases of AIH and Down syndrome. The first case was a 29-year-old male, reported by McCulloch *et al*[33] in 1982 while the second was a 21-year-old male with autoimmune PSC by Mehta *et al*[34], in 1995. In 1990, another case of a 12-year-old child is described with Down syndrome and AIH[35]. Considering the known risk of autoimmune complications in Down syndrome, we thought we would find more cases of AIH. On the contrary, literature data showed many cases of viral hepatitis occurring in Down syndrome, due to immunodeficiency condition.

Other unbalanced genomic diseases: They are rare genetic syndromes caused by deletion and/or duplication of chromosomes. The correlation of symptoms is variable of cognitive deficit and multiorgan involvement. Monosomy and trisomy for different regions in chromosomes account for about 1% of cases of developmental delay and intellectual disability. Some of them are noted to have immunodeficiency and immune-mediated complications. In our review, we found description of a 24-year-old woman with AIH and SMS (OMIM 182290), due to a 17p11.2 deletions (16,660,721-20,417,975, GRCh37/hg19)[36], another 3-year-old girl patient with 22q13.3 deletion syndrome (Phelan-McDermid syndrome) (OMIM 606232)[37], finally a 12-year-old girl with de novo heterozygous 11.6 Mb chromosome 2q33.1-q34 deletion (197,942,576-209,522,220, GRCh37/hg19)[37].

We think that AIH is due to haploinsufficiency of key genes located in the deleted region. Lymphocyte-specific member of the TNF receptor superfamily (*TACI* gene) located within the SMS region, plays a crucial role in humoral immunity. So we might speculate that *TACI* haploinsufficiency, in this condition, could cause hyperactive B cells and increased capacity for antigen-specific antibody production. In similar manner, the loss of one copy in one or more of the 55 genes, from *NUP50* to *RABL2B*, in 22q13.3 region in Phelan-McDermid syndrome; and of the *CD28/CTLA4/ICOS* gene cluster in 2q33.1-q34 deletion, similar to *ALPS5* due to *CTLA4* haploinsuffiency, would be predisposing AIH. In this case, probably the deletion of the *CD28/CTLA4/ICOS* gene cluster induced a multi-organ inflammation and exhibited a Treg suppressive defect.

Group-3 includes

NS/RASopathies: NS (OMIM 163950) is characterized by short stature, typical facial dysmorphology and congenital heart defects. The incidence of NS is estimated to be between 1:1000 and 1:2500 live births. The syndrome is transmitted as an autosomal dominant trait. In more than 50% of patients with NS, mutations in the Protein Tyrosine Phosphatase Non-Receptor Type 11 (*PTPN11*) gene are identified.

We found two patients with the association of NS and AIH. In 2012, Quaio *et al*[38] published the first case of patient with AIH and NS. Another case is a 6 year-old girl, that we reported in 2015, with heterozygous mutation c.923A>G (Asn308Ser) in exon 8 of *PTPN11* gene[5]. Autoimmune diseases and autoantibodies were frequently present in patients with RASopathies, even if the etiopathogenesis is still unknown.

The *PTPN11* are clustered in the interacting portions of the amino N-SH2 (Src homology 2) domain and the phosphotyrosine phosphatase (PTP) domains, which are involved in switching the protein between its inactive and active conformations. Missense mutation causes a gain-of-function changes resulting in excessive *SHP2* activity, that underlie the pathogenesis of NS. We hypothesize that *SHP2* modulates *ERK/MAPK* pathway and its involvement in cytokine/inflammatory signaling. In an interesting article published in 2016, it was highlighted that inhibition of *SHP2* activity blocks T cell proliferation, leading to decreased IFN-γ and IL-17 Levels, ultimately normalizing SLE associated pathogenicity in target tissues. These data suggest SHP2 activity is integrally involved in SLE and that its normalization may be a potent and targeted therapy for treatment of patients with SLE[39].

WD: In our research on PubMed, we found two articles about AIH and WD[40,41], that is a disorder of copper metabolism (OMIM 277900). The diagnosis is established by a combination of low serum copper and ceruloplasmin concentrations, increased urinary copper excretion and detection of biallelic *ATP7B* pathogenic variants by molecular genetic testing. The manifestations include neurologic, psychiatric or liver diseases. These include recurrent jaundice, simple acute self-limited hepatitis-like illness, autoimmune-type hepatitis, fulminant hepatic failure, or chronic liver disease. The AIH in WD patients responds well to chelation therapy with D-penicillamine. There were reported a 6-year-old boy and a 25-year-old female patients, presented with clinical symptoms suggestive of AIH, with a mutation in *ATP7B* gene, confirming the diagnosis of WD. In patients who showed chronic hepatopathy resembling AIH, the differential diagnosis with WD is mandatory, because resolving the dilemma allows the clinician to prescribe the appropriate therapy.

H syndrome: H Syndrome (OMIM 612391) is an autosomal recessive disorder characterized by cutaneous hyperpigmentation, hypertrichosis and induration with numerous systemic manifestations. The syndrome is caused by homozygous or compound heterozygous mutations in *SLC29A3* a gene on chromosome 10q22 that encodes a nucleoside transporter (hENT3). There is one case report that described a 17 mo-old male with mild to moderate autoimmune chronic active hepatitis, confirmed with biopsy and treated with prednisone and immunosuppressor[42].

FHL: In 2015, Esmaeilzadeh *et al*[43] described a patient with FHL5 (OMIM 613101) caused by *STXBP2* gene mutation presenting with AIH. This syndrome is a rare disorder characterized by immune dysregulation, defective function of natural killer cell, proliferation and infiltration of hyperactivated macrophages and T-lymphocytes, cytopenia and hepatosplenomegaly. It was the first description of AIH.

Tuberous sclerosis complex: TSC (OMIM 191100) is a rare autosomal-dominant neurocutaneous disorder, with prevalence of 1:6000, characterized by multisystem hamartomas and benign tumors developing. This condition is caused by heterozygous loss-of-function mutations in the *TSC1* or *TSC2* tumor suppressor genes coding for hamartin and tuberin, respectively.

We found an article about a 47 year-old woman, affected by TSC, with a mutation identified in the *TSC1* gene [c.682C>T (p.Arg228*)] and lymphangioleiomyomatosis, sarcoidosis, primary biliary cirrhosis and AIH[44]. This was the first report of this coexistence, and we might speculate that this is related with the dysregulation of the pathway involving *mTOR* and *MAPK* and their interaction.

In literature, *PI3K/AKT/mTOR* signaling has been implicated in SLE pathogenesis. Its activity is increased in SLE mice models as well as in human lupus patients. The expression of this signaling pathway exists broadly in immune cells, including T cells, B cells, monocytes, macrophages, neutrophils and dendritic cells[39].

Sickle cell disease: It is a chronic hemolytic disease (OMIM 603903) that may induce acute accidents, like severe anemia, bacterial infections, and ischemic vaso-occlusive accidents caused by sickle-shaped red blood cells obstructing small blood vessels and capillaries. The patients have beta globin variant (Hb S). Our PubMed research found three articles.

In 2017, a retrospective review reported 7 patients of median age of 9 years with sickle cell disease (SCD) and AIH. The patients were treated with standard immunosuppressive therapy[45]. Previous case reports described two patients with SCD and AIH[46,47].

The occurrence of AIH may be due to a complex interaction with the underlying liver disease in altered immunoregulatory mechanisms. AIH is common in patients with SCD and they respond satisfactorily to immunosuppressive treatment. The authors reported how liver biopsy may be helpful in confirming the diagnosis and to exclude acute vaso-occlusive sickling episodes[45].

Gaucher disease type 1: It is the chronic non-neurological form of Gaucher disease autosomal recessive (OMIM 230800), characterized by prevalence of 1:100000 organomegaly, bone involvement and cytopenia, caused by a mutation in the *GBA* gene. The hepatomegaly (80% of cases) in rare cases can progress towards fibrosis followed by cirrhosis. We found an article, who described one gaucher disease type 1 patient with autoimmune chronic active hepatitis[48].

Primary cutaneous amyloidosis: It refers to a variety of skin diseases characterized by the extracellular accumulation of amyloid. They have genetic heterogeneity and may be caused: Primary cutaneous amyloidosis (PLCA)-1 by heterozygous mutation in the gene encoding oncostatin-M-receptor-beta (*OSMR*) (OMIM 105250), PLCA-2 by heterozygous mutation in the *IL31RA* gene (OMIM 613955), PLCA-3 by mutation in the *GPNMB* gene (OMIM 617920). There were two case reports which described one patient each other, a 36 year-old male and a 50 year-old female, with PLCA and AIH [49,50]. These reports in the literature have been associated to autoimmune disorders, which suggests the possibility of a common underlying immune-mediated mechanism.

PFIC3: The PFIC3 is a heterogeneous group of autosomal recessive liver disorders (OMIM 602347), with childhood predominance, which causes cholestasis of hepatocellular, caused by a genetic defect in the *ABCB4* gene. In literature there is the first interesting association of PFIC3 and AIH type 1[51]. It regards a 22 year-old patient with diagnosis of PFIC3 caused by an allele with a previously described mutation and a new genetic variant (c.3680T>C; p.Ile1227Thr), transmitted by his mother, which is associated with AIH. The authors reported the importance of genetic testing of the *ABCB4* gene in patients with autoimmune liver disease with incomplete response to immunosuppressive treatment.

CONCLUSION

In this review, we performed a research of literature, during the last 10 years, from 2010 to 2020, to collect all clinical cases reporting the association between AIH and genetic syndromes. We observed that AIH is a frequent complication of group-1 syndrome, that includes disease whose causative gene have a role in immunoregulation. AIH is more rarely present in other group of genetic syndromes. If we consider a single disease, the number of articles is very limited, but we suppose that this could be related to rarity of genetic syndrome.

We hypothesize that AIH and genetic syndromes are combination of rare manifestation. Over the last decade, the attention of AIH diagnosis is increased and there is evidence that many triggers are involved for AIH pathogenesis, such as

familiarity, genetic predisposition, drug and infections. This paper suggests that genetic syndromes, as observed in the reported clinical cases, are a trigger for AIH, whose pathogenetic mechanism could be specific for each other, also related to genetic factors.

Genetic syndromes could contribute to the risk of developing AIH with a primitive gene mutation that compromises an immune response. For examples, it is demonstrated role of some gene products such as, *FOXP3*, *ICOS*, *TIGIT*, *CTLA4*, in pro-inflammatory/pro-B helper profile[10].

We suggest that the association between AIH and genetic syndrome might be not casual and claim that there might be an etiopathogenetic correlation between the causative genetic mutation and the immune imbalance, that is expressed as AIH. Considering that we have dealt with rare diseases and sometimes very rare, having found 34 articles in 10 years, we think there are not a few. On the other hand, it is fair to observe that when the clinical cases described are few, it is difficult to exclude that it is a coincidence. Much attention should be paid by clinicians to AIH diagnosis, with periodical autoantibody detection and identification of AIH manifestations and interpretation of liver autoimmune serology, to minimize the problem of underestimation of AIH diagnosis. Moreover, we underly the severity of AIH complication and in these cases the time of diagnosis should be crucial in order to start, as soon as possible, an appropriate therapy.

We suggest that when a patient presents a clinical picture of cryptogenic chronic hepatitis, that is unexplained, it is useful to explore differential diagnosis of AIH associated with genetic syndrome. Given the clinical relevance of this topic, further reports are needed to demonstrate our hypothesis and collect new evidence in this field.

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MINIREVIEWS

Assessing the prognosis of cirrhotic patients in the intensive care unit: What we know and what we need to know better

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Abstract

Critically ill cirrhotic patients have high in-hospital mortality and utilize significant health care resources as a consequence of the need for multiorgan support. Despite this fact, their mortality has decreased in recent decades due to improved care of critically ill patients. Acute-on-chronic liver failure (ACLF), sepsis and elevated hepatic scores are associated with increased mortality in this population, especially among those not eligible for liver transplantation. No score is superior to another in the prognostic assessment of these patients, and both liver-specific and intensive care unit-specific scores have satisfactory predictive accuracy. The sequential assessment of the scores, especially the Sequential Organ Failure Assessment (SOFA) and Chronic Liver Failure Consortium (CLIF)-SOFA scores, may be useful as an auxiliary tool in the decision-making process regarding the benefits of maintaining supportive therapies in this population. A CLIF-ACLF > 70 at admission or at day 3 was associated with a poor prognosis, as well as SOFA score > 19 at baseline or increasing SOFA score > 72. Additional studies addressing the prognostic assessment of these patients are necessary.

Key Words: Cirrhosis; Extrahepatic organ failure; Organ replacement therapy; Mortality; Prognostic scores; Chronic Liver Failure Consortium-Sequential Organ Failure Assess-

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Core Tip: Assessing the potential benefits of maintaining or suspending supportive therapies for cirrhotic patients who are not eligible for liver transplantation is a major challenge at the bedside, especially in those admitted to general intensive care units (ICUs). In this article, we identify the main causes of ICU admission, analyze the main factors associated with prognosis, and provide a tool to assist the decision-making process.

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INTRODUCTION

Liver cirrhosis (LC) accounts for more than 7000 deaths per year in France and more than 25000 deaths per year in the United States[1]. The World Health Organization recently estimated that cirrhosis is the 12th leading cause of mortality in the world, with alcohol, hepatitis B virus and hepatitis C virus being the main causes of cirrhosis [2,3]. Cirrhotic patients account for 2.3% and 4.5% of all intensive care units (ICUs) admissions[1], and their mortality is traditionally high-approximately 34% to 69% depending on the reason for admission[2]. The increased effectiveness of supportive treatments and the spread of liver transplantation programs have improved the prognosis of these patients[1,4-6]. Nonetheless, the prognosis of cirrhotic patients admitted to the ICU remains poor[7], especially among those admitted to the general ICU who are ineligible for transplantation. The prognosis is determined by the extent of hepatic and extrahepatic organ dysfunction[8]. The occurrence of three or more organ failures in cirrhotic patients has an almost certain fatal outcome[6,9]. For ethical reasons and due to limited resources, physicians need to be able to quickly identify cases that benefit from aggressive treatment and ICU admission, discriminating good candidates for ICUs from those for whom the prognosis is poor despite strong therapeutic interventions.

CIRRHOTIC PATIENTS ADMITTED TO THE ICU – AN OVERVIEW

Hemodynamic changes in patients with cirrhosis, linked to sodium retention, the development of ascites, and alterations in systemic and splanchnic hemodynamics and coagulation, are linked to systemic impairments in organ function, especially cardiomyopathy and renal dysfunction in this population[10]. A systemic inflammatory response has been observed in these patients, with complex immune dysfunction that increases the complexity of treatment and mortality in comparison with the general population[6,11]. High-grade hepatic encephalopathy (HE), septic shock, acute-on-chronic liver failure (ACLF), variceal bleeding, the need for mechanical ventilation and acute kidney injury (AKI) are clinical decompensations that most commonly motivate admission to the ICU[6].

Sepsis and septic shock

Infections are among the main reasons for admission of these patients to the ICU, as 30%–50% of patients with cirrhosis either present with infection during admission or develop infection during hospitalization[2,12]. Sepsis is a consequence of the host response to infection[13] and it is characterized by the release of pro- and anti-inflammatory cytokines and pro- and anti-coagulant substances in response to pathogens [14]. Several studies have highlighted the major influence of cirrhosis on the susceptibility to severe bacterial infections, with higher in-hospital mortality rates as a result

of septic shock in cirrhotic relative to noncirrhotic patients (71% *vs* 49%, respectively) [15,16]. Cirrhotic patients have an altered defense against bacteria associated with reduced bacterial clearance. This immune defect facilitates bacterial translocation induced by the increased intestinal permeability and gut bacterial overgrowth observed in cirrhosis[17]. Sepsis leads to the production of various inflammatory mediators that are increased in cirrhotic patients compared to noncirrhotic septic patients[6]. This state leads to complex organ alterations that often lead to the development of extrahepatic organ dysfunction, including HE and renal, respiratory, and circulatory failure during sepsis, a syndrome referred to as ACLF, which is also associated with a deterioration in hepatic function[18]. Commonly encountered infections in cirrhosis include spontaneous bacterial peritonitis, pneumonia, urinary tract infection, and cellulitis[19]. Sepsis is more common in cirrhotic than in noncirrhotic ICU patients, and it is also associated with a higher mortality rate[15]. Variables associated with mortality in septic cirrhotic patients are the presence of more than one site of infection, Child C status and elevated Model for End-stage Liver Disease (MELD) score[12].

Variceal bleeding

Cirrhotic patients with variceal bleeding are usually transferred to the ICU for hemodynamic stabilization. The fate of variceal bleeding in cirrhotic patients has changed over the last two decades[14]. Overall hospital mortality decreased from 42% in 1980 to 14% in 2000[20]. ICU admissions for variceal bleeding fell significantly in the last decade and were associated with a decrease in mortality over time[21]. Although overall mortality rates have decreased in cirrhotic patients with variceal bleeding, it is still high in the first 6 wk after the initial episode, and could exceed 30% in those with more severe disease and in those with multiorgan failure[5,6,22]. Rebleeding occurs in up to 20% of patients during the first 6 wk, and in this case, the mortality rate can exceed 50%. Patients with Child C or MELD ≥ 18, portal vein thrombosis, bacterial infections, and renal failure have a high likelihood of recurrence or death[6].

AKI

Cirrhosis-associated AKI is usually multifactorial and commonly involves bacterial infections, hypovolemia (secondary to overdiuresis, hemorrhagic shock, large-volume paracentesis or diarrhea), drug-induced nephrotoxicity, parenchymal renal disease and, in the absence of these causes, hepatorenal syndrome (HRS)[5,23]. With a yearly rate of 8%–12%, HRS-AKI is quite common in decompensated cirrhosis with ascites[10,23]. In hospitalized patients, it is approximately 25% and it increases up to 40%–60% in those admitted to the ICU[14,24]. AKI is associated with a poor prognosis and represents an important predictor for short-term mortality in patients with cirrhosis [25].

Encephalopathy

HE is a brain dysfunction caused by liver failure and/or portosystemic shunts and it manifests as a wide spectrum of neurological and/or psychiatric abnormalities[26]. Approximately 30%–40% of patients with cirrhosis present with an episode of HE at some time of their illness, with a poor prognosis and a mortality increase of 50% within 1 year after the episode of HE[6]. Patients with more severe grades (grade III-IV) could require admission to the ICU and orotracheal intubation and eventually prolonged MV, variables that are associated with increased mortality in this scenario [27,28].

Short and long-term mortality in ICU-cirrhotic patients

Short-term mortality in ICU-cirrhotic patients ranges from 42% in the ICU to 54% during hospitalization[29]. There is variability between different studies due to different selection criteria for patient admission between centers, differences between therapeutic strategies (including liver transplantation) and the low number of patients studied in each cohort in this short period of time[30]. During the ICU stay, prolonged MV is an important prognostic marker for ICU mortality[28]. Among the long-term mortality data for cirrhotic patients, there is high in-hospital mortality with reduced survival rates at 6 mo and 1 year. Thus, the one-year survival rate was 32% among patients alive at discharge from the ICU[9]. In another large study of short- and long-term survival, we found a comparable reduction in survival, with 8%–21% patients dying shortly after ICU discharge. In the ICU, 28-d, 3-, 6-mo, and 1-year mortality rates were 47%, 53% (116/218), 66%, 74%, and 77%, respectively[7]. The Glasgow coma scale, mean arterial pressure, bilirubin, and albumin determined on admission to the ICU have independent prognostic significance for assessing 6-month mortality. Severe

sepsis had the strongest association with increased 6-month mortality among the primary ICU admission reasons[29].

PROGNOSTIC SCORES IN CIRRHOTIC PATIENTS ADMITTED TO THE ICU

Liver cirrhosis is characterized by a long phase of compensated disease until the first episode of decompensation occurs. The time elapsed until such an event is variable and unpredictable; however, it marks a change in the progression of the liver disease [30]. Upon acute decompensation, some of these patients develop organ failure and need to be admitted to the ICU for optimal treatment. Historically, the in-hospital mortality rates of these patients are very high, promoting the idea that admitting them to the ICU would be a futile measure[22]. More current series show that the hospital mortality of these patients is quite heterogeneous, reflecting the varying degrees of hepatic involvement that these patients may present on admission to the ICU, as well as their different reasons for admission to the ICU[31].

Even so, the nonnegligible mortality rates of critically ill patients with liver cirrhosis, associated with scarce and expensive intensive care resources, make the indication of ICU admission of this population a matter of debate. Prognostic scores are helpful in this decision-making, aiming at therapeutic proportionality at the individual level and an adequate allocation of resources at the institutional level. The prognostic scores can be specific to each pathology. In the case of liver cirrhosis, we can mention Child–Pugh (CP), the MELD, and the Chronic Liver Failure-Consortium ACLF (CLIF-ACLF) score, for example, or assessments common to all patients admitted to the ICU, such as the Sequential Organ Failure Assessment (SOFA) score and the Acute Physiology and Chronic Health Evaluation II (APACHE II) score. These scores can be performed immediately upon admission to the ICU (first 24 h) or during the first days of hospitalization, leading to an evolutionary assessment over this short period of time. We can also evaluate the prognosis of decompensated liver cirrhosis taking into account the number of organic disorders at its presentation. The most relevant studies regarding prognostic scores are summarized in Table 1.

General ICU scores have been frequently used in the evaluation of cirrhotic patients. However, these scores do not include the complexity of chronic liver disease, including the heterogeneity of its clinical stages and possible etiologies, thus imposing caution in the use of these tools. On the other hand, CP and MELD incorporate limited information about extrahepatic organic dysfunction. Next, the main scores will be discussed, as well as comparisons of their performances.

HEPATIC-SPECIFIC SCORES

CP and MELD

The chronic liver disease severity score described by Child in 1964 and modified by Pugh in 1973 was used to describe the prognosis of patients undergoing surgical ligation of esophageal varices, demonstrating that patients with less perioperative liver dysfunction had lower mortality in six months[32]. It is currently used to assess the severity of chronic liver disease. The MELD score was described to predict mortality at 3 mo in patients electively submitted to the placement of portosystemic shunts[33] and later used to prioritize patients listed for liver transplantation because it proved to be a reliable mortality risk index[34].

Specific scores for cirrhosis, such as CP and MELD, seem ideal for prognosis in cirrhotic patients with slow decompensation but do not perform well in those with acute decompensation accompanied by multiple organ and system dysfunction (DMOS). DMOS is a clinical condition where there are multiple acute systemic failures (renal, circulatory, neurologic, hematological, pulmonary, hepatic) associated with an initial injury, most commonly sepsis, trauma or shock[35,36]. They show moderate results[37], with the MELD score showing slightly better results than the CP[3]. The MELD score has reasonable discriminatory power (AUROC = 0.81) in predicting mortality in cirrhotic patients admitted to the ICU, approaching the SOFA score (AUROC = 0.83)[31].

Variations of MELD: MELD-sodium

Dilutional hyponatremia is common in patients with advanced cirrhosis, and the inclusion of natremia in the MELD score has been suggested to increase its prognostic

Table 1 Accuracy of prognostic scores in intensive care units cirrhotic patients

Ref.	Year	*n*	ICU/hospital mortality	APACHE II	SAPS II	SOFA	CP	MELD	MELD-Na	RFH	CLIF-SOFA
Cholongitas *et al*[31], 2006	2006	312	65%	0.78		0.83	0.72	0.81		0.83	
Das *et al*[41], 2010	2010	138	54%		0.78	0.84	0.76	0.77	0.75		
Levesque *et al*[42], 2012	2012	377	43%		0.89	0.92	0.79	0.82	0.79		
Cholongitas *et al*[44], 2008	2012	412	61%	0.74		0.85	0.67	0.80	0.75		
Emerson *et al*[45], 2014	2014	59	48%	0.72		0.76	0.70	0.74			0.75
Campbell *et al*[46], 2015	2015	115	46%	0.71		0.71	0.68	0.70		0.77	0.74
McPhail *et al*[51], 2015	2015	971	52%	0.76	0.78	0.79		0.78			0.81

ICU: Intensive care units.

capacity for mortality, with greater importance when the MELD scores are lower[38, 39]. The MELD-Na score was better than the MELD score for predicting mortality in some studies[40] but less accurate than the SOFA score[41-43].

CP variation: CP + L

More recent data suggest that lactate, a component of the prognostic model of fulminant hepatitis, is an independent marker of mortality in patients with cirrhosis admitted to the ICU[44] and it seems to significantly improve the CP score's ability to predict ICU mortality[45]. Serum lactate and ascites are independent predictors of ICU mortality, as proposed by the CTP + L score. This score incorporates serum lactate levels into CP, increasing its discriminatory ability as a prognostic stratification tool. Subsequently, a retrospective cohort study with a total of 199 cirrhotic patients admitted to a general ICU at two different centers validated the CP + L score as a predictor of mortality, showing results superior to the original CP: AUC CP + L 0.75 and AUC CP 0.68. In this work, the MELD and SOFA scores had AUCs of 0.7 and 0.71, respectively[2].

Royal free hospital score

Studies have suggested that an alternative approach to predict mortality in patients with decompensated cirrhosis could be the number of organ dysfunctions at its presentation, ranging from 4% in patients without DMOS to 90% in those with three or more organ dysfunctions and thus in a DMOS scenario[31]. In this context, a specific score for cirrhosis was developed and subsequently modified[43], taking into account possible organic failures involved during acute decompensation, the Royal Free Hospital Score (RFH). This score was shown to have a performance similar to the SOFA score and superior to APACHE II, MELD, and CP.

A retrospective cohort study by Campbell *et al*[46], with a total of 199 cirrhotic patients admitted to the ICU, validated the RFH score as a predictor of mortality in the ICU with an accuracy of 0.77, which was higher than the other scores evaluated: CP, CP-L, MELD, SOFA and CLIF-SOFA. The RFH score is the first liver-specific score to be matched, in terms of mortality predictive ability, to the general ICU scores used in these patients. In addition to the fact that it includes hepatic and extrahepatic parameters of organ dysfunction associated with higher mortality in this subset of patients, the inclusion of lactate levels in this score should be highlighted. Despite the well-known relationship between serum lactate levels and worse outcomes[2], no other hepatic-specific score proposed thus far has included this parameter.

ICU mortality and morbidity scores (dysfunction)

ICU-specific mortality scores were created to assist the intensive care physician in predicting the outcome of patients admitted to the ICU. Among these scores, the most important are the APACHE II and SOFA scores. APACHE II uses the worst physiological variables of the patient in the first 24 h of ICU stay for its elaboration, in addition to previous comorbidities and age[47]. The SOFA score assesses the severity of patients admitted to the ICU according to the number of organ dysfunctions. The score is graded in five levels (from 0 to 4 points) for six organ systems: neurological, hemodynamic, respiratory, renal, hematological and hepatic, with a score greater than or equal to 3 in any organ system constituting organ failure[48]. Unlike the APACHE II

score, which is performed at a specific time in the ICU (24 h of admission), the morbidity scores allow for an evolutionary assessment throughout the days of ICU admission[48].

These scores have already been evaluated in specific populations of cirrhosis[15]. When compared to each other and with specific scores for cirrhosis, the SOFA score shows moderate to high accuracy, higher than the other scores, even for long-term mortality[3,45,49]. Lindvig *et al*[3], in their systematic review, found that the SOFA score has better accuracy for death prediction, with an AUROC between 0.81% and 0.95%, a value higher than the APACHE II score (AUROC 0.66-083), MELD (AUROC 0.77–0.93) and CP (AUROC 0.71–0.87).

ACLF

ACLF is a clinical syndrome characterized by acute liver cirrhosis decompensation associated with one or more organic disorders and a high short-term mortality rate. The European Association for Study of Liver/CLIF (EASL-CLIF Consortium) has established diagnostic criteria for ACLF with a view, above all, to identify patients at greater risk of death in the short term. For the establishment of the ACLF diagnostic criteria, the presence of organic dysfunction and a high mortality rate at 28 d (> 15%) in cirrhotic patients with acute decompensation were considered. The assessment of organ dysfunction, in turn, was based on the SOFA score, but with modifications taking into account the pathophysiological and clinical characteristics of cirrhosis, giving rise to the CLIF-SOFA score[50].

CLIF-SOFA improves the hematological, neurological, cardiovascular, and renal domains by considering commemoratives usually present in chronic liver disease patients, as well as the peculiarities of the clinical manifestations and therapy used during acute decompensation. Objectively, the hematological parameter is no longer the platelet count giving rise to the measurement of INR. The neurological parameter now includes the presence of HE stratified under West Haven criteria, and in the cardiovascular and renal domains, it takes into account the use of terlipressin and renal replacement therapy, respectively. There is also a change in the hepatic domain with elevation of the total bilirubin threshold to characterize this organ dysfunction.

McPhail *et al*[51] demonstrated the validity of the CLIF-SOFA score in terms of its ability to predict mortality with a slight improvement over the SOFA score and other prognostic scores. Aiming at a better performance than CLIF-SOFA, the CLIF-C ACLF score was developed based on CLIF organ failure score scores, the latter also a derivation of SOFA and CLIF-SOFA[52]. However, the CLIF-C ACLF showed a slightly higher prognostic accuracy for 28-d mortality than the CLIF-SOFA scores and it was moderately higher than MELD, MELD-Na and Child–Pugh: agreement index of 0.76; 0.72; 0.68; 0.68; 0.66, respectively[52].

Evolutionary assessment of scores-what we need to know better?

Most prognostic scores in critically ill populations are constructed with data collected over the first 24 h of ICU admission. However, multiorgan failure seems to be related to a worse prognosis among patients with acute cirrhosis decompensation[1,4,22]. Seeking to increase the accuracy of prognostic scores in cirrhotic patients admitted to the ICU, a baseline assessment of the score followed by its reanalysis in a short period of time seems to be more accurate in predicting hospital mortality. The SOFA score seems to be the score with the best discrimination power when compared to the CTP, MELD, APACHE II scores, both at the initial time and when reassessed at 48 h: AUC for mortality, after 48 h of 0.88; 0.78; 0.86 and 0.78, respectively[44]. The modified SOFA score (removing the hepatic component from the score) was also shown to be highly accurate and with better discriminative power when compared to CP, MELD, and APACHE II scores both on the first day of ICU admission (AUC 0.84) and on the third day (AUC 0.83)[41]. It is interesting to note that the presence of 3 to 4 organ dysfunctions after 72 h of admission to the ICU is related to an important increase in mortality during hospitalization[41].

A limitation of the prognostic scores evaluated on admission to the ICU is to neglect the continuum of physiological changes in critical patients with decompensated cirrhosis[53]. The serial assessment of the SOFA score throughout the ICU stay contemplates the dynamics of the occurrence of organic dysfunctions, including the effects of the offered therapy[44,54]. Both the analysis of the variation in the SOFA score (Δ-SOFA) and access to the mean and maximum SOFA values during ICU admission are good prognostic indicators, regardless of the value of the score accessed

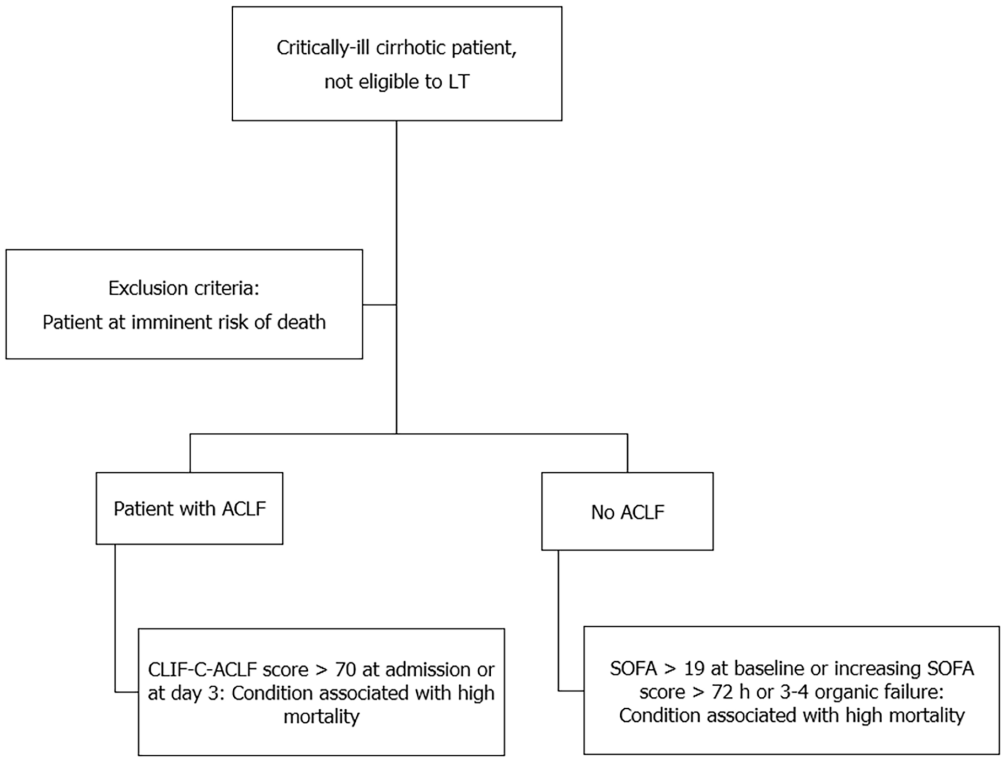

Figure 1 Proposed algorithm for prognostic scores in critically-ill cirrhotic patients. ACLF: Acute-on-chronic liver failure; CLIF: Chronic Liver Failure Consortium; SOFA: Sequential Organ Failure Assessment.

at the time of admission[54]. In a retrospective cohort study comprised of 971 patients, the CLIF-SOFA score seemed to have a slightly higher accuracy than the SOFA score for mortality (AUC 0.81 *vs* 0.79) when evaluated during the first day of hospitalization and an improvement in death prediction at 48 h after ICU admission. However, the results seem overlapping when evaluated on the seventh day of ICU stay, with both showing good discriminatory power[51]. Dynamic prognostication seems to be the most promising strategy when establishing the prognosis of this population, especially in those with ACLF, septic shock and multiorgan failure[55]. A proposed algorithm is summarized in Figure 1. A trial of unrestricted intensive care for a few days could be proposed as a reasonable strategy in this population[41]. There are also opportunities for novel biomarkers of ACLF to improve existing models and potentially reflect information not currently captured in the conventional clinical and biochemical data [56].

An important limitation of prognostic studies in this field is that the interpretation of ROC curves is necessary because the criteria for therapeutic limitations or even the removal of supports are not reported in these studies, which leads to falsely high areas under the curves. Another limitation of prognostic scores is that they were not designed to predict outcomes beyond mortality, such as cost-effective treatment, recovery of physical activity or the quality of life after the ICU stay. In addition, some organ dysfunction scores may give similar weights for organ dysfunction with very different prognoses[57]. Alteration of the level of consciousness due to HE after bleeding from esophageal varices and even chronic thrombocytopenia, common in advanced cirrhosis, has a better prognosis than that of vasopressor or acute loss of renal function. Figure 1 outlines a structured assessment model based on prognostic scores in this population. A condition associated with high mortality, based on these scores, does not necessarily mean that therapeutic efforts should be stopped but that patients, family members and staff can have a better understanding of the prognosis, in light of current knowledge. Knowledge of the patients' wishes, beliefs and desires is fundamental to establish future therapeutic strategies.

CONCLUSION

In critically ill cirrhotic patients who are not awaiting liver transplantation, there is no

"gold standard" for predicting their short- and long-term prognosis. Several variables are associated with a worse prognosis, such as the presence of sepsis, the number and intensity of associated organ failures, and the duration of MV. Baseline severity scores, as well as the sequential assessment of organ failure scores, provide more certainty regarding the impact of critical illness on the prognosis of this population.

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MINIREVIEWS

Liver transplantation for pediatric inherited metabolic liver diseases

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Abstract

Liver transplantation (LT) remains the gold standard treatment for end stage liver disease in the pediatric population. For liver based metabolic disorders (LBMDs), the decision for LT is predicated on a different set of paradigms. With improved outcomes post-transplantation, LT is no longer merely life saving, but has the potential to also significantly improve quality of life. This review summarizes the clinical presentation, medical treatment and indications for LT for some of the common LBMDs. We also provide a practical update on the dilemmas and controversies surrounding the indications for transplantation, surgical considerations and prognosis and long terms outcomes for pediatric LT in LBMDs. Important progress has been made in understanding these diseases in recent years and with that we outline some of the new therapies that have emerged.

Key Words: Pediatric metabolic liver disease; Liver transplantation; Liver based metabolic disorders; Inherited; Cell therapy; Gene therapy

Core Tip: The decision for liver transplantation (LT) in liver based metabolic disorders (LBMDs) is not straightforward. As outcomes from pediatric LT continue to improve, transplantation is no longer merely life saving, but also potentially significantly improves the child's quality of life. We herein discuss the clinical presentation, medical and surgical treatment for some of the common LBMDs. We provide a practical update on the indications, dilemmas and controversies for LT and the long-term outcomes for children with LBMDs.

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INTRODUCTION

Liver transplantation (LT) remains the standard of care for children with end-stage liver disease. With advances in the perioperative transplant management, the outcomes after pediatric LT continue to improve-with better survival rates[1] and quality of life measures[2].

Indications for pediatric LT can be broadly divided in to three main groups (Figure 1). (1) Cholestatic diseases, such as biliary atresia and other conditions leading to biliary cirrhosis are the most common indications for LT in the pediatric population [3]; (2) Inherited metabolic liver diseases constitutes a wider group of diseases, in which inborn errors of liver metabolism lead to severe intra- or extra-hepatic manifestations. Within this group of conditions, LT results in a cure in some, whilst others have an improved quality of life after transplantation, without necessarily being cured from their primary illness; and (3) The third group is more varied, with indications of acute liver failure, tumors and re-transplantations.

Some of the more common liver based metabolic disorders (LBMDs) are exemplified below.

LBMDS CURED BY LT

Crigler-Najjar syndrome type 1

Crigler-Najjar syndrome (CNS) type 1 is secondary to a total deficiency of the uridine diphosphoglucuronate glucuronosyltransferase activity[4]. This results in a severe indirect hyperbilirubinaemia from birth, with an otherwise normal liver biochemistry. It is an extremely rare familial disease affecting one *per* 600000-1000000 live births worldwide. It has an autosomal recessive inheritance pattern and is caused by biallelic mutations of the *UGT1A1* gene[5].

Natural history and medical treatment: The build up of unconjugated bilirubin, which deposits in the brain, eventually leads to kernicterus, which is irreversible in most cases. Exchange transfusion in the neonatal period and plasmapheresis in older children, may be indicated for acute episodes of severe hyperbilirubinaemia.

Intensive phototherapy is the mainstay of treatment for CNS type 1, particularly in the newborn period. It is less effective in older children and adults due to skin thickness, pigmentation and lower body surface are to body mass[6].

Other treatments include bilirubin-binding agents such as orlistat–a lipase inhibitor which works better in tandem with calcium phosphate. Both of these agents help in the excretion of bilirubin through the gut[7,8]. Other pharmacological agents with limited evidence for efficacy include enzyme-inducing agents (phenobarbital), choleretics (ursodiol) and heme-oxygenase inhibitors (tin-protoporphyrin and zinc-protoporphyrin).

LT: At present, the only definitive treatment for CNS type 1 is LT. The two main types of LT include orthotopic LT (OLT) and auxiliary partial OLT (APOLT). The host liver is replaced with a whole or partial liver graft in OLT, whilst in APOLT only part of the native liver is removed and replaced with the graft. APOLT has the theoretical advantage for future novel therapies directed at native hepatocytes, such as gene replacement and genome editing[4].

The transplant provides the child with a normal liver with normal *UGT1A1* enzymatic activity, thereby completely normalizing bilirubin levels and providing the child with a normal quality of life. LT is advisable before neurological damage occurs [9]. As the outcomes of transplantation in infants are now similar to children, transplantation is indicated in the first few years of life to prevent prolonged impairment to the child and family.

Future research implications: In recent years, allogenic hepatocyte transplantation has become an attractive alternative to LT[10]. Normal hepatocytes are transplanted *via* the portal vein or peritoneal space. Encouraging results have been observed with a reduction in bilirubin levels and reduced need for phototherapy[11]. Issues still exist around the longevity of the transplanted cells–which decreases after a few months, limited supply and cell quality. Mesenchymal stem cell therapy has shown some promise in animal models and may provide a new alternative treatment in the future [12].

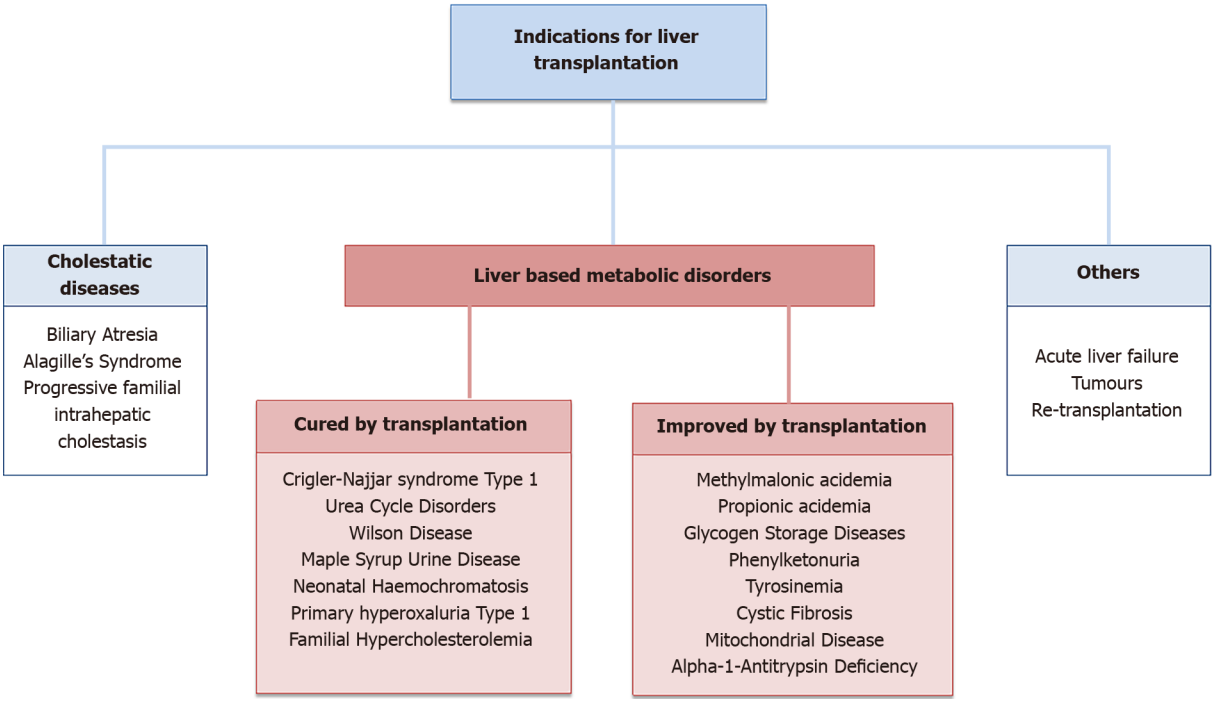

Figure 1 Indications for liver transplantation.

Ex vivo and *in vivo* gene therapy is another new avenue for treatment of CNS type 1. Different approaches including infusing autologous liver or induced pluripotent stem cells into the liver and *in vivo* gene replacement using a vector delivery system have been proposed, but there remain little safety and efficacy data[4].

Urea cycle disorders

Urea cycle disorders (UCDs) are a group of disorders secondary to defects of urea synthesis and related metabolic pathways. UCDs result from a deficiency in either one of the six enzymes [n-acetylglutamine synthetase (NAGS), carbamoylphosphate synthetase I (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthase, argininosuccinate lyase (ASL), and arginase 1] or two mitochondrial transporters of the urea cycle pathway or metabolites of the amino acids related to the urea cycle[13]. The liver is central to these metabolic pathways, and plays a key role in removing waste from protein catabolism. The defect in the pathway leads to life threatening hyperammonaemia[14]. It is the most common IEM based in the liver with an incidence of 1 in 30000–46000 Live births. All UCDs are inherited in an autosomal recessive manner apart from OTC deficiency, which is inherited in an X-linked manner.

Natural history and medical treatment: Clinical findings are secondary to hyperammonaemia including seizures, coma, cerebral edema and death, with long-term neurodevelopmental implications in survivors. The severity of symptoms can be variable, with some presenting with fatal hyperammonaemia in infancy to asymptomatic adults. In the neonatal period, symptoms occur within hours to days after birth. Initially, neonates with UCD may present with non-specific features such as poor feeding, vomiting, lethargy and tachypnea, but quickly progress to coma and death secondary to hyperammonaemia. NAGS, CPS1 and OTC deficiencies, have the poorest outcomes with neonatal onset of hyperammonaemia and death within the first year of life[15]. Some children may have a delayed presentation with less severe features such as mild gastrointestinal or neurological symptoms. The long-term outcome is dependent on the number of episodes of hyperammonaemia (due to non-adherence, infections and lack of compliance to diet).

The medical management of UCDs requires multidisciplinary input and is complex. The treatment strategy for acute hyperammonaemia is three-fold[16]: (1) Reduce blood ammonia levels through hemodialysis or hemofiltration; (2) Reversal of the catabolic state through caloric and arginine supplementation; and (3) Elimination of excess nitrogen pharmacologically (*e.g.* benzoate and phenylbutyrate)

In the long term, a diet restrictive of protein, alongside supplementation with essential amino acids is key. Medications to increase waste nitrogen excretion are also important[17]. Despite aggressive and prompt medical treatment, not all episodes of acute hyperammonaemia can be avoided, and the risk of neurological damage remains.

LT: LT offers a practical cure for UCDs as the metabolic defect is predominantly or exclusively within the liver. A long waiting list duration is associated with long-term risk of cognitive delay[18]. As such LT should be considered in children with UCD to prevent progressive neurologic injury and improve cognitive outcomes. Post-transplantation, patients are allowed a normal diet without taking nitrogen scavengers [19]. LT should be offered early to patients with severe UCDs, poorly controlled with medical interventions to prevent long term neurological damage. Living related transplantation offers the advantage of optimal timing after confirmation of the donor phenotype[20].

Future research implications: Allogenic hepatocyte transplantation has been shown to have a sustained partial correction of the metabolic defect in OTC and ASL deficiency patients[21,22]. Another promising treatment for UCD is gene therapy and has seen many years of preclinical evaluation, but concerns still remain around the safety of the application[23].

Maple syrup urine disease

Maple syrup urine disease (MSUD) is an autosomal recessive disease, secondary to mutations in six gene loci where branched-chain alpha-ketoacid dehydrogenase complex is encoded. This results in the inability of the body to fully breakdown the essential amino acids valine, leucine and isoleucine. It has an estimated incidence of 1 in 185000 live births[24].

Natural history and medical treatment: There are five distinct clinical phenotypes of MSUD, without clear correlation of genotype-phenotype. Classic MSUD manifests in the neonatal period with delayed development, feeding difficulties, failure to thrive, opisthotonus, "bicycling" movements and maple syrup odor[25]. Metabolites accumulate and are excreted in the urine, sweat and ear cerumen, leading to the sweet odor of maple syrup. If left untreated, irreversible neurological damage and metabolic crisis occurs.

The most common medical treatment for patients with MSUD is dietary restriction of the affected amino acids, with supplementation[26]. Despite aggressive treatment, many patients will still experience episodes of metabolic decompensation during acute illness or stress, with risk of developing cerebral edema. Acute metabolic decompensation management includes effectively treating the underlying stressor, restricting protein intake, ample caloric support, supplementation with cofactors, elimination of toxic metabolites and correcting metabolic abnormalities[27].

LT: In patients with recurrent metabolic crises and high risk of cerebral edema, despite optimal medical treatment, LT should be considered[28]. LT is curative and significantly improves quality of life in children with MSUD. Patients can immediately cease protein-restricted diet and are safe from catabolic crisis[28]. Preexisting neuro-disability does not get reversed but LT offers neurological function stability and risk of cerebral edema is greatly reduced[29].

Domino transplantation where the explanted liver is used for another recipient without the underlying disease, has been used successfully in MSUD[30-32]. The new liver provides the metabolic protection in the MSUD patient, whilst the domino recipient has a normal systemic metabolism of branched amino acids and can counter the effects of an MSUD liver. This helps with organ allocation and diminishes the impact of the original transplant in the overall pool of organs[33].

Future research implications: Sodium phenylbutyrate (NaPBA) is commonly used for treatment in patients with UCD. In a cohort of 533 patients with UCD, Burrage *et al*[34] showed a reduction in branched chain amino acids and suggested follow up studies to investigate it's utility in MSUD[34]. Studies are currently ongoing to assess its efficacy in MSUD patients.

Animal studies have shown encouraging therapeutic results using hepatocyte transplantation with partial metabolic correction of MSUD in a murine model[35]. Whilst promising, this intervention still warrants further clinical investigation.

Wilson disease

Wilson disease (WD) is secondary to mutations of the gene *ATP7B* on chromosome 13, which codes for the transmembrane ATP7B transporter, involved in the transport of copper, incorporation of copper to the protein caeruloplasmin and excretion of excessive copper into bile. Excess copper in the liver leads to liver destruction, diffusion in to blood and eventually deposition in the other organs[36]. It is an autosomal recessive condition with a prevalence of 1 in 30000 people. An age-phenotypic presentation has been observed with hepatic presentations seen in the younger age groups (< 10 years: 83%, 10-18 years: 52%, > 18 years: 24%), whilst a neuropsychiatric presentation was more common in the older age groups (> 18 years: 74%, 10-18 years: 48%, < 10 years: 17%). The median age of presentation is 13.2 years (range 3–74 years), but children are rarely symptomatic before the age of 5 years[37].

Natural history and medical treatment: The clinical features in the pediatric population depend mainly on the predominant organ involved (liver and brain). The deposition of copper in various site of the body leads to the plethora of clinical presentations.

The majority of children present with liver disease, ranging from an asymptomatic rise in transaminases, acute hepatitis, acute liver failure, acute on chronic liver failure, chronic hepatitis, cirrhosis, fatty liver disease or malignancy[38]. It is important to remember that the finding of another cause of liver dysfunction such as acute viral hepatitis or non-alcoholic steatohepatitis, does not necessarily rule out Wilson's disease[39].

Up to 25% of children and adolescents present with acute or decompensated liver failure[40]. The presentation is similar to that of acute hepatitis, but the condition leads to rapid deterioration, with a high mortality. Symptoms include severe jaundice, Coombs- negative hemolytic anemia, deranged coagulation, ascites, encephalopathy and renal failure. Children present with very high serum bilirubin, rise in liver enzymes, low serum alkaline phosphatase and defective synthetic functions.

By the time children present with neurological symptoms, most already have liver disease, although may not be overtly symptomatic. Subtle signs may start from a young age such as deterioration of school performance or handwriting and dysarthria. Neurological signs tend to be wide-ranging and variable. Behavioral and psychological changes are very common in WD and make up for roughly one-third of presenting symptoms.

Medical therapy is mainly focused around the copper chelation. Main drugs currently in use include D penicillamine, trientine, zinc and ammonium tetrathiomolybdate. Treatment should be commenced as soon as the child is diagnosed, as untreated WD can be fatal. In patients with acute liver failure or advanced liver disease, LT is the only effective therapy.

LT: The liver disease is cured by LT and extra-hepatic symptoms generally improve after LT, particularly neurological signs. LT is the only option for patients with acute liver failure with encephalopathy secondary to WD. In children with liver dysfunction without encephalopathy, but are unresponsive to medical treatment, the indications are less clear. The Wilson Index is helpful in identifying children with decompensated liver failure, with a 93% sensitivity and 98% specificity[41].

Future research implications: Animal models have shown that restoration of 30%-50% of metabolic function may protect the rest of liver cells. This raises the possibility of gene therapy and hepatocyte transplantation as a potential therapeutic option in children with WD[42]. For patients with acute liver failure secondary to WD, hepatocyte transplantation may be used as transient support until chelation treatment shows its effect or as a definitive cure through repopulation of the liver by healthy donor cells as seen in animal models of WD[43].

LBMDS IMPROVED BY LT

Methylmalonic acidemia and propionic acidemia

Methylmalonic acidemia (MMA) and propionic acidemia (PA) are the commonest forms of organic acidemias resulting from defective catabolism of the amino acids[44]. MMA is an autosomal recessive disorder secondary to the complete or partial deficiency of methylmalonyl-CoA mutase. MMA is also caused by several inborn errors of cobalamin or B12 metabolism. It is rare with an incidence of 1 in 80000 live births[45]. PA is also an autosomal recessive disorder due to a defect in the enzyme

propionil-CoA carboxylase[46].

Natural history and medical treatment: The presentation can be divided in to three categories: (1) Neonatal presentation with signs of sleepiness, encephalopathy, coma, hypotonia and hepatomegaly; (2) Infantile form with recurrent metabolic crisis and neurological changes; and (3) Chronic presentation with developmental delay, failure to thrive and recurrent infections[44]. Neurological signs include epilepsy, developmental delay and dystonia resulting from lesions in the basal ganglia. Methylmalonate is also nephrotoxic and can lead to progressive renal disease and end-stage renal failure by adolescence. Investigations in these patients show ketoacidosis, hyperammonaemia and hyperglycinemia. Urine organic acids will reveal propionyl-CoA derivative or methylmalonate.

Each metabolic crisis requires correction and maintenance therapy is dependent on dietary restriction of protein (low protein and high caloric diet with continuous overnight feeding), supplementation with amino acids, carnitine, metronidazole (reduce production of prioprionate in the gut) and cobalamine[47]. Intensive clinical management with aggressive treatment with dialysis and haemofiltration is often needed to minimize neurological sequelae. Despite early detection and maximal medical therapy, many children develop significant neurological, renal and cardiac complications.

LT: LT provides the deficient enzyme in MMA, but the overall biochemical defect is not entirely corrected as the enzyme is expressed in most cells in the body. Neurological and renal function may deteriorate further after LT in some MMA patients[48]. Kidney transplantation has had long-term success in reducing MMA levels and avoiding metabolic crisis in moderate forms of the disease[49]. In more severe forms, combined liver and kidney transplantation may reduce frequency of metabolic crises, severity of illness and probably decreases risk of further neurological deterioration, but does not abolish it entirely[45]. With that in mind, the indications for liver and/or kidney transplantation are still unclear in MMA.

The idea behind transplantation is to provide the deficient enzyme through the graft and correct renal failure if present. It is important to remember that transplantation does not cure MMA but may reduce the frequency of crisis and improve the child's quality of life. Pre-transplant assessment should include a thorough neurological assessment as transplantation does potentially have the risk of further neurological deterioration. Dietary restriction of protein should be continued as a precaution against future metabolic decompensation and late complications after transplantation [45].

In PA, LT also only partially corrects the metabolic defect[50]. However, the improvement seen appears to be more significant in PA compared to MMA–diet can usually be normalized, no further metabolic crises and neurocognitive function remains as it is pre-transplantation[51]. LT should be indicated in patients with recurrent metabolic crises despite optimal medical therapy, with a view of preventing further neurological deterioration and cardiac complications[50].

Future therapies: The role of new and novel treatments such as genetic modification, hepatocyte transplantation and chronic medical therapies remains uncertain[52].

Glycogen storage diseases

Glycogen storage diseases (GSD) constitutes a group of mainly autosomal recessive metabolic disorders, caused by the accumulation of either an abnormal amount or type of glycogen. It has an incidence of 1 in 20000 to 40000 live births. Various enzymes of glycogen metabolism are potentially involved, with 12 types of GSD recognized – seven of which have an enzymatic defect in the liver. Types I, III, IV, VI and IX are associated with severe liver disease[53-55].

Natural history and medical treatment: Typically, it presents with fasting hypoglycemia, hepatomegaly and growth retardation. In the GSD type I, hepatocellular adenomas with risk of transformation to hepatocellular carcinoma has been found[55, 56], particularly in those with pre-existing adenomatous nodules. In GSD type III, some patients may progress to liver cirrhosis, whilst some develop hepatocellular carcinoma[54]. GSD IV patients have a variable phenotype and some develop liver cirrhosis and hepatocellular carcinoma early on. Extrahepatic manifestations such as renal dysfunction in GSD type 1, myopathy in GSD type III and IV may also be present. It is important to distinguish between subtypes for optimal management. Diagnosis is through enzyme assays in the liver other tissues and mutation analysis. Presence of PAS-positive glycogen staining in liver biopsy samples is useful in

confirming the diagnosis.

Treatment for liver GSD includes dietary changes and medical treatment when symptoms are not corrected by diet. In GSD type I, continuous overnight enteral drip-feeding is used to avoid fasting hypoglycemia and regular oral cornstarch intake is used for prolonged glucose release and have significantly improved metabolic control [57]. Other pharmacotherapy may be needed such as allopurinol for hyperuricaemia, angiotensin converting enzyme inhibitors for proteinuria and granulocyte-colony stimulating factor for neutropenia in GSD type Ib[58]. In patients with GSD types III, VI and IX, a high protein diet alongside uncooked cornstarch is standard therapy. Whilst metabolic control is generally successful with medical therapy, long-term complications still occur[15]. Adherence is also a common issue in children and adolescents and may not be tolerated in many, results in a higher rate of complications.

LT: In patients with very poor metabolic control despite optimal medical therapy, those with multiple recurrent adenomas with increasing size, progressive liver cirrhosis and/ or hepatic failure, LT should be considered. In children with GSD type IV, LT is generally the best option for treatment, particularly in those that develop liver cirrhosis[59]. Children with GSD are also living longer and despite medical treatment, many develop long-term complications. With the outcomes of LT improving, including better biochemical and clinical parameters, LT offers the potential to be both preventative and curative for patients with GSD.

Indications for LT in GSD can be summarized as: (1) Correction of LBMD when medical therapy is unsuccessful or impairs quality of life; (2) Cirrhosis and complications; and (3) Liver tumors such as adenoma and hepatocellular carcinoma.

LT corrects the enzymatic defect, but the extrahepatic manifestations often complicate post-transplantation management[59].

Future therapies: There has been limited experience with hepatocyte transplantation, but initial reports are positive[60,61]. Gene therapy has been developed in animal models, but there remains insufficient data for clinical trials[62].

Phenylketonuria

Phenylketonuria (PKU) is a rare autosomal recessive condition secondary to mutations in the phenylalanine hydroxylase gene (PAH). This results in a deficiency of PAH, an enzyme in the liver that converts phenylalanine (Phe) to tyrosine. The incidence is roughly 1 in 10000 and does vary by ethnic group, being higher in Caucasians.

Natural history and medical treatment: The lack of this enzyme results in abnormally high levels of phenylalanine in the brain, causing intellectual problems, developmental delay and psychiatric issues. Universal newborn screening in most developed nations has led to early detection and significantly reduced the number of children with intellectual disability secondary to PKU. Despite ongoing and early treatment of patients with PKU, majority of patients will still have a lower intellectual ability compared to family members and suffer from mental health issues[63,64].

Medical therapy consists of restriction of phenylalanine intake and supplementation with phenylalanine-free amino acid mixtures to ensure adequate protein intake[65]. The diet needed is extremely restrictive and include mainly fruits, vegetables and low protein modified foods such as bread, rice and pasta[66]. Dietary treatment, when maintained in childhood and well into adulthood has been shown to result in markedly improved outcomes at a cognitive and psychiatric level for patients. However, adherence to this strict regime is not ideal, particularly in adolescents and in adulthood.

Dietary modification has evolved with the introduction of glycomacropeptides (GMP), which are proteins contained in "whey". These contain very little phenylalanine, which makes them suitable for replacing amino acid substitutes. Compliance has been shown to be improved with GMP compared to traditional amino acid foods[67]. The medication sapropterin, a form of tetrahydrobiopterin cofactor of phenylalanine hydroxylase has a success rate of up to 55% in PKU patients[68]. Patients with milder form of disease are more likely to respond to this drug. Another recent pharmaceutical drug known as peglyated phenylalanine ammonia lyase or pegvaliase, an enzyme substitute therapy has been assessed in Phase 2 and Phase 3 clinical trials[69]. Over 24 mo, patients showed a 69% decrease in Phe levels from baseline but almost all patients had mild to severe adverse events[70].

LT: Whole liver LT is not thought to be acceptable in majority of patients and physicians due to the availability of non-surgical treatment options.

Future therapies: Gene therapy has been shown to be successful in mouse models but no studies have reported trials in patients yet[71]. A variation of gene therapy is gene-editing techniques (Crispr/Cas9 or TALENS) to repair common mutations or insert active gene into "safe" areas of the gene. The development of an expressive synthetic RNA for the *PAH* gene is in development, but not with human subjects[72].

Cell-based therapies including hepatocyte and stem cell transplantation have been considered viable alternatives[73]. One patient has received hepatocyte transplantation with temporary improvement in Phe levels[74].

DILEMMAS AND CONTROVERSIES

The decision for transplantation in LBMDs remains a complex one. Whilst the distinctions between each group of LBMDs is relatively arbitrary and may overlap, the indication for LT is one that must be carefully considered.

In a disease process such as biliary atresia, the risk-benefit decision for LT may be relatively simple. In a child with failed portoenterostomy, with progressive liver disease and poor survival beyond 36 mo of life, LT offers long-term survival of over 80% in biliary atresia patients[75]. Therefore the risk/benefit decision is based on quantitative improved survival outcomes.

Indications for LT for LBMDs however, are based on a different set of paradigms. Some LBMDs result in progressive liver disease, leading to cirrhosis and liver failure, therefore making LT a life-saving procedure, whilst some LBMDs do not cause liver injury, but the toxic intermediary metabolites have significant extra-hepatic effects.

LT remains the mainstay of treatment for LBMDs causing life-threatening illness such as the neonatal form of the UCD OTC deficiency, primary hyperoxaluria and CNS type 1[6,76,77]. The enzymatic defects in these conditions are well documented and present with severe clinical phenotypes manifesting in life-threatening complications. LT offers a replacement for the hepatic enzymes, therefore providing a life-saving metabolic cure.

With improvement in the outcomes and reduced risks associated with LT, LT has become an attractive treatment strategy for a significant number of other LBMDs with a considerably more complicated phenotype and risk/benefit profile. The utility of LT as a life improving *vs* life saving treatment modality raises a number of important questions. This paradigm shift of improving quality of life as opposed to saving lives has dramatically changed the plethora of diseases for which LT may be considered appropriate therapy. The blurring of lines between standard medical therapy and more aggressive surgical intervention, increasingly poses complex decisions for the transplant community[78].

Furthermore, LBMDs are relatively rare, and a detailed understanding in to the natural progression/history is still lacking. There is also a diverse genotype and phenotype correlation for many of these rare disorders. The risk/benefit consideration is made even more complicated for a given individual as the inherent risks of a condition are not always well-defined.

SURGICAL CONSIDERATIONS

As more children receive transplants for LBMDs, organ allocation is an important consideration. In the United States, the Pediatric end-stage liver disease score and Model for End Stage Liver Disease score are used to prioritize candidates for LT. These scoring systems are centered mainly on worsening biochemical parameters which progress with advancing liver failure. In many LBMDs, there is typically no evidence of progressive liver disease and as such predicting risk of which candidate is most likely to benefit for LT can be challenging. As we expand the indication for LT for metabolic conditions, the issue of organ allocation must also be addressed.

The issue of scarcity of donor organs has led to optimization of the available grafts through various surgical techniques such as reduction of an adult donor graft in children, particularly through split liver grafts[79], auxiliary transplantation and the use of heterozygous donors.

Auxiliary transplantation[80]–where the whole or partial left lobe of a living or deceased donor is transplanted in an orthotopic site whilst preserving the right lobe of the recipient[81] is increasingly being used (Figure 2). Whilst technically challenging, advantages are two fold; (1) It allows the native liver to continue functioning normally, aside from the enzymatic defect, serving as a safety net should the graft fail; and (2) It may serve as a bridge to gene therapy, a new and novel developing area of metabolic medicine. Despite the initial discouraging results with higher mortality and morbidity, more recent studies[82,83] from experienced centers have shown comparable outcomes to whole LT and successful weaning of immunosuppression with native liver regeneration[84]. Study by Sze *et al*[85], showed that of the 96 paediatric LT patients with LBMDs, 14 (13%) children had auxiliary transplantation. Of these, 11 children had noncirrhotic LBMDs (CNS type 1, OTC, familial hypercholesterolism, proprionic acidemia). Long term patient and graft survival was not statistically different to standard orthotopic LT at 1 and 10-years post-transplantation[85]. Cautious selection of patients for auxiliary transplantation is vital as LBMDs that lead to cirrhosis or produce abnormal enzymes or proteins such as primary hyperoxaluria should not be treated with auxiliary transplantation as the underlying abnormality results in disease progression[86].

Living related living transplant using relatives as donors has emerged as a solution to the scarcity of donor organs. In Japan, where there are no deceased donors, living related donor LT for metabolic disorders is a key option[87]. As described above, most metabolic disorders have an autosomal recessive inheritance pattern. Parents, who are obligate carriers of the recipient's disorders, become potentially heterozygous donors. Kasahara *et al*[20], conducted an extensive review from a Japanese multicenter registry of living related LT[20]. Among the patients transplanted for metabolic conditions, 95% of donors were parents who were carriers of the recipients' disorders. Indications for transplantation were WD in 30%, UCD in 29%, MMA in 10% and GSD 7.7%[88]. The outcome reported after using heterozygous donors was excellent with better long-term survival rate, especially in WD and UCD. Other studies have also demonstrated the safety of heterozygous donors for LT in LBMDs with excellent metabolic correction [89,90].

As previously discussed, LT for organic acidurias is not curative, but may improve quality of life. Combined liver and kidney transplantation can be considered in patients with MMA and PA with frequent metabolic decompensation episodes in spite of rigorous medical therapy, based on highly individualized criteria[47]. The experience with combined liver and kidney transplantation in this cohort of patients remains limited. In MMA patients specifically, it has become an effective treatment modality with favorable graft survival and short-term outcomes, and good survival rates[45,91,92]. Combined liver and kidney transplantation does not cure the disease, but leads to partial correction of the metabolic derangement and improvement in clinical features. Medical therapy is generally continued, although less stringent than pre-transplantation, in order to lower the risk of renal and neurological worsening [47]. Choice of immunosuppressive therapy that is renal-sparing is encouraged and neurological side effects from medication need to be carefully monitored[93].

OUTCOMES AND PROGNOSIS

With LBMDs constituting roughly 15%-25% of LT in the pediatric population, it is important to consider the outcomes of these children. Single and multicenter studies have suggested that their outcomes are comparable if not better than those transplanted for decompensated cirrhosis or other forms of chronic liver disease with excellent survival rate of > 82% at 10 years[85,94] (Figure 3) (Graph data from King's College Hospital, 2009).

Some studies, however, have shown that chronic rejection is a common problem in LT for LBMDs, often leading to re-transplantation[85,95]. Re-transplantation is associated with higher morbidity and mortality. Immunosuppression regimes are important in maintaining long-term allograft health, but may also contribute to potentially serious complications over time.

Optimization of immunosuppression can be challenging and is not standardized[85,96]. In children receiving LT for LBMDs, the optimal use of immunosuppressive agents is to achieve a balance between minimizing risks of allograft rejection and secondary toxicity[97]. Renal impairment specifically is frequently seen in these children. Thus, choosing an immunosuppressive agent with minimal nephrotoxic potential is important. The use of basiliximab, a chimeric anti-IL2 receptor antibody,

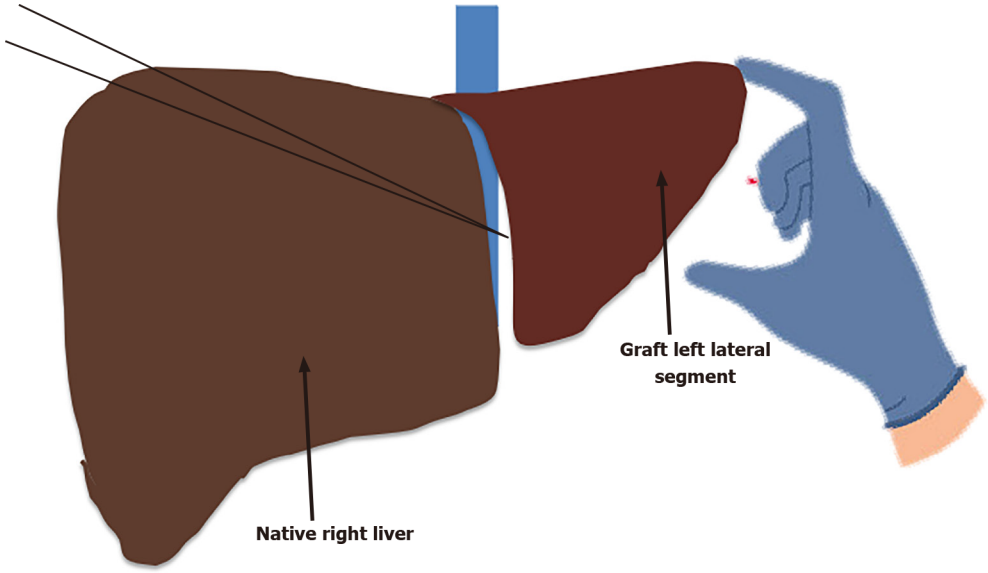

Figure 2 Auxiliary liver transplantation for select liver based metabolic disorders.

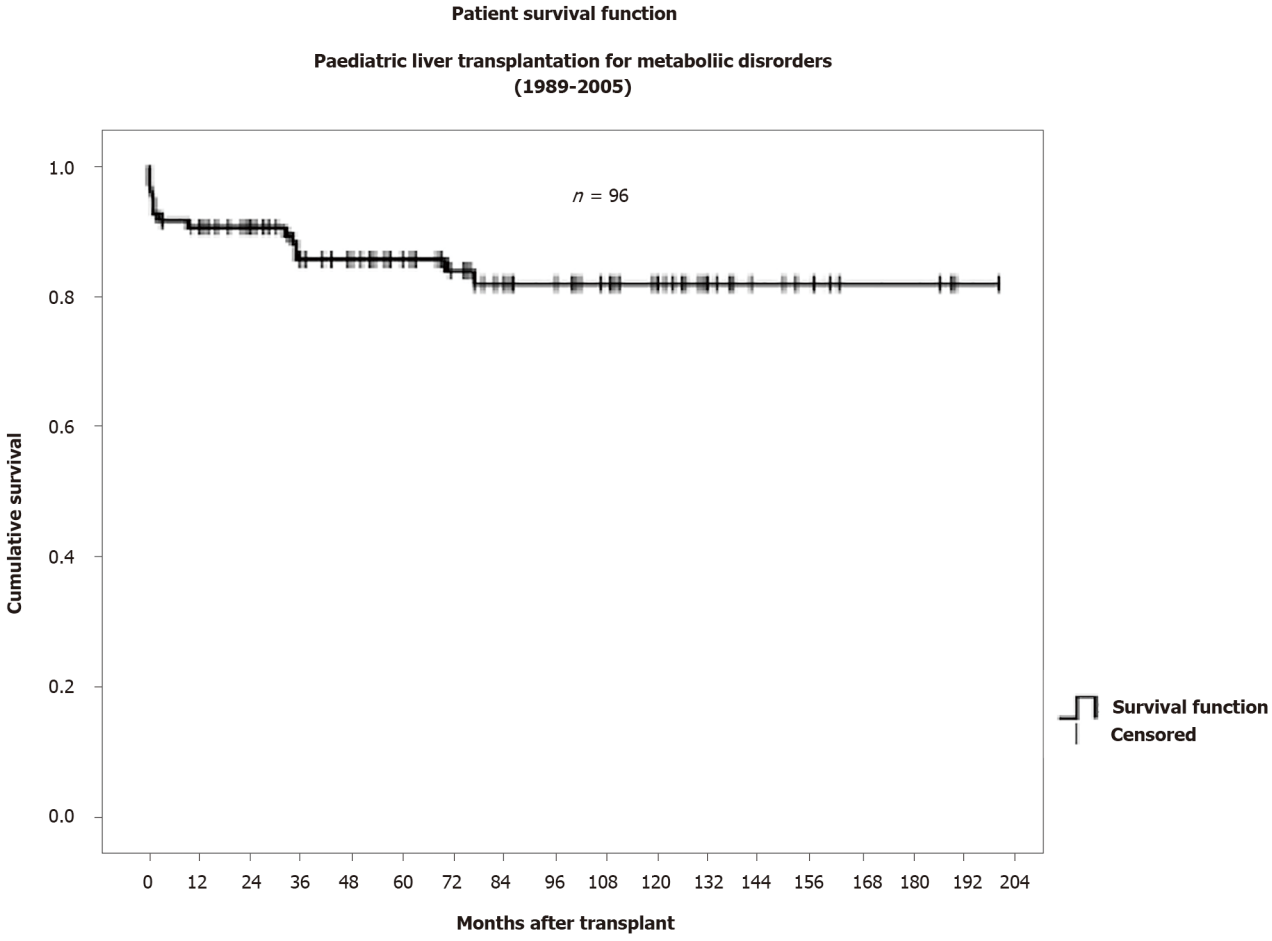

Figure 3 Patient survival for pediatric liver transplantation for metabolic disorders (data from King's College Hospital, London).

has been shown to be an effective renal-sparing agent with delayed entry and lower early target trough levels of calcineurin inhibitors (CNI) in children with renal impairment[98]. Mycophenolate mofetil (MMF) is also a CNI sparing agent, useful in children with CNI toxicity. Induction with monoclonal antibodies such as Basilixumab as an induction, followed by the use of MMF may be a helpful renal-sparing strategy in children with renal dysfunction.

The overall prognosis for children receiving transplantation for LBMDs must account for both allograft and extrahepatic complications. Meaningful survival in all pediatric LT recipients should be a state of complete physical, mental and social wellbeing[99]. Long-term management of children transplanted for LBMDs must include aspects such as growth and nutrition, neurological outcomes and psychosocial well-being.

CONCLUSION

Pediatric LT has come leaps and bounds in the treatment of children with LBMDs. Where it has been previously viewed only as life-saving for some LBMDs, there is good reason to consider a shift in the utility of LT beyond metabolic rescue. It remains the gold standard for children with end stage liver disease. The success rate in most LBMDs is promising but the clinician plays a vital role in determining which patients are most suited for LT. The care pre- and post-transplantation is especially important. Pre-transplantation, identifying the most appropriate candidate for transplant will involve assessment of the severity of the primary disease, neurological status, and comorbidities which may affect transplant survival and ensuring that all alternative treatment modalities have be explored. It is important to remember that good metabolic control including ongoing dietary management and medical therapy supplements often results in better post-transplantation outcomes. A multidisciplinary network of professionals is key in the management of these children post LT, to ensure all aspects including growth and development, psychosocial well-being and nutrition are considered.

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MINIREVIEWS

Liver and COVID-19: From care of patients with liver diseases to liver injury

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Abstract

The global pandemic of coronavirus disease 2019 (COVID-19) changed dramatically all priorities on medical society and created several challenges for clinicians caring for patients with liver diseases. We performed a comprehensive review about how COVID-19 can affect the liver, the influence of liver diseases on the risk of developing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and COVID-19 severity and also some strategies to overcome all the challenges clinicians have to face in the management of patients with liver diseases in a period of time when all the focus turned on COVID-19. We analyze the relationship between COVID-19 and non-alcoholic fatty liver disease, alcoholic liver disease, viral hepatitis, autoimmune liver disease, cirrhosis, hepatocellular carcinoma and liver transplantation, as well as the approach to SARS-CoV-2 vaccination.

Key Words: COVID-19; Liver diseases; Vaccination; SARS-CoV-2

Core Tip: Coronavirus disease 2019 (COVID-19) has become a major health problem worldwide in the last few months, affecting the health system dramatically. Apart from the respiratory system, associated liver injury is one of the main concerns in severe acute respiratory syndrome coronavirus 2 infection and several mechanisms could explain liver abnormalities. In this mini-review, and different from other papers, we not only analyze liver injury by COVID-19, the effect of COVID-19 in liver diseases, its pathophysiology and strategies to keep an adequate care of liver patients, but also

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highlight the potential higher risk of severe disease or risk of infection in patients with different etiologies of liver disease. We also analyze the recent recommendations and prioritization regarding vaccination in patients with liver disease.

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INTRODUCTION

Coronaviruses are single-stranded RNA viruses that mainly cause upper respiratory tract infections in humans. Two coronaviruses were previously described, severe acute respiratory syndrome coronavirus (SARS-CoV), causing an epidemic in 2003, and middle eastern respiratory syndrome coronavirus (MERS-CoV), causing an epidemic in 2012[1].

The new SARS-CoV-2 is responsible for one of the most important and devastating pandemic in the human history - the first case of severe pneumonia caused by SARS-CoV-2 was described on 3rd January 2020 in Wuhan, China, the first epicenter of the disease[2]. Since then, SARS-CoV-2 have widespread across the world, causing a global pandemic - in the beginning of May 2021, World Health Organization reported more than 150000000 infected patients and more than 3000000 deaths[3].

Coronavirus disease 2019 (COVID-19) has a variety of clinical presentations, with the majority of patients remaining asymptomatic or with mild symptoms, such as cough, anosmia, fatigue, diarrhea, headache or fever. However, 10%-15% will present acute hypoxemia or respiratory distress syndrome that might progress to multi-organ failure and death[4-7].

The respiratory tract is the main target of SARS-CoV-2 but several reports revealed a systemic involvement of the disease, including liver and the gastrointestinal tract[8].

In this review, we will highlight the relationship between COVID-19 and the liver.

LIVER INJURY IN COVID-19

It is well established that the respiratory tract is involved in the majority cases of SARS-CoV-2 infections but several studies reported COVID-19 associated liver injury, defined as liver damage during disease progression or treatment[9].

Elevated serum liver biochemistries in patients with COVID-19 was first described by Chen *et al*[10] in Wuhan where 43.9% of patients had elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Overall, the incidence of liver injury ranged from 14.8% to 78% and the most common changes are mild elevations of AST and/or ALT (mainly within 3 times the upper limit of normal)[11-13]. The wide range of incidence could be explained by the different cut-off values of upper limit of normal and geographical variability in prevalence and type of underlying chronic liver disease[7,14].

It was also described a possible relationship between liver injury and severity of the disease: Abnormalities in liver function were significantly higher in critically ill patients and associated with poorer outcome. One large Chinese study showed that 18% of non-severe COVID-19 patients had elevated ALT *vs* 56% in the group of severe COVID-19[1,15,16].

Liver biopsies in COVID-19 patients did not show any typical pattern of hepatic lesions and liver injury is probably associated with multiple mechanisms (Table 1)[1,7,9,17-26]: (1) Direct cytotoxicity by active replication of SARS-CoV-2 in hepatic cells due to abundance of its receptor in cholangiocytes - however, the major COVID-19 induced liver function abnormalities are in aminotransferases that might be explained by others factors such as mitochondrial dysfunction, SARS-CoV-2 induced hepatic steatosis, transaminase release due to breakdown of skeletal and cardiac muscle and venous and arterial thromboses; (2) Hyper-inflammatory reaction to COVID-19: Substantial elevations in serum ALT are usually associated with high levels of C reactive protein, D-dimer, ferritin and interleucin-6 and result from the development of the cytokine

Table 1 Mechanisms of coronavirus disease 2019 liver injury

Mechanisms	Pathophysiology
Direct cytotoxicity	Active replication of SARS-CoV-2 in hepatic cells
Hyper-inflammatory reaction	Cytokine storm and activation of immune system
Systemic hypoxia	COVID-19 cardiomyopathy
Drug-induced liver injury	Liver toxicity to medication used to treat COVID-19

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019.

storm and activation of the innate and adaptive immune system; (3) Systemic hypoxia and hepatic congestion related to cardiomyopathy (hypoxia hepatitis is frequent in the severe cases); and (4) Drug-induced liver injury: Mainly with lopinavir-ritonavir, tocilizumab and remdesivir.

COVID-19 AND LIVER DISEASES

The presence of previous liver disease could influence the prognosis of COVID-19 and SARS-CoV-2 could also pose some difficult challenges in care of liver diseases' patients (Table 2).

Non-alcoholic fatty liver disease and metabolic associated fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is one of the most common etiologies of liver disease in the world and the most emerging cause in developed countries, being expected to become the leading cause of liver transplantation worldwide[27,28]. Recently, a new concept has merged, metabolic associated fatty liver disease (MAFLD), diagnosed in the presence of hepatic steatosis and any of the following metabolic conditions: Diabetes mellitus, obesity/overweight or evidence of metabolic dysregulation in lean patients[29].

Several studies investigated the possible relationship between NAFLD/MAFLD and the outcome of COVID-19. Ji *et al*[30] reported liver abnormalities in 50% at admission of COVID-19 and NAFLD patients and in 75% during hospitalization and NAFLD was an independent risk factor for COVID-19 progression[27]. Another study, a meta-analysis by Pan *et al*[31], showed that NAFLD increased the risk of disease progression among patients with COVID-19.

NAFLD patients may also suffer from comorbidities known to be important risk factors for severity of COVID-19 and that could negatively influence prognosis, such as hypertension, obesity or diabetes[27]. However, Zhou *et al*[32] established a synergic effect of NAFLD for severe COVID-19 in patients less than 60 years-old and independent of other comorbidities, showing that NAFLD alone could be an important prognostic factor. This might be explained by metabolically active fat, which is associated with[17,33]: (1) Chronic inflammatory changes and higher cytokine levels, making NAFLD patients more vulnerable to cytokine storm in COVID-19; and (2) Imbalance in host inflammatory and tolerance response to SARS-CoV-2. On the other hand, it was also demonstrated that COVID-19 patients exhibited higher levels of monocyte chemoattractant protein-1 that is associated with steatohepatitis exacerbation, increasing the risk of NAFLD progression[34].

Therefore, it is of paramount importance to carefully follow NAFLD and COVID-19 patients due to the higher risk of poorer outcomes in both diseases.

Alcoholic liver disease: Alcoholic liver disease is one of the main causes of liver disease and its patients were considered one of the most affected groups during the pandemic as they present[35-37]: (1) Higher risk of developing SARS-CoV-2 infection due to reduced immunity to bacterial and viral infection (due to heavy alcohol consumption) and also willingness to adopt prevention measures; (2) Worse COVID-19 outcomes with a study reporting to be the only liver disease with a significant odds ratio for death; and (3) Higher alcohol consumption during the time of social isolation, increasing the risk of decompensation.

Strategies to overcome all these difficulties should be implemented and include social and psychological support (locally or *via* telemedicine), educational sessions to deal with the risk of COVID-19 as well as regular appointments with hepatologists.

Table 2 Influence of liver diseases in risk of infection or outcome of coronavirus disease 2019

Higher risk of infection or severe outcome of COVID-19	Apparently non-higher risk of infection or severe outcome of COVID-19
Non-alcoholic fatty liver disease	Hemochromatosis
Alcoholic liver disease	Wilson's disease
Alpha-1 antitrypsin deficiency	Autoimmune liver disease
Cirrhosis	Hepatitis B infection
Hepatocellular carcinoma	

COVID-19: Coronavirus disease 2019.

A debatable question is the use of corticosteroids in alcoholic hepatitis: There are some recommendations suggesting to avoid steroids in this situation as it may delay viral clearance but benefits must be weighed against risks and there are some reports showing that prednisolone might be an effective and safe treatment in patients with SARS-CoV-2 infection and alcoholic hepatitis[38,39].

Other metabolic liver diseases: There is no data on the risk of infection and severity of COVID-19 in patients with hemochromatosis and Wilson's disease. It is always important to search for iron overload in patients with SARS-CoV-2 and abnormal liver tests as elevated ferritin levels could be associated to viral infection and mask an underlying hemochromatosis[40].

Alpha-1 antitrypsin might inhibit infection by SARS-CoV-2, has anticoagulation effects and protect against inflammation[41]. Therefore, patients with alpha-1 antitrypsin deficiency seems to have increased risk of infection and COVID-19 severity, mainly Pi*ZZ and/or low alpha-1 antitrypsin levels.

Autoimmune liver diseases: Autoimmune liver diseases are a group of diseases that include autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC).

The management of autoimmune liver diseases was one of the main concerns of hepatologists during COVID-19 pandemic due to the use of immunosuppressive therapy. Previous reports with other coronaviruses (SARS-CoV or MERS-CoV) did not show worse outcomes in patients who were undergoing transplantation, chemotherapy or other immunosuppressive treatments and there was also some evidence that imunossupressive therapy might have a protective effect against severe COVID-19[42,43]. Therefore, the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) were against stopping immunosupressive therapy as it may lead to disease flares that will need high doses of steroids, which will increase the susceptibility for SARS-CoV-2 infection. More recently, a multicenter study evaluated the outcomes of COVID-19 in patients with AIH and showed that the overall outcome of SARS-CoV-2 disease was favorable in patients without cirrhosis and that ongoing immunosuppression was not associated with increased risk of severe COVID-19[44]. Efe *et al*[44] also described that the risk of AIH relapse may be related with hyperstimulation of the immune system by COVID-19[45]. There is scarce information about COVID-19 and PBC or PSC - an Italian study found an incidence of SARS-CoV-2 infection of 5.6% in AIH patients but only 1.5% in PBC patients - the higher incidence in AIH might be related with the use of immunosuppressive therapy (not used in PBC)[46]. Another important finding, also described in other autoimmune and inflammatory conditions, is the development of new-onset PBC after COVID-19, where SARS-CoV-2 triggered the development of PBC in a genetically predisposed individual[45,47,48].

Viral hepatitis: COVID-19 did not seem to influence the course of hepatitis C virus (HCV) or hepatitis B virus (HBV) infection. A meta-analysis by Mantovani *et al*[49] reported an incidence in COVID-19 patients of only less than 0.1% HCV infection and 0.1% of HBV infection. In fact, the major effect of COVID-19 is the impact in HCV elimination efforts. A Spanish study showed that the interruption of HCV screening, linkage to care and harm reduction programs, would substantially decrease HCV diagnosis and treatment, consequently, increase the number of HCV liver-related deaths, hepatocellular carcinoma (HCC) and decompensated cirrhosis[50]. Thus, it is of paramount importance to keep HCV elimination a major health priority through

innovative programs as telehealth or home-delivery HCV drugs[50].

Akin to HBV infection, one of the largest cohorts of patients with COVID-19 and past or current HBV infection did not show an association with acute liver injury. Patients that fulfill the criteria for HBV treatment or under corticosteroid therapy should receive antiviral treatment but it may not be necessary in all patients with COVID-19 and current or past HBV infection[51]. A study by Liu *et al*[52] also reported that HBV infection did not predispose COVID-19 patients to more severe outcomes. There is also a report of COVID-19 accompanied by HBV infection causing a fulminant hepatitis[53].

Cirrhosis: Cirrhosis is one of the major causes of morbidity and mortality in the entire world and the second leading cause of digestive disease mortality[54].

Patients with cirrhosis have multiple mechanisms of immune dysfunction and are more susceptible to infection, not only to severe bacterial infection but also to viral and fungal-related disease[7,22,55]. However, data about risk of COVID-19 in this population is controversial, with Richardson *et al*[56] not suggesting a higher prevalence of cirrhotic patients in COVID-19 population while Kushner *et al*[57] reporting higher risk of infection, severity of the disease and hepatic decompensation. In cirrhotic patients, there is also a relationship between severity of liver disease and SARS-CoV-2 morbidity and mortality, with Child-Pugh C patients presenting higher frequency of Intensive Care Unit admission, renal replacement therapy and mortality [58].

Bajaj *et al*[59] showed that cirrhotic patients hospitalized with COVID-19 had similar mortality rates of patients admitted with cirrhosis alone but higher than patients with COVID-19 alone. An Italian study also demonstrated that cirrhotic patients that develop COVID-19 present a worse prognosis due to respiratory complications but also worsening of liver function leading to end-stage liver disease. They also found that the 30-d mortality in non-cirrhotic patients was significantly lower[60].

A very interesting finding in a multicenter cohort is that COVID-19 is associated with hepatic decompensation and, in this study, 24.3% had no respiratory symptoms at the time of diagnosis[4]. As so, testing to SARS-CoV-2 infection is advisable in patients with hepatic decompensation and early admission should be considered due to high rates of mortality.

SARS-CoV-2 infection can also cause acute-on-chronic liver failure characterized by hepatic decompensation events, extrahepatic organ failure and high rates of mortality.

EASL and World Gastroenterology Organization recommend that care should be maintained as this fragile population have a very high risk of decompensation. Prophylaxis of spontaneous bacterial peritonitis and encephalopathy, therapeutic paracentesis and variceal banding in high risk patients should be always performed in a COVID-19 free environment and following all the protective measures, as this will reduce the risk of further decompensation and hospitalization[37,61].

Cancer and hepatocellular carcinoma: Patients with COVID-19 and cancer are at increased risk of infection and worse outcomes[62]. A nationwide Chinese study that included 1590 patients (18% with history of cancer) reported higher risk of adverse events in patients with active or past history of cancer. This might be explained as cancer patients are more susceptible to infection (due to their systemic immunosuppressive state associated with malignancy but also with its treatment) and have increased risk of COVID-19 related serious events[63,64].

HCC is the sixth most commonly diagnosed and the fourth leading cause of cancer-related death in the world, being one of the major health challenges in liver clinic[65,66]. There is scarce information on the impact of COVID-19 in patients with HCC - in a small study, Zhang *et al*[67] reported poorer outcomes in patients with HCC but also with other malignancies when compared to the general population.

The major impact of COVID-19 on HCC is related to the delay on the proper management of HCC. A French multicenter study reported a significant decrease in the rate of HCC patients referred for first diagnosis or treatment[68]. Several interpretations could be made but may be related to the increase delay of referral by other professionals, patients' fear to search for healthcare services, delay in the Hepatology appointments and limited assessment to diagnostic and therapeutic tools. They also found a higher rate of treatment delay longer than one month when compared 2019 to 2020[68].

Currently, AASLD and EASL recommend to continue HCC surveillance and treatment with an acceptable delay of a maximum of two months to reduce the number of patients presenting with HCC not amenable to curative treatment[43,69]. Whenever possible, telemedicine could replace clinic visits and multidisciplinary team

meetings, and all diagnostic and therapeutic procedures should be performed according to the COVID-19 prophylactic measures to avoid nosocomial spread on infection[60].

The real effect of COVID-19 on HCC management is still undetermined and only the middle-term follow-up will clarify the pandemic impact on HCC morbidity and mortality.

Liver transplantation: The risk and severity of COVID-19 in liver transplant patients is still unclear[70]. A multinational cohort reported a similar risk to the general population of contracting infection with SARS-CoV-2[71]. The proportion of liver transplant recipients hospitalized with COVID-19 was 82% and 19% died and advanced age, presence of non-liver cancer and elevated baseline creatinine were associated with higher mortality rates, while the type of immunosuppression and time since transplantation were not associated[71]. However, the European Liver and Intestine Transplantation Association established a registry and suggested that longer time of transplantation might have higher rates of mortality[72].

Liver transplantation programmes were heavily affected by COVID-19 pandemic by several reasons: Limited access to intensive care unit (ICU) due to the number of COVID-19 patients needing ventilation support, reduced number of organs because all major guidelines recommended against using organs from donors with SARS-CoV-2 infection and also limited access of patients to liver transplant centers[58].

It is crucial to maintain liver transplant programs to reduce liver diseases mortality, facing all the new challenges through innovative tools, in which telemedicine might play a key role.

The postoperative period is also a challenge and should follow a SARS-CoV-2 free pathway, with proper free-SARS-CoV-2 ICU to ensure high transplant success rates and preventing nosocomial infection[5]. In the perioperative period, patients' follow-up should be preferably through telemedicine and, in case of symptoms, the threshold for testing for SARS-CoV-2 infection should be low[5]. In case of COVID-19, patients should always present to the hospital for medical evaluation[5].

Regarding immunosuppression after liver transplants, all liver associations recommend to maintain medication as there is no data suggesting a higher risk of COVID-19 severity, while stopping will increase the risk of graft rejection[43,61,73]. However, in case of COVID-19, immunosuppression should be reduced, particularly antimetabolite dosages[43].

Vaccination: The development of SARS-CoV-2 vaccine is one of the major advances to mitigate all the health and economic issues. This development started in January 2020 and progressed very rapidly, being now available more than 5 vaccines. The process of vaccination is moving forward worldwide in order to achieve herd immunity as soon as possible.

Despite some concerns about vaccines' adverse events, the safety profile is excellent and, based on current knowledge, there is no contra-indication for vaccination of liver disease patients, as the potential benefits are higher than the risks[74]. However, there is a report of auto-immune hepatitis developing post-COVID-19 vaccination[75].

Vaccination should also be prioritized in[74]: (1) Cirrhotic patients or with liver decompensation; (2) Hepatobiliary malignancies patients; (3) Chronic liver disease patients and risk factors for severe COVID-19; (4) Liver transplant recipients (prior to liver transplant whenever possible or 3-6 mo after transplantation); and (5) Healthcare professionals caring for these patients.

CONCLUSION

Liver abnormalities in COVID-19 patients are common and may result from direct cytotoxicity, hyper-inflammatory status or DILI. In addition, a direct relationship between grade of liver injury and severity of the disease was also established.

The existence of previous liver disease could influence the prognosis, with patients with NAFLD, cirrhosis and HCC presenting higher risk of severe COVID-19 and death (Table 2). In this population, vaccination should be considered a priority. On the other hand, the focus on SARS-CoV-2 infection lead to reduced access to care for patients with liver disease that must be reestablished to improve the outcome of these diseases.

In conclusion, the consequences of COVID-19 on liver ranges from its direct liver injury to the profound negative effect on liver disease patients' care which might increase liver disease burden and negatively influence prognosis.

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ORIGINAL ARTICLE

Basic Study

Direct modulation of hepatocyte hepcidin signaling by iron

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Abstract

BACKGROUND

Liver-secreted hepcidin is the systemic master switch of iron homeostasis and decreased levels of hepcidin are considered to cause iron overload not only in hereditary hemochromatosis but also in hemolytic anemia and chronic liver diseases. The regulation of hepcidin is complex and its response to iron is still not completely understood.

AIM

To study the direct effect of iron on various established hepcidin signaling pathways in hepatoma cells or primary hepatocytes.

METHODS

Hepcidin mRNA expression was studied by quantitative real-time (qRT)-PCR in the presence of various forms of iron including ferric ammonium citrate (FAC) in hepatoma cells (Huh7), murine primary hepatocytes and an established co-culture model of phorbol myristate acetate-differentiated THP-1 monocytes and Huh7 cells. To analyze hepcidin signaling, the response to bone morphogenetic protein 6 (BMP6), interleukin (IL)-6, IL-1β, hypoxia and lipopolysaccharide (LPS) were studied. Hepcidin and small mothers against decapentaplegic 6 (SMAD6) mRNA levels were assessed by qRT-PCR and the expression of phosphorylated signal transducer and activator of transcription 3 (phospho-STAT3), STAT3, phospho-SMAD1/5/8 and SMAD1 proteins were analyzed by western blot.

RESULTS

All iron III forms including FAC efficiently blocked hepcidin mRNA expression at non-toxic dosages in Huh7 cells or primary hepatocytes in a time and dose-

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dependent manner ($P < 0.001$; $P < 0.05$). Hepcidin blockage could be efficiently blunted by iron chelators salicylaldehyde isonicotinoyl hydrazone (SIH) and Desferal ($P < 0.001$). FAC also inhibited BMP6, hypoxia, IL-1β and IL-6-mediated hepcidin induction ($P < 0.001$; $P < 0.001$; $P < 0.05$; $P < 0.001$), and FAC also inhibited LPS-mediated hepatic hepcidin induction in co-culture model ($P < 0.001$). Moreover, FAC reduced SMAD6 mRNA and p-SMAD1/5/8 protein expression at basal or upon stimulation by BMP6 ($P < 0.05$; $P < 0.01$), and FAC also reduced SMAD6 and p-SMAD1/5/8 expression under hypoxia ($P < 0.01$; $P < 0.05$). However, FAC has no significant effect on p-STAT3 protein expression at basal or upon stimulation by various stimuli. Notably, in the presence of the BMP/SMAD signaling pathway inhibitor LDN193189 Hydrochloride (LDN), FAC was unable to further decrease hepcidin, SMAD6 and p-SMAD1/5/8 expression compared with LDN alone.

CONCLUSION
Iron directly blocks hepatocellular hepcidin signaling through the BMP/SMAD pathway but independent of STAT3. This mechanism may contribute to continued iron overload in many pathophysiological conditions ultimately causing a vicious cycle of continued hepcidin suppression.

Key Words: Hepcidin/iron metabolism; Iron overload; Inflammation; Hypoxia; BMP/SMAD; STAT3

Core Tip: Hepcidin is paradoxically and strongly suppressed during hemolytic iron overload. Although various upstream regulators of hepcidin have been discovered, the direct iron sensing mechanisms by hepcidin remain obscure. This study investigated the direct effect of iron on hepcidin signaling and for the first time to show that iron directly blocks hepcidin transcription *via* bone morphogenetic protein/small mothers against decapentaplegic but not the STAT3 signaling in various established *in vitro* models of hepcidin signaling.

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INTRODUCTION

Excess iron causes cancer and severe tissue damage and chronic iron overload is not only driving the rather rare hereditary iron overload diseases but also secondary iron overload diseases due to hemolysis or common chronic liver diseases such as alcoholic liver disease or hepatitis C[1]. In most of these diseases, suppression of hepcidin, the systemic master switch of iron homeostasis in mammals, has been identified to play a key role. Hepcidin is primarily expressed in hepatocytes as a precursor pro-peptide and to a lesser extent in macrophages or cardiomyocytes[2-4]. It is regulated at the transcription side, and its mRNA levels correspond well with concentrations of the peptide[5]. By binding to and degrading the iron exporter ferroportin 1 (Fpn1) which is localized at the basolateral membranes of duodenal enterocytes, macrophages and hepatocytes[6], circulating hepcidin efficiently blocks iron absorption, iron recycling and iron storage[7,8]. Consequently, its overexpression leads to hypoferremia and anemia[9], while the reduction of hepcidin levels causes iron overload[10,11].

The regulation of hepcidin is complex and the direct mechanisms of iron sensing are still not completely understood. Bone morphogenetic protein 6 (BMP6) released from endothelial cells (ECs) can efficiently induce hepcidin transcription *via* the SMAD pathway[12]. BMP6 binds to the BMP receptor on the liver cell membrane and its co-receptor hemojuvelin to promote the phosphorylation of the receptor-associated proteins small mothers against decapentaplegic (SMAD) 1/5/8. The latter interacts

with SMAD4 to form the SMAD complex, translocates into the nucleus and binds to the hepcidin promoter[13]. In addition, inflammation mediators (*e.g.*, IL-6, IL-1β, hypoxia or ROS/H_2O_2) can also induce hepcidin transcription by promoting the phosphorylation of STAT3 to initiate STAT3-mediated hepcidin signaling[14]. Cytokines namely IL-6 and microbial molecules such as lipopolysaccharide (LPS) represent an important evolutionary conserved mechanism during infection/inflammation to strongly induce hepatic hepcidin secretion leading to a rapid decrease of serum iron, which is thought to function as anti-bacterial defense mechanism[15]. More recently, the central redox signaling molecule H_2O_2 has been also identified as a potent inducer of hepcidin[16] with hypoxia further enhancing hepcidin-expression *via* the STAT3 signaling pathway[17]. Further data suggest that intracellular oxidases such as NOX4 may play an important upstream role in controlling hepcidin *via* the STAT3 pathway[17].

C/EBPα, BMP6, SMAD 1, 5, 8 and 4, TMPRSS6, IL-6, CREBH, CHOP and TLR4), an overall and conclusive regulatory network regarding the control of iron is not yet fully understood. This includes the experimental and clinical finding that hepcidin responds differentially to iron overload *in vitro* and *in vivo*[18-20]. Although recent data suggest important intercellular crosstalks *e.g.*, between hepatocytes and endothelial cells or macrophages[14,21-23], the direct iron sensing mechanisms by hepcidin remain obscure. It has been reported that TfR1, ERFE or GDF15 overexpression contributes to iron overload by suppressing hepcidin *in vivo*[24-28]. However, there are examples that the seemingly paradox direct negative impact of iron on hepcidin, identified *in vitro*[19], may have direct clinical implications. For instance, in the most common human liver disease, alcoholic liver disease[29], hepatic iron overload is one of the key factors that drive the diseases and determine survival[30] with alcohol directly suppressing hepcidin[31]. In thalassemia, hepcidin is also strongly suppressed during hemolysis. While repetitive blood transfusions have been long thought to cause iron overload[32], a recently established thalassemia mouse model could demonstrate that hepatic iron overload occurs without additional blood supply through suppressed hepcidin levels[33].

These considerations prompted us to study the direct effect of iron in an *in vitro* setting on various established hepcidin signaling pathways including the BMP/SMAD signaling pathway and STAT3-mediated hepcidin signaling *via* cytokines, hypoxia, and LPS using a recently established macrophage-hepatocyte co-culture model[14]. Our data show that iron inhibits primarily the BMP/SMAD pathway but does not affect the STAT3 pathway. In conclusion, direct exposure of hepatocytes to pathophysiological iron deposits is a strong suppressor of BMP-mediated hepcidin signaling that could initiate a vicious cycle of continued hepcidin suppression.

MATERIALS AND METHODS

Cell culture

Huh7 cells from the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan) were grown under standard conditions using Dulbecco's modified Eagle medium (Sigma-Aldrich, Taufkirchen, Germany), 25 mmol/L glucose and 10% fetal calf serum under 210 mL/L O_2 (21% O_2) and 50 mL/L CO_2 (5% CO_2)[16]. Murine primary hepatocytes kindly provided by Dr. Sai Wang (University of Heidelberg, Germany) were grown under standard conditions using Williams' medium (Sigma-Aldrich, Taufkirchen, Germany), 10% fetal bovine serum, 1% P/S (Penicillin and Streptomycin), 1% L-Glutamine, 0.5% ITS (Insulin-Transferrin-Selenium), 0.1% Dexamethasone, and were seeded at a cell density of 2 x 10^5 cell/well in 12-well plates for experiment. The immortalized human monocyte THP-1 cells from the American Type Culture Collection (ATCC, Manassas, VA, United States) were grown in RPMI-1640 medium with 25 mmol/L glucose (Gibco, Thermo Fisher Scientific, Waltham, MA, United States) Supplementary Figureed with 10% fetal bovine serum. THP-1 cells were seeded in 12-well plates and treated with phorbol myristate acetate (PMA) at 100 ng/mL for 24 h to induce differentiation. After differentiation, cells were washed and incubated in fresh media for 24 h before experiment[14].

Chemicals and reagents

PMA, LPS, LDN, FAC, $FeCl_3$, FC, $FeSO_4$, Hemin, Desferal, human recombinant IL-6 were all purchased from Sigma-Aldrich. Ferrlecit (sodium ferric gluconate) was obtained from a commercial pharmacy in its retail packaging. Human recombinant IL-1β was purchased from Enzo Lifesciences (Lörrach, Germany) and human

recombinant BMP6 was purchased from R&D, Germany. SIH was a gift of Dr. P. Ponka (McGill University, Montreal, Canada).

Macrophage differentiation and co-culture

THP-1 monocytes were differentiated to macrophages and co-cultured as described recently[14]. Briefly, THP-1 cells were seeded for differentiation with PMA (100 ng/mL) at a density of 0.25 × 10^5 cells/well in 12-well plates. After 48 h of differentiation, Huh7 cells were seeded on the top of macrophages at a density of 0.7 × 10^5 cells/well and incubated overnight for attachment. The co-culture was conditioned to LPS (0.5 μg/mL) and/or FAC (50 μmol/L) under 21% O_2 and 5% CO_2 for 24 h. Aiming at studying the effects of macrophage-conditioned medium, differentiated THP-1 macrophages were conditioned to LPS and/or to FAC for 24 h. Huh7 cells were exposed to the macrophage-conditioned medium for 24 h. In the co-culture experiments, a pathophysiological hepatocytes-to-macrophages ratio of 4 to 1 was used as described previously[14].

Hypoxia experiments

Huh7 cells were seeded at a cell density of 0.7 x 10^5 cell/well in 12-well plates. Huh7 cells were treated with or without FAC. Hypoxia was induced as described recently using a hypoxia chamber[14]. Briefly, cell culture plates were placed in the hypoxia chamber and flushed with a gas mixture of 1% O_2, 5% CO_2 and 940 mL/L N_2 (94% N_2) for 3 min and incubated at 37 °C for 24 h[16].

RNA isolation, cDNA synthesis and quantitative real-time PCR analysis

Total RNA was isolated with Trifast (Peqlab biotechnology GmbH, Erlangen, Germany) according to the manufacturer specifications. Reverse transcription and quantitative real-time PCR (qRT-PCR) reactions were performed as previously described[16]. Primers and probes were designed using the Probefinder software (Roche, Mannheim, Germany) and the sequences are shown in Table 1. Primarily, levels of hepcidin mRNA were assessed since they correspond well to the levels of the propeptide. The levels of secreted peptide are only used in clinical studies where liver biopsies are not available[5].

Immunoblotting

Cells were washed in ice-cold 1xPBS and harvested in RIPA buffer plus 1 × Complete® protease inhibitor with EDTA (Roche Applied Sciences, Penzberg, Germany) on ice. Western Blotting was performed as described previously[16]. Following the transfer, the proteins immobilized on nitrocellulose membranes were incubated overnight with the antibodies anti-pSTAT3, anti-STAT3 (1:1000 dilution; Cell Signaling Technology, Frankfurt am Main, Germany); anti-pSMAD1/5/8, anti-SMAD1 (1:1000 dilution; Cell Signaling Technology, Frankfurt am Main, Germany) or anti-GAPDH (1:2000 dilution; Cell Signaling Technology, Frankfurt am Main, Germany). After incubation with the IRDye-conjugated 680 anti-mouse or 800 anti-rabbit antibodies (1:10000 dilutions; LI-COR, Inc., Lincoln, NE, United States), the membranes were scanned using an infrared imaging system (Odyssey CLx; LI-COR, Inc., Lincoln, NE, United States).

Statistical analysis

All the data were expressed as mean ± SD. Significant differences ($P < 0.05$) between means of data sets were assessed by one-way ANOVA with Tukey's test or two-way ANOVA with Sidak's test using GraphPad Prism 6 software.

RESULTS

Efficient suppression of hepatocellular hepcidin by higher iron levels

Although iron injection *in vivo* causes strong induction of hepcidin[34,35], direct exposure of isolated hepatoma cells or murine primary hepatocytes to various forms of iron causes an efficient suppression of hepcidin mRNA expression (Figure 1A and B; $P < 0.001$ and $P < 0.05$ *vs* control). The inhibiting effect of iron was observed over a wide concentration range (Supplementary Figure 1) and could be efficiently blocked by two iron chelators (SIH and Desferal) (Figure 1C; $P < 0.001$ *vs* FAC group). While this "paradox" response towards iron may be explained by the absence of co-factors or other neighboring cells *in vitro*, the direct inhibition of hepcidin by iron may have important pathophysiological implications for hepatic iron overload in the context of

Table 1 Primer list of the genes analyzed by quantitative real-time polymerase chain reaction

Gene	Primer sequence
human β2-mg	forward: 5′-tga ctt tgt cac agc cca aga ta-3′
	reverse: 5′-aat cca aat gcg gca tct tc-3′
	probe: FAM-tga tgc tgc tta cat gtc tcg atc cca-TAM
human GAPDH	forward: 5′-gaa ggt gaa ggt cgg agt-3′
	reverse: 5′-gaa gat ggt gat ggg att tc-3′
	probe: FAM-caa gct tcc cgt tct cag cc-TAM
human hepcidin	forward 5′-cag gac aga gct gga gcc a-3′
	reverse: 5′-gca gca cat ccc aca ctt tg-3′
	probe: FAM-ctg ctg cgg ctg ctg tca tcg a-TAM
human SMAD6	forward: 5′-tgc aac ccc tac cac ttc a-3′
	reverse: 5′-cga gga gac agc cga gag t-3′
	probe UPL # 10 (Roche)
mouse HPRT	forward: 5′-ggt cca ttc cta tga ctg tag att tt-3′
	reverse: 5′-caa tca aga cgt tct ttc cag tt-3′
	probe UPL # 22 (Roche)

chronic liver diseases or due to hemolysis. We further demonstrate that the suppression of hepcidin mRNA expression is not due to toxic or subtoxic effects as even five times higher FAC concentration did not affect growth or cell division (see Supplementary Figure 2A). Moreover, a significant suppression of hepcidin mRNA expression by FAC was observed at 6 h and continued over the observed time interval of 24 h (Supplementary Figure 2B; $P < 0.001$ *vs* control). In summary, *in vitro* exposure of hepatocytes to high levels of iron suppresses hepcidin, which may have important pathophysiological implications by initiating a vicious iron overloading cycle. Further experiments were carried out with FAC as a standard model for iron exposure.

Iron efficiently blocks BMP6 to induce hepatocellular hepcidin

We next studied the influence of iron (FAC) on BMP6-mediated hepcidin signaling, one of the major pathways in basal and iron-responsive expression of hepcidin. As shown in Figure 2A, recombinant BMP6 efficiently increased hepcidin mRNA levels by almost four times ($P < 0.001$ *vs* control). However, the presence of iron FAC not only blocked basal hepcidin expression under control conditions but completely inhibited BMP6-mediated hepcidin induction (Figure 2A; $P < 0.001$ *vs* BMP6 group). In fact, even in the presence of BMP6, FAC inhibited hepcidin mRNA levels by ca. 50% (Figure 2A; $P < 0.05$ *vs* control). Notably, BMP6 was unable to induce SMAD6 mRNA and p-SMAD1/5/8 protein expression under FAC conditions (Figure 2B, C and D; $P < 0.01$ *vs* BMP6 group), while no effect on p-STAT3 protein expression was seen (Figure 2E and F). In conclusion, *in vitro*, external iron has a profound inhibitory effect of basal hepcidin expression and completely abolished BMP6-mediated hepcidin signaling through SMAD but not the STAT3 pathway.

FAC inhibits hypoxia-mediated hepcidin induction in a STAT3-independent manner

Recently, hypoxia and hydrogen peroxide have been identified as important modulators of hepcidin expression predominantly through the STAT3 pathway and involving oxidase enzymes of the NOX family[16,17]. To avoid direct interactions between iron and *e.g.*, peroxide, we therefore next focused on hypoxia to study the role of FAC in a STAT3-mediated hepcidin signaling. In confirmation of previous experiments[14], Figure 3A demonstrates that hypoxia is able to significantly increase hepcidin mRNA levels ($P < 0.05$ *vs* normoxia control). However, hypoxia was unable to induce hepcidin mRNA expression under FAC conditions (Figure 3A; $P < 0.01$ *vs* normoxia control and $P < 0.001$ *vs* hypoxia control). Expectedly, hypoxia did not have any significant effect on SMAD6 mRNA and p-SMAD1/5/8 protein expression (Figure 3B, C and D), but efficiently upregulated p-STAT3 protein expression as shown previously (Figure 3E and F; $P < 0.05$ *vs* normoxia control). In contrast, FAC

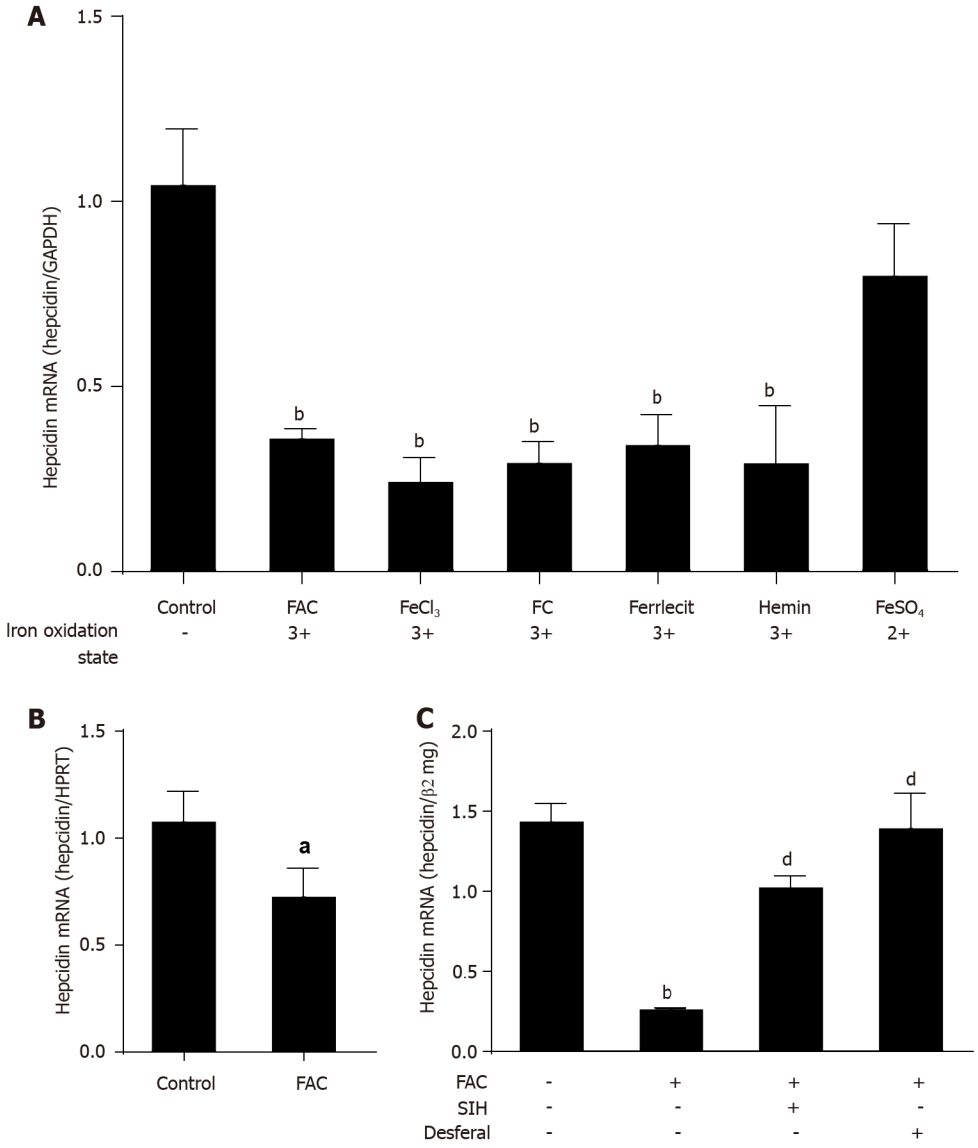

Figure 1 Efficient suppression of hepcidin by higher iron levels. A: Huh7 cells were treated with 50 μmol/L of FAC, $FeCl_3$, FC, ferrlecit, hemin or $FeSO_4$ for 24 h; B: Murine primary hepatocytes were treated with FAC (50 μmol/L) for 24 h; C: Huh7 cells were treated with FAC (50 μmol/L) in the presence or absence of SIH (100 μmol/L) or Desferal (50 μmol/L) for 24 h. Total RNA was extracted from Huh7 cells or murine primary hepatocytes. Hepcidin mRNA levels were determined by quantitative real-time PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase or hypoxanthine phosphoribosyltransferase or β2mg. Data are presented as mean ± SD. $^aP < 0.05$, $^bP < 0.001$ *vs* control; $^dP < 0.001$ *vs* FAC group. FAC: Ferric ammonium citrate; $FeCl_3$: Ferric chloride; FC; Ferric citrate; $FeSO_4$: Ferrous sulfate; SIH: Salicylaldehyde isonicotinoyl hydrazine; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; β2mg, β2-microglobulin; HPRT: Hypoxanthine phosphoribosyltransferase.

still decreased SMAD6 mRNA and p-SMAD1/5/8 protein expression under hypoxia (Figure 3B, C and D; $P < 0.01$ and $P < 0.05$ *vs* hypoxia control), but had no effect on p-STAT3 protein expression even under hypoxia (Figure 3E and F). These results demonstrate that FAC also and primarily affects hepcidin even in a typical STAT3-signaling setting through basal modulation of the SMAD pathway.

FAC efficiently blocks cytokine-mediated hepcidin expression

Cytokines such as IL-6 and IL-1β are important upstream regulators of hepcidin playing an important role in the so-called anemia of chronic disease response[36]. For instance, they are primarily responsible for the general hypoferremia observed during infections[37,38]. To study the effect of iron on cytokine signaling, hepatoma cells were exposed to FAC and/or IL-1β or IL-6 *in vitro* for 24 h and hepcidin mRNA was assessed by qRT-PCR. As shown in Figure 4A and B, both cytokines efficiently increased hepcidin mRNA levels while FAC blocked IL-1β-mediated induction by about 50% and IL-6-mediated induction completely ($P < 0.05$ *vs* IL-1β group and $P < 0.001$ *vs* IL-6 group). FAC not only decreased the basal but also the SMAD6 mRNA and p-SMAD1/5/8 protein expression induced by IL-1β (see Supplemen-

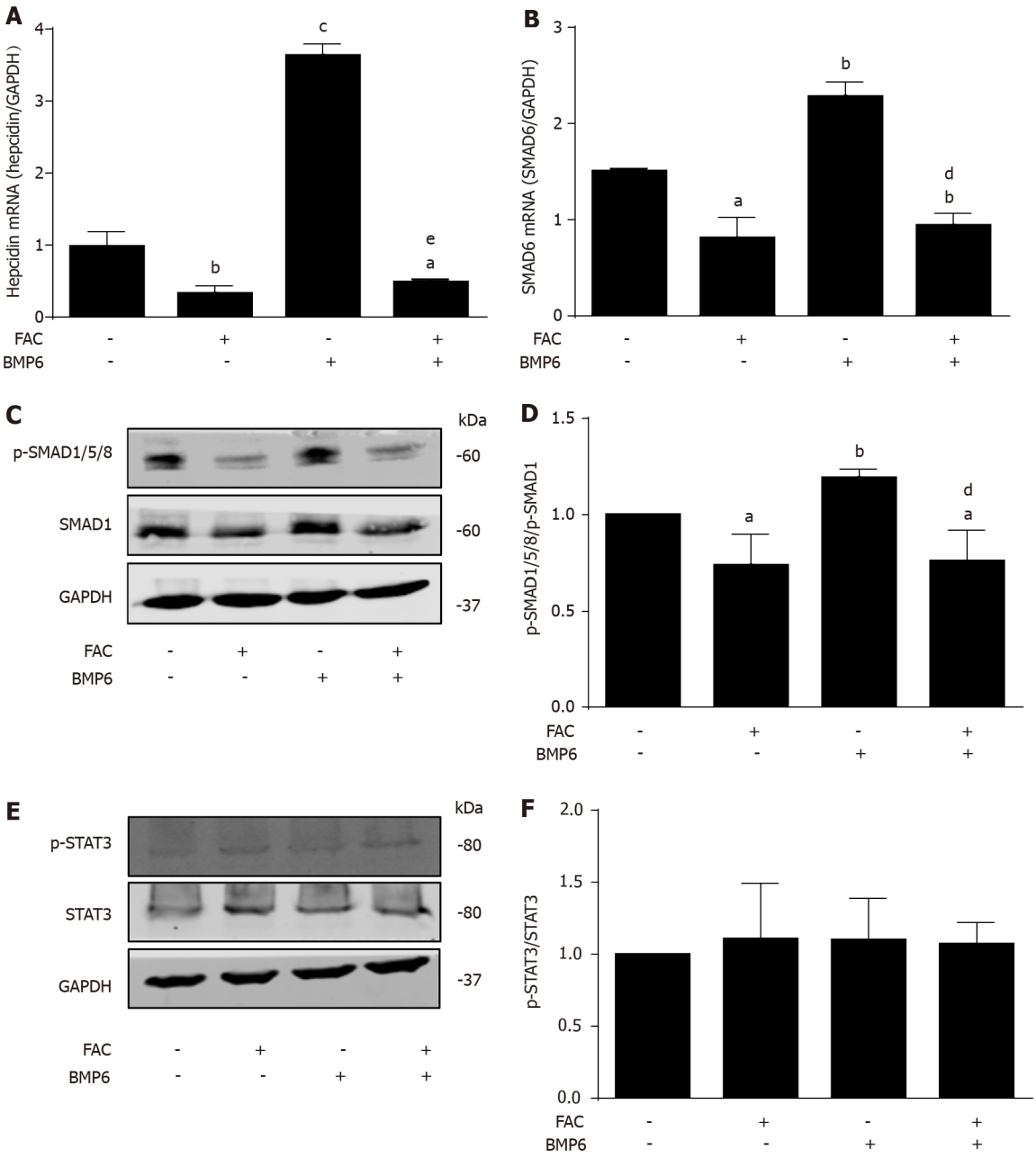

Figure 2 Ferric ammonium citrate profoundly blocks bone morphogenetic protein 6-mediated hepcidin signaling. Huh7 cells were treated with or without bone morphogenetic protein 6 (BMP6) (40 ng/mL) in the presence or absence of ferric ammonium citrate (FAC) (50 μmol/L) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC decreased the hepcidin mRNA expression in the presence or absence of BMP6; B: FAC decreased small mothers against decapentaplegic 6 (SMAD6) mRNA expression in the presence or absence of BMP6; C, D: FAC decreased p-SMAD1/5/8 protein expression in the presence or absence of BMP6; E, F: Both BMP6 and FAC have no significant effect on phosphorylated signal transducer and activator of transcription 3 (p-STAT3) protein expression. SMAD1, p-SMAD1/5/8, STAT3, p-STAT3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. Hepcidin and SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western Blots are representatives of three independent experiments. Data are presented as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control; $^{d}P < 0.01$, $^{e}P < 0.001$ *vs* BMP6 group. FAC: Ferric ammonium citrate; BMP6. Bone morphogenetic protein 6; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

tary Figure 3A). FAC still decreased the SMAD6 mRNA and p-SMAD1/5/8 protein expression in the presence of IL-6 (see Supplementary Figure 4A). In addition, while both cytokines induced p-STAT3 protein expression (see Supplementary Figure 3B or Supplementary Figure 4B; $P < 0.01$ *vs* IL-1β group or IL-6 group), FAC had significant effect on p-STAT3 protein expression neither in the presence nor absence of IL-1β or IL-6. Notably, IL-6 was still able to induce hepcidin under FAC conditions (See Figure 4B). Taken together, these findings suggest that the presence of FAC significantly attenuates hepcidin response to cytokines, which is SMAD dependent but does not involve STAT3.

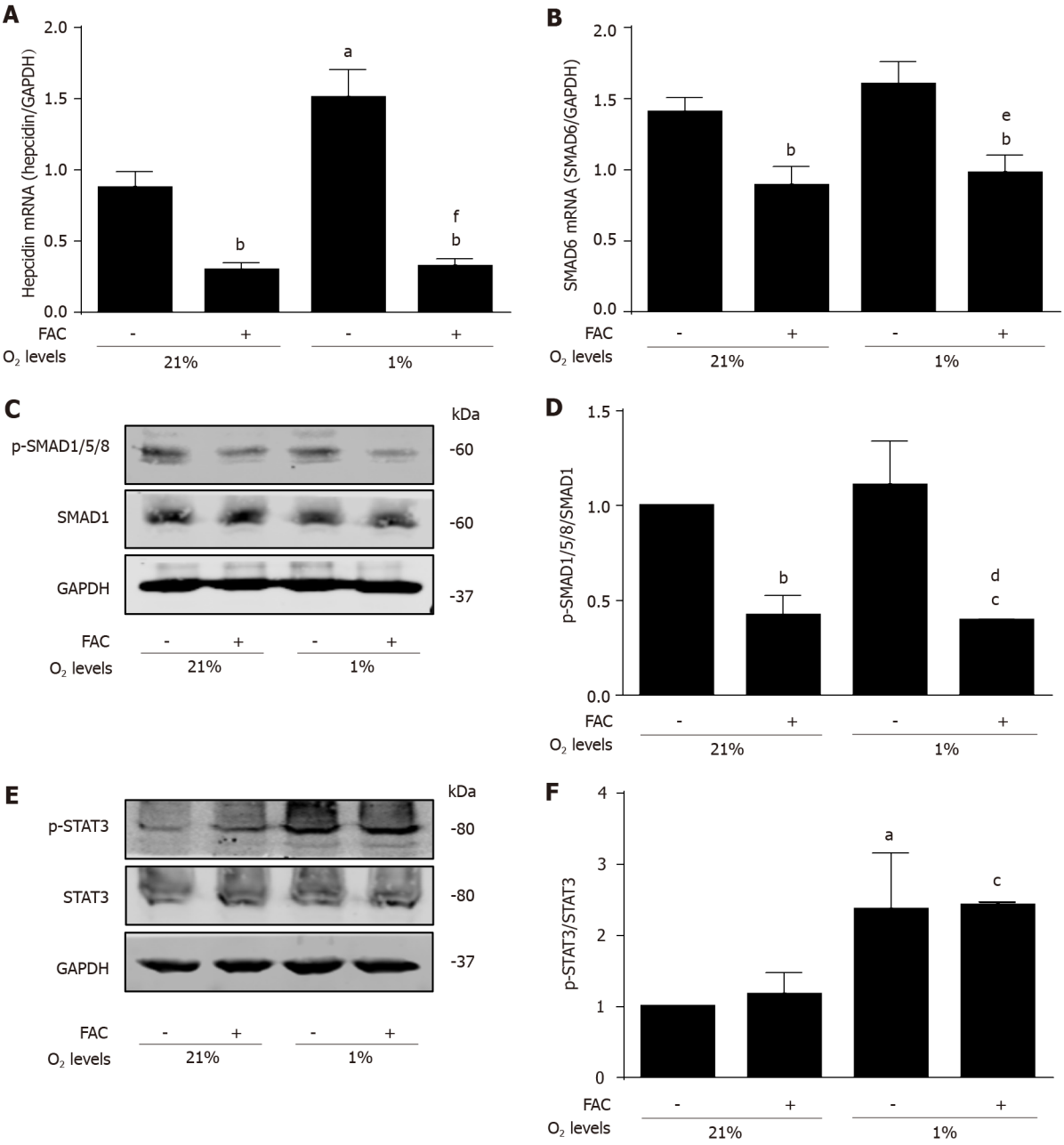

Figure 3 Ferric ammonium citrate efficiently inhibits hypoxia-mediated hepcidin response independent of signal transducer and activator of transcription 3. Huh7 cells were exposed to normoxia (210 mL/L O_2, 21% O_2) or hypoxia (10 mL/L O_2, 1% O_2) in the presence or absence of ferric ammonium citrate (FAC) (50 μmol/L) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC decreased the basal and hypoxia-induced hepcidin mRNA expression; B: Hypoxia has no obvious effect on small mothers against decapentaplegic 6 (SMAD6) mRNA expression, but FAC decreased SMAD6 mRNA expression in the presence or absence of hypoxia; C, D: Hypoxia has no significant effect on p-SMAD1/5/8 protein expression, while FAC decreased p-SMAD1/5/8 protein expression in the presence or absence of hypoxia; E, F: Hypoxia increased phosphorylated signal transducer and activator of transcription 3 (p-STAT3) protein expression, while FAC has no significant effect on p-STAT3 protein expression in the presence or absence of hypoxia. SMAD1, p-SMAD1/5/8, STAT3, p-STAT3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. Hepcidin and SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western Blots are representatives of three independent experiments. Data are presented as mean ± SD. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ *vs* control (21% O_2); $^dP < 0.05$, $^eP < 0.01$, $^fP < 0.001$ *vs* control (1% O_2). FAC: Ferric ammonium citrate; O_2: oxygen; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

Inhibition of hepatocellular hepcidin by FAC requires BMP/SMAD signaling

We next studied the role of BMP/SMAD signaling in the modulation of hepatocellular hepcidin by FAC using a BMP/SMAD signaling inhibitor LDN193189 (LDN)[39]. LDN suppressed the basal hepcidin mRNA expression (Figure 5A; $P < 0.001$ *vs* control), while FAC in combination with LDN could not further suppress hepcidin mRNA expression compared with LDN alone (Figure 5A). FAC in combination with LDN could not further suppress SMAD6 mRNA and p-SMAD1/5/8 protein expression compared with LDN alone (Figure 5B, C and D). Neither FAC nor LDN had a

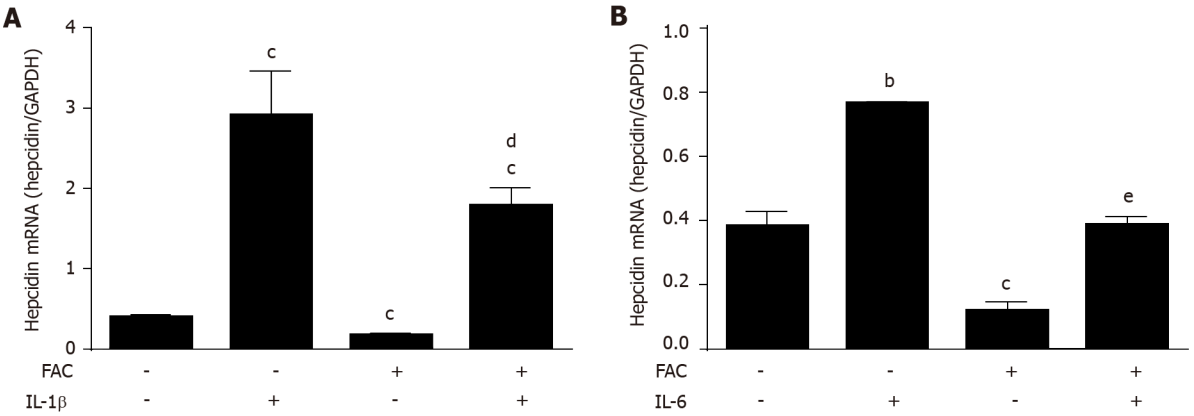

Figure 4 Ferric ammonium citrate efficiently blocks cytokine-mediated hepcidin expression. Huh7 cells were treated with or without IL-1β (10 ng/mL) or IL-6 (10 ng/mL) in the presence or absence of ferric ammonium citrate (FAC) (50 μmol/L) for 24 h. Total RNA was extracted from Huh7 cells. A: FAC significantly decreased IL-1β-induced hepcidin mRNA expression; B: FAC efficiently blocks IL-6-induced hepcidin mRNA expression. Hepcidin mRNA levels were determined by qRT-PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase. Data are presented as mean ± SD. $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control; $^{d}P < 0.05$, $^{e}P < 0.001$ *vs* IL-6 group. IL-1β: Interleukin 1β; IL-6: Interleukin 6; FAC: Ferric ammonium citrate; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

significant effect on p-STAT3 protein expression (Figure 5E and F). In conclusion, these data suggest that the BMP/SMAD signaling is necessary for FAC to inhibit hepcidin expression.

FAC decreases hepatic hepcidin expression induced by LPS in a macrophage-hepatocyte co-culture model

We finally studied the effect of FAC on a more complex and recently established co-culture model of macrophages and hepatocytes to mimic an inflammatory bacterial response by LPS under crosstalk conditions of both cell lines. Human THP-1 monocytes were differentiated into macrophages using PMA as described recently [40]. We examined the effect of LPS on hepatocellular hepcidin mRNA expression in the presence or absence of macrophages. A co-culture model of macrophages and hepatocytes was established according to the cell ratio of 4 to 1 of hepatocytes to macrophages in order to mimic pathophysiological cell ratios in the liver microenvironment[14]. In a normal experimental setting, THP-1 monocytes were differentiated with PMA for 24 h, washed with PBS, and then cultured in fresh medium for another 24h followed by co-cultivation for another 24h with huh7 cells. Huh7 cells were treated by LPS for 24h, and Huh7 cells were co-cultured with THP-1 macrophages in the presence of LPS or exposed to LPS-conditioned macrophage medium for 24 h. LPS slightly induced hepcidin mRNA expression in Huh7 cell monoculture. Co-culture with macrophages induced hepcidin mRNA expression (Figure 6A; $P < 0.001$ *vs* Huh7 control), which was further enhanced by LPS (Figure 6A; $P < 0.001$ *vs* co-culture control) in line with recent studies[14,41]. Notably, the effects of macrophages on hepcidin mRNA expression are even stronger than direct LPS-stimulation (Figure 6A; $P < 0.001$ *vs* Huh7 LPS group). FAC also significantly decreased hepatic hepcidin mRNA expression in our co-culture model (see Figure 6B; $P < 0.05$ *vs* control), and the presence of FAC also significantly attenuated the LPS-mediated expression of hepatic hepcidin mRNA in our co-culture model (see Figure 6B; $P < 0.001$ *vs* LPS group). As demonstrated in Supplementary Figure 5A, FAC decreased the LPS-induced SMAD6 mRNA and p-SMAD1/5/8 protein expression ($P < 0.05$ *vs* LPS group). Moreover, LPS induced p-STAT3 protein expression (see Supplementary Figure 5B; $P < 0.05$ *vs* control), while FAC had no significant effect on p-STAT3 (see Supplementary Figure 5B). Similar results to the directly co-culture model were also observed by using the macrophage-conditioned medium (data not shown). In conclusion, iron also significantly blocks hepcidin expression in a more complex macrophage-hepatocyte co-culture model upon LPS stimulation in SMAD but not STAT3 dependent fashion.

DISCUSSION

We here show that iron suppresses hepatocellular hepcidin signaling directly under *in*

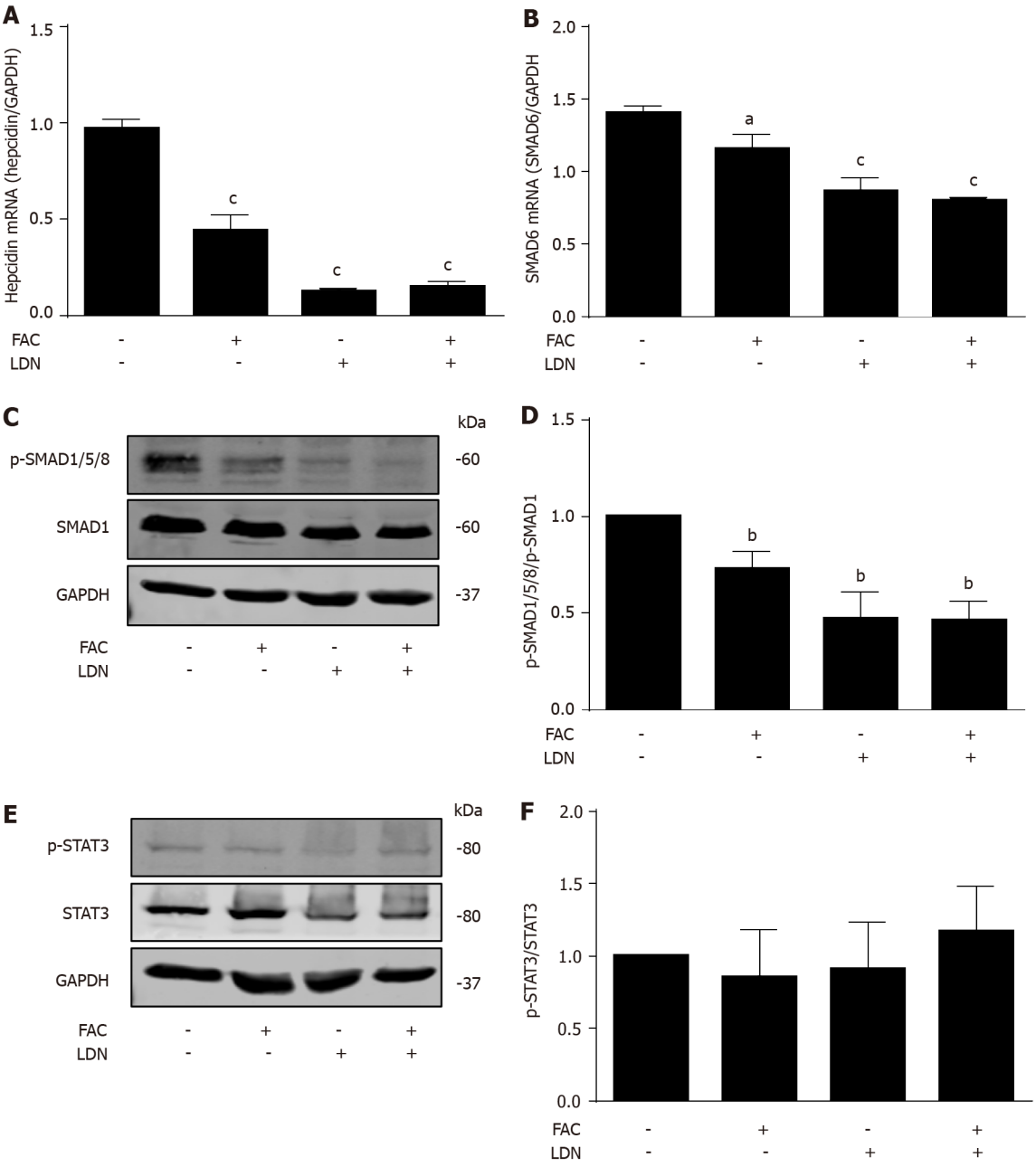

Figure 5 Inhibition of hepatocellular hepcidin by ferric ammonium citrate requires bone morphogenetic protein/small mothers against decapentaplegic signaling. Huh7 cells were treated with or without ferric ammonium citrate (FAC) (50 μmol/L) in the presence or absence of LDN193189 Hydrochloride (LDN) (20 nmol/L) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC or LDN decreased the basal hepcidin mRNA expression, but FAC in combination with LDN did not further suppress hepcidin mRNA expression compared with LDN alone; B-D: FAC or LDN decreased the basal small mothers against decapentaplegic (SMAD)6 mRNA and p-SMAD1/5/8 protein expression, but FAC in combination with LDN did not further suppress SMAD6 and p-SMAD1/5/8 expression compared with LDN alone; E, F: Both FAC and LDN had no significant effect on phosphorylated signal transducer and activator of transcription 3 (p-STAT3) protein expression. SMAD1, p-SMAD1/5/8, STAT3, p-STAT3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. Hepcidin and SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western Blots are representatives of three independent experiments. Data are presented as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control. FAC: Ferric ammonium citrate; LDN: LDN193189 Hydrochloride; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

vitro conditions. By exploring several established *in vitro* models of hepcidin signaling, we further demonstrate that this direct inhibitory effect of iron on hepcidin transcription unanimously affects the BMP-SMAD pathway but not the STAT3 pathway. Since iron-mediated blockage of hepcidin mRNA expression is also observed in primary hepatocytes at higher iron dosages and can be prevented by iron chelators, we suggest that this mechanism could contribute to hepcidin suppression in various iron overload diseases including hemolytic iron overload.

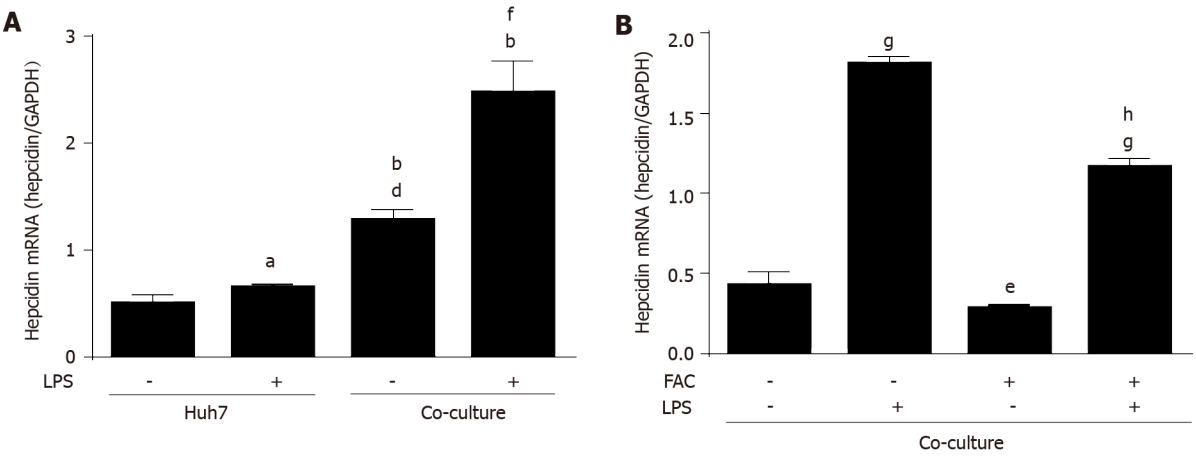

Figure 6 Ferric ammonium citrate decreases hepatic hepcidin expression induced by lipopolysaccharide in a macrophage-hepatocyte co-culture model. Huh7 cells were treated with or without lipopolysaccharide (LPS) (500 ng/mL) for 24 h. Huh7 cells were directly co-cultured with THP-1 macrophages according to pathophysiological macrophage/hepatocyte cell ratio (1:4) and then treated with or without LPS (500 ng/mL) for 24 h in the presence or absence of ferric ammonium citrate (FAC) (50 μmol/L). Total RNA was extracted from Huh7 cells or Huh7 cells and THP-1 macrophages. A: Hepcidin mRNA levels were slightly increased by LPS in monoculture of Huh7 cells, and macrophages increased hepcidin mRNA levels compared with monoculture control and the presence of LPS further markedly increased hepcidin mRNA levels; B: FAC decreased the basal and LPS-induced hepcidin mRNA levels in the co-culture model. Hepcidin mRNA levels were determined by qRT-PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase. Data are presented as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.001$ *vs* Huh7 control; $^{d}P < 0.001$ *vs* Huh7 LPS group; $^{e}P < 0.05$, $^{f}P < 0.01$, $^{g}P < 0.001$ *vs* co-culture control; $^{h}P < 0.001$ *vs* co-culture LPS group. LPS: Lipopolysaccharide; FAC: Ferric ammonium citrate.

Although not widely gained attention, it has already been known for many years that hepatocellular hepcidin rapidly loses its responsiveness to iron under cultured conditions[19,41]. While this could be due to the loss of serum factors, the "*in vivo* liver microenvironment", altered oxygen conditions or loss of metabolic demand ex vivo, the absence of an essential intercellular crosstalk could be another explanation. Namely with the identification of the BMP6-SMAD pathway, the role of endothelial released BMP6 has been identified as a major upstream event of the hepcidin response [23,26]. Indeed, and also shown here, exposure of cultured hepatocytes to recombinant BMP6 is able to efficiently recover the hepcidin response.

On the other hand, such paradox responses of hepcidin towards iron levels have been also well documented in patients with severe thalassemia. These patients show pronounced hemolytic anemia and require repeated blood transfusion[32]. Patients with severe disease typically show progressive liver damage and cirrhosis due to serious iron toxicity[42]. The recent establishment of a murine thalassemia model clearly demonstrates that hepatic iron overload occurs also in the absence of additional blood supply under continued hemolysis-mediated suppression of hepcidin[33].

The mechanisms behind this hepcidin suppression in hemolytic diseases are still controversially discussed. Erythropoietin (EPO) has been proposed as an important factor although the underlying mechanisms are not completely understood and cannot be recapitulated by direct exposure of hepatocytes to EPO[43]. The recent identification of bone marrow-derived erythroferrone (ERFE) and Growth Differentiation Factor-15 (GDF15) in response to EPO stimulation suggests that these factors at least partly contribute to hepcidin suppression during hemolysis[28,44-46]. However, our data on the direct inhibiting effect of iron on hepcidin signaling *in vitro* suggest that iron per se could also contribute to hepcidin suppression.

Chronic liver diseases represent another important model of chronic iron overload and ca. 50% of chronic liver diseases show hepatic iron overload with an inadequate hepcidin response[30]. While primary liver damage either through alcohol damage or viral replication could account for the total loss of hepcidin response[47-49], iron itself could also play a regulatory role. In our various *in vitro* models of hepcidin signaling, we here demonstrate that iron efficiently blocks hepcidin response primarily through the SMAD pathway. Although this seems rather counteractive towards the iron-mediated BMP-hepcidin response, this experiment deserves serious consideration especially during pathophysiological conditions such as severe hemolysis or damage to the liver sinus-endothelial layer. It may explain why continued hepatic iron overload would initiate a vicious cycle of hepcidin suppression and further iron uptake through the duodenal brush border[50]. It would also implicate that besides pharmacological approaches to re-introduce hepcidin or increase hepcidin peptide

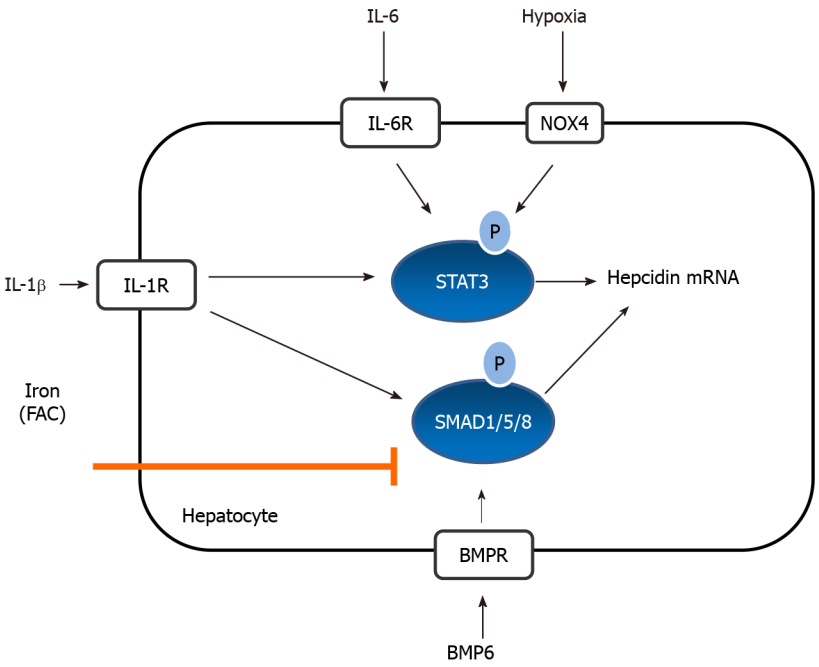

Figure 7 Scheme of iron-mediated blockage of hepcidin transcription *via* bone morphogenetic protein/small mothers against decapentaplegic but independent of signal transducer and activator of transcription 3 signaling. Iron (ferric ammonium citrate) primarily blocks hepcidin transcription *via* the bone morphogenetic protein (BMP)/small mothers against decapentaplegic pathway while no effect on signal transducer and activator of transcription 3 signaling was observed. The scheme also shows all studied hepcidin signaling pathways including BMP6, interleukin (IL)-6, IL-1β, hypoxia or a complex co-culture model with macrophages. IL-1β: Interleukin 1β; IL-6: Interleukin 6; BMP6: Bone morphogenetic protein 6; FAC: Ferric ammonium citrate; IL-1R: IL-1 receptor; IL-6R: IL-6 receptor: NOX4: NADPH Oxidase 4; BMPR: BMP receptor; p-STAT3: Phosphorylated signal transducer and activator of transcription 3; p-SMAD1/5/8: Phosphorylated small mothers against decapentaplegic 1/5/8.

levels (*e.g.*, mini hepcidins), removal of iron remains the cornerstone of the treatment. Not only would it remove the primary toxic agent iron but it would interrupt the suppressing effect of hepcidin on iron. It may also stimulate a mechanistic discussion on the therapeutic usage of iron chelators *vs* phlebotomy.

Although our data clearly show an exclusive effect of *in vitro* iron on the SMAD signaling cascade, the direct molecular mechanisms still remain elusive. Notably, hepcidin signaling was inhibited by iron in all explored models including the co-culture model with macrophages. Even in primary STAT3-mediated processes such as cytokines, hypoxia or LPS, iron efficiently blocked hepcidin transcription underlining the important role of the SMAD pathway for basal hepcidin expression. In line with this is the observation that efficient SMAD blockage by the SMAD inhibitor LDN could not be further enhanced by iron. Second, experiments with membrane permeable or non-permeable iron chelators (SIH or Desferal) show that iron chelators efficiently counteract the inhibitory effect of iron on hepcidin. Although do not provide definite answers to the underlying mechanisms of the iron-mediated hepcidin inhibition, the almost immediate effect restricted to the SMAD pathway and the fact that only oxidized forms of iron are effective suggests to us that iron may directly act through the BMP receptor or associated molecules such as TfR1 or TfR2[30].

On a final note, we were surprised not to see any interaction of iron with the STAT3 pathway. Since STAT3 is responsive to peroxide and iron and H_2O_2 are known for decades to chemically interfere *via* the Fenton chemistry[30], it would have been no surprise to see direct effects on hepcidin transcription. However, it remains open whether compensating mechanisms exist to counteract decreased peroxide levels *e.g.* by upregulating oxidases *etc*.

In summary, to our knowledge, this work is the first to show that iron directly blocks hepcidin transcription, at baseline or upon stimulation by different stimuli, through the BMP/SMAD but not STAT3 signaling *in vitro*. A summarizing scheme is shown in Figure 7. We think that in addition to potential hepcidin suppressing factors such as GDF15 or ERFE, iron could directly block hepcidin transcription under conditions of either excess iron or a liver endothelial fenestration with larger access to the hepatocellular membrane. Specifically under pathological conditions such as severe hemolysis or chronic iron overload as observed in alcoholic liver disease, this novel mechanism may contribute to further iron overload and initiate a vicious cycle.

To interrupt this cycle, the removal of iron should be the most efficient therapeutic goal. It will not be an easy task to validate this concept in *in vivo* models since iron levels in the direct environment of hepatocytes are not easy to quantitate.

CONCLUSION

In conclusion, iron including FAC per se, directly blocks hepcidin transcription and the inhibitory effect could be observed over a large concentration range involving all forms of iron-III, which was not caused by toxicity or inhibition of cell growth. FAC has a profound inhibitory effect on hepcidin expression at baseline or upon stimulation by stimuli in various cell models, which was controlled through the BMP/SMAD pathway but independent of STAT3. We suggest that this mechanism may contribute to continued iron overload in many pathophysiological conditions ultimately causing a vicious cycle of continued hepcidin suppression. Anyway, this study provides a new idea for in-depth exploration of iron overload diseases and provides an experimental basis for the underlying therapeutic goal.

ARTICLE HIGHLIGHTS

Research background

Excess iron causes cancer and severe tissue damage and chronic iron overload is not only driving the rather rare hereditary iron overload diseases but also secondary iron overload diseases due to hemolysis or common chronic liver diseases such as alcoholic liver disease or hepatitis C. In most of these diseases, suppression of hepcidin, the systemic master switch of iron homeostasis in mammals, has been identified to play a key role. Hepcidin is primarily expressed in hepatocytes as a precursor pro-peptide and to a lesser extent in macrophages or cardiomyocytes. Elevated hepcidin causes hypoferremia and anemia by efficiently blocking iron absorption, iron recycling and iron storage by binding to and degrading the major iron export pump ferroportin 1.

Research motivation

The direct iron sensing mechanisms by hepcidin remain obscure and seemingly paradox response of hepcidin have been observed in various clinical scenarios. Thus, direct intravenous injection of iron causes rapid induction of hepcidin, iron release in the context of hemolytic diseases such as thalassemia efficiently block hepcidin expression and cause further detrimental iron accumulation. Moreover, it still remains largely unexplained why hepatocellular hepcidin is downregulated under *in vitro* conditions. These observations prompted us to study in detail the direct effect of iron in cultured hepatocytes.

Research objectives

The authors here aimed to study the direct effect of iron on various established hepcidin signaling pathways including the bone morphogenetic protein (BMP)/small mothers against decapentaplegic (SMAD) signaling pathway and signal transducer and activator of transcription 3 (STAT3)-mediated hepcidin signaling *via* cytokines, hypoxia, and lipopolysaccharide (LPS) using a recently established macrophage-hepatocyte co-culture model.

Research methods

Hepcidin mRNA expression in presence of various forms of iron was studied, using hepatoma cells (Huh7), murine primary hepatocyte and a co-culture model of phorbol myristate acetate-differentiated THP-1 monocytes and hepatoma cells. The response to BMP6, interleukin (IL)-6, IL-1β, hypoxia and LPS were studied in order to analyze hepcidin signaling. Hepcidin and SMAD6 mRNA levels were assessed and the expression of phospho-STAT3, STAT3, phospho-SMAD1/5/8 and SMAD1 proteins were analyzed.

Research results

All iron III forms including ferric ammonium citrate efficiently blocked hepcidin mRNA expression at non-toxic dosages in hepatoma cells or primary hepatocytes. Using iron chelators, the blockage of hepcidin by iron could be efficiently blunted. Iron

also had a profound inhibitory effect of basal hepcidin expression and completely abolished BMP6-mediated hepcidin signaling through SMAD but not the STAT3 pathway. Iron also and primarily affected hepcidin even in a typical STAT3-signaling setting through basal modulation of the SMAD pathway and iron significantly attenuated hepcidin response to cytokines, which is SMAD dependent but does not involve STAT3. In the co-culture model, iron inhibited LPS-mediated hepcidin induction.

Research conclusions

In conclusion, iron directly blocks hepatocellular hepcidin transcription involving all forms of iron III and the effect was not caused by toxicity or reduced cell growth. Iron also inhibits hepcidin upregulation in various models of hepcidin stimulation primarily through the BMP/SMAD pathway but independent of STAT3 signaling. We propose that his mechanism may contribute to continued iron overload at least under pathophysiological conditions of iron release ultimately causing a vicious cycle of continued hepcidin suppression and further iron overload.

Research perspectives

This study provides a new concept for better understanding the seemingly paradox response of hepcidin in *in vivo* and *in vitro* settings. Moreover, understanding the direct inhibitory effects of iron on hepcidin signaling at the hepatocellular side could help to identify novel molecular targets for future therapies.

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ORIGINAL ARTICLE

Basic Study

Serum zonulin levels in patients with liver cirrhosis: Prognostic implications

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Author contributions: Voulgaris TA was involved in the design of the trial, collected the clinical, biochemical and endoscopic data, performed the statistical analyses and wrote the initial draft; Karagiannakis D helped plan the study, developed the detailed study protocol, collected the clinical, biochemical and endoscopic data, and reviewed the manuscript; Hadziyannis E contributed to serum samples analysis; Karamanolis GP and Manolakopoulos S helped in the design of the study and reviewed the manuscript; Papatheodoridis GV was involved in the improvement of the protocol as well as in the finalization of the manuscript; Vlachogiannakos J contributed to the design of the study, the interpretation of the data, and review and finalizing of the manuscript.

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Abstract

BACKGROUND

Increased gut permeability and bacterial translocation play an important role in liver cirrhosis. Zonulin is a recently recognized protein involved in the disintegration of the intestinal barrier.

AIM

To investigate possible differences in serum zonulin levels among patients with different cirrhosis stages and their potential prognostic implications.

METHODS

Consecutive cirrhotic patients who attended our liver clinic were included in the study. Serum zonulin levels, clinical, radiological and biochemical data were collected at baseline. Patients who accepted participation in a regular surveillance program were followed-up for at least 12 mo.

RESULTS

We enrolled 116 cirrhotics [mean Child-Turcotte-Pugh (CTP) score: 6.2 ± 1.6; model for end-stage liver disease score: 11 ± 3.9]. The causes of cirrhosis were viral hepatitis (39%), alcohol (30%), non-alcoholic fatty liver disease (17%), and other (14%). At baseline, 53% had decompensated cirrhosis, 48% had ascites, and 32% had history of hepatic encephalopathy. Mean zonulin levels were significantly higher in patients with CTP-B class than CTP-A class (4.2 ± 2.4 ng/dL *vs* 3.5 ± 0.9 ng/dL, $P = 0.038$), with than without ascites ($P = 0.006$), and

with than without history of encephalopathy (P = 0.011). Baseline serum zonulin levels were independently associated with the probability of decompensation at 1 year (P = 0.039), with an area under the receiving operating characteristic of 0.723 for predicting hepatic decompensation. Higher CTP score (P = 0.021) and portal vein diameter (P = 0.022) were independent predictors of mortality.

CONCLUSION

Serum zonulin levels are higher in patients with more advanced chronic liver disease and have significant prognostic value in identifying patients who will develop decompensation.

Key Words: Zonulin; Cirrhosis; Intestinal barrier; Bacterial translocation; Decompensation; Permeability

Core Tip: Zonulin is a protein that appears to play a significant role in gut barrier integrity. Increased zonulin levels and deregulation of intestinal permeability have been demonstrated in patients suffering from celiac disease or type 2 diabetes. However, the role of zonulin as a promoting factor of intestinal barrier disruption in patients with liver cirrhosis has not been studiedadequately. We evaluated serum zonulin levels in patients with different stages of advanced liver disease. According to our findings, serum zonulin levels are increased in patients with more advanced liver disease and are independently associated with progression to decompensation.

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INTRODUCTION

Bacterial translocation (BT) is defined as the passage of viable endogenous bacteria and endotoxins from the intestinal lumen through the mucosa into the mesenteric lymph nodes and other organs[1]. In patients with liver disease, BT has been demonstrated to play a pivotal role on the occurrence or aggravation of serious complications[2]. Bacterial overgrowth, decreased intestinal peristalsis with concomitant increased permeability, as well as immunological alterations that have been found in patients with chronic liver diseases appear to be the main causative factors of BT[3-6]. Among them, the exact pathophysiological mechanism leading to increased intestinal permeability is the most difficult to investigate and remains to be thoroughly explained.

Recently, Fasano[7] identified zonulin, a novel 47-kDa protein precursor of haptoglobin-2 (pre-HP2), which is synthesized by the intestinal and liver cells and mayplay a significant role in disruption of the gut barrier. Evidence exist to support that small intestine epithelial cells exposed to enteric bacteria, secret zonulin, which in turn attaches to special receptors located on the membrane of intestinal epithelial cells, leading to a disconnection of occludin from ZO-1. This disrupts the tight junctions and consequently increases the gut permeability[8].

Currently, the connection between increased zonulin levels and the deregulation of intestinal permeability has been observed in patients suffering from celiac disease, type 2 diabetes, obesity and inflammatory bowel disease (IBD)[9-13]. However, the role of zonulin and its possible involvement in the dysfunction of intestinal barrier function in patients with cirrhosis has not been studiedthoroughly.

The aim of our study was to assess the serum zonulin levels in patients with cirrhosis and investigate their possible impact on patients' prognosis.

Institutional review board statement: The study was reviewed and approved by the Greek Committee for the Protection of Personal Data (approval No. 1990) after the official request of the "Laiko" General Hospital Athens Attiki (Laiko Hospital Institutional Review Board Decision: 712;8/5/2017).

Conflict-of-interest statement: The authors declare having no real or potential conflicts to disclose.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at jvlavhog@hotmail.com. Consent was not obtained but the presented data are anonymized and risk of identification is low.

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MATERIALS AND METHODS

Over a period of 12 mo (February 2017-January 2018), all cirrhotic patients, aged from 18 years to 80 years, who attended our outpatient liver clinics were considered eligible for inclusion in the study, regardless of the etiology and severity of their liver disease. We excluded patients with alcoholic hepatitis, porto-splenic vein thrombosis, non-cirrhotic portal hypertension, hepatocellular carcinoma (HCC), transjugular intrahepatic portosystemic shunt (TIPS), chronic kidney disease, celiac disease, acute infection, IBD, or any other chronic intestinal disease.

The diagnosis of cirrhosis was based on clinical and laboratory findings, imaging studies or liver histology, when available. All patients had liver stiffness measurement (LSM) of ≥ 14 kPa (by elastography). At baseline, all patients underwent abdominal ultrasound with spleen and portal diameter measurements and baseline LSM and spleen stiffness measurement (SSM) by shear wave elastography (SWE). In addition, all patients underwent clinical examination and laboratory testing every 3 mo, and abdominal ultrasound every 6 mo.

The study protocol was approved by the Ethics Committee of "Laiko" General Hospital of Athens, Greece. A written consent was obtained from each patient with respect to all ethical guidelines issued by the 2000 revision (Edinburgh) of the 1975 Declaration of Helsinki.

Clinical and laboratory data

Clinical and laboratory data, routine blood parameters, including platelet count, prothrombin time, serum albumin, serum creatinine, international normalized ratio (INR), serum aspartate aminotransferase, alanine aminotransferase, and bilirubin, were measured at the time of patient enrollment. Likewise, the existence of ascites or hepatic encephalopathy (HE) was noted. The severity of liver disease was determined by Child-Turcotte-Pugh (CTP) scoring, and the model for end-stage liver disease (MELD) score calculated according to the UNOS formula. Study end-points included death, liver transplantation and liver decompensation in patients with compensated cirrhosis at baseline.

Two-dimensional SWE

All patients underwent LSM and SSM by two-dimensional (2D)-SWE performed by a single experienced operator (> 500-exam experience) in fasting patients. The Aixplorer® ultrasound system (Supersonic Imagine S.A., Aix-en-Provence, France) with an abdominal 3.5 MHz curved array probe was used, as recommended. 2D-SWE measurements were performed at each patient's initial assessment. Ten reliable LSM and ten reliable SSM values were obtained from each patient and the mean values were then calculatedrespectively. The SD was < 20% of the mean values of LSM and SSM, respectively.

Sample collection-zonulin measurement

A venous blood sample was collected from each patient, with or without precooled anticoagulant (heparinized/EDTA)-coated tube. The serum or plasma was then separated from the blood by centrifugation at 3000 rpm for 10 min at room temperature. The samples were stored at -80 °C.

Serum levels of zonulin were measured using an enzyme-linked immune-sorbent assay (Immundiagnostik AG, Bensheim, Germany); the sensitivity of the assay was 0.01 ng/mL.

Statistical analysis

Statistical analysis was performed by SPSS V23 (IBM Corp., Armonk, NY, United States). Data were expressed as frequencies, mean with SD, or median with interquartile range, as appropriate. Quantitative variables were compared with Student's *t*-test or Mann-Whitney test for normally distributed and non-normally distributed variables, respectively. Qualitative variables were compared with chi-squared test or Fisher's exact test, as appropriate. The relationship between parameters was assessed by using Spearman's correlation coefficient. Multivariate logistic regression analysis models were used to identify independent, significant, predictive factors of a poor outcome. Only parameters with a significant or a trend for significant associations ($P < 0.10$) with the dependent variable in the univariate analysis being included in the multivariate analysis models. The area under the receiving operating characteristic (AUROC) curves for zonulin predictability, as well as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were

calculated. The c-statistics of AUROC curves were provided with their 95% confidence intervals (CIs). Diagnostic accuracy was considered to be poor when a c-statistic was 0.65-0.75, good when a c-statistic was 0.76-0.85, and excellent when a c-statistic was > 0.85. The optimal cut-off was selected from the AUROC curves as the point which provided the maximum sum of sensitivity and specificity. All tests were two-sided and P values < 0.05 were considered to be significant.

RESULTS

In total, 127 consecutive cirrhotic patients were initially assessed. Eleven patients were excluded, due to HCC (n = 5), acute infection (n = 4) or portal vein thrombosis (n = 2). Therefore, 116 patients were finally included in the study. Mean age was 59 ± 13 years, and 71 (61.2%) were male. Viral hepatitis was the main cause of liver disease (38.8%). Compensated and decompensated liver disease were marginally equally distributed in our cohort, while a significant proportion of patients had ascites at the time of enrollment. Esophageal or gastric varices were documented in 65 (55.2%) of the patients and 60 (51.7%) were under treatment with b-blockers. Patient characteristics are presented in Table 1.

Compared to patients with compensated liver disease, those with decompensated liver disease had significantly lower platelet counts (106 ± 37 × 10^9/L *vs* 137 ± 55 × 10^9/L, P = 0.006), higher INR values (1.3 ± 0.28 *vs* 1.2 ± 0.2, P = 0.003) and lower albumin levels (3.5 ± 0.5 g/Dl *vs* 4.8 ± 0.6 g/dL, P < 0.001) as well as higher MELD (12.6 ± 4.1 *vs* 9.2 ± 1.6, P < 0.001) and CTP scores (7 ± 1.7 *vs* 5.3 ± 0.5, P < 0.001).

Zonulin levels

Mean serum zonulin levels were 3.6 ± 1.5 ng/dL. Patients with CTP-B had significantly higher serum zonulin levels compared to those with CTP-A cirrhosis (4.2 ± 2.4 ng/dL *vs* 3.5 ± 0.9 ng/dL, P = 0.038). On the other hand, patients with CTP-C cirrhosis had lower levels of serum zonulin compared to the two other groups. Specifically, CTP-C patients had lower levels of zonulin than CTP-A (2.6 ± 0.7 ng/dL *vs* 3.5 ± 0.9 ng/dL, P = 0.035) or CTP-B patients, although the latter difference did not reach statistical significance (2.6 ± 0.7 ng/dL *vs* 4.2 ± 2.4 ng/dL, P = 0.157) (Figure 1).

Serum zonulin levels were higher in patients with than without ascites (4.16 ng/dL *vs* 3.26 ng/dL, P = 0.006). Similarly, patients with a history of HE had higher zonulin levels compared to those without history of HE (4.17 ng/dL *vs* 3.39 ng/dL, P = 0.011). The presence of varices was also associated with numerically higher levels of zonulin but this difference did not reach statistical significance (Figure 2).

No significant correlation was observed between serum zonulin levels and platelets, serum albumin, bilirubin, INR, MELD score, age or body mass index. Moreover, treatment with b-blockers was not found to affect the levels of zonulin (patients on treatment: 3.6 ± 1.5 ng/dL *vs* no treatment with b-blockers: 3.4 ± 0.9 ng/dL, P = 0.513).

Follow-up

Sixty-three out of the one-hundred and sixteen patients were followed for at least 12 mo or until death/Liver transplantation, whichever occurred first. Their mean age was 60 ± 15 years and 30 (48%) were male. The majority of patients (n = 36, 57%) had compensated cirrhosis at baseline. Forty-four (69.8%) patients had CTP-A and nineteen had CTP-B (30.2%) cirrhosis. Mean MELD score was 11.3 ± 3.2. Thirty-nine (61.9%) patients had no varices or small varices without red spots and twenty-four (38.1%) patients had high-risk varices (large varices or small varices with red spots). Twenty of the twenty-seven (74%) patients with decompensated cirrhosis had ascites at baseline. No patient with compensated liver disease who was in the follow-up group was under rifaximin treatment, while 8/27 patients with decompensated disease were receiving rifaximin. Patients with decompensated cirrhosis receiving rifaximin on baseline and followed up for at least 12 mo showed numerically higher serum zonulin levels at baseline, though not statistically significant (patients onrifaximin treatment: 4.49 ± 2.37 ng/dL *vs* no rifaximintreatment: 3.41 ± 1.08 ng/dL, P = 0.144)

Specific treatment was received by 32 (50.8%) of the 63 patients. Among them, mean baseline LSM was 22.9 ± 9.3 kPa and mean baseline SSM was 35.3 ± 8.6 kPa.

Twelve (33.3%) of the thirty-six patients with compensated cirrhosis at baseline progressed to decompensated disease [11/36 (30.5%) developed ascites and 1/36 (2.8%) developed variceal bleeding]. Patients who progressed to liver decompensation (n = 12) had higher baseline serum zonulin levels at (3.98 ± 0.79 ng/dL *vs* 3.18 ± 1.02 ng/dL, P = 0.011) and lower albumin levels (3.64 ± 0.53 g/dL *vs* 4.10 ± 0.51 g/dL, P =

Table 1 Baseline clinical and laboratory characteristics of the patients

Examined parameter		Baseline value
Sex as M/F, *n* (%)		71/45 (61.2)
Age in yr[1]		59 ± 13
BMI, kg/m^2		27.5 ± 5.0
Liver disease etiology, *n* (%)		
	Chronic hepatitis B	25 (21.6)
	Chronic hepatitis C	20 (17.2)
	Alcohol abuse	35 (30.2)
	NAFLD	20 (17.2)
	Autoimmune hepatitis	5 (4.3)
	Other	11 (9.5)
CTP class, *n* (%)		
	A	78 (67.2)
	B	33 (28.4)
	C	5 (4.4)
	CTP score	6.2 ± 1.6
MELD score		11.0 ± 3.9
Decompensated cirrhosis, *n* (%)		61 (52.6)
History of HE, *n* (%)		37 (31.9)
Ascites, *n* (%)		56 (48.3)
Bilirubin in mg/dL		1.3 ± 0.9
Creatinine in mg/dL		1.0 ± 1.2
Albumin in g/L		41.0 ± 4.0
Platelet count as × 109/L		121 ± 49
INR		1.3 ± 0.3

[1]Quantitative variables are expresses as mean ± SD.

BMI: Body mass index; CTP: Child-Turcotte-Pugh; F: Female; HE: Hepatic encephalopathy; INR: International normalized ratio; M: Male; MELD: Model for end-stage liver disease; NAFLD: Nonalcoholic fatty liver disease.

0.013) as well as a trend for lower platelet counts (104 × 10^9/L *vs* 138 × 10^9/L, P = 0.094) and higher SSM (36.1 ± 9.3 kPa *vs* 31.1 ± 7.4 kPa, P = 0.087) compared to patients who remained compensated during follow-up (Table 2). In multivariate logistic regression analysis, progression to liver decompensation within 12 mo was independently associated with higher serum zonulin [odds ratio (OR): 6.53, 95%CI: 1.08-39.57, P = 0.041] and lower albumin at baseline (OR: 0.03, 95%CI: 0.002-0.92, P = 0.044). Baseline serum zonulin levels offered an AUROC of 0.723 (P = 0.039) for predicting development of decompensation within 1 year (Figure 3). The cut-off point that could better predict progression to decompensation was 3.65 ng/dL, with specificity 73%, sensitivity 73%, NPV 84% and PPV 57%.

In total, 7 (11.3%) patients died (6 due to liver related causes and 1 due to non-liver related malignancy), while 2 patients (2.9%) underwent liver transplantation. Patients who died or underwent liver transplantation (n = 9) had lower baseline albumin levels compared to patients (n = 54) who survived (3.20 ± 0.62 g/dL *vs* 3.87 ± 0.62 g/dL, P = 0.010), higher CTP score (7.4 *vs* 5.9, P < 0.001) and greater portal vein diameter (1.55 cm *vs* 1.27 cm, P = 0.002) (Table 3). In multivariate logistic regression analysis, higher CTP score (OR: 2.06, 95%CI: 1.02-4.16, P = 0.021) and portal vein diameter (OR: 71.54, 95%CI: 1.56-329.52, P = 0.022) were independently associated with mortality.

Table 2 Univariate analysis of factors associated to liver disease decompensation

Examined parameter, baseline values	Patients who remained compensated during follow-up, *n* = 24	Patients who proceed to decompensated disease during follow-up, *n* = 12	*P* value
Sex as M/F	13/11	3/9	0.157
Age in yr[1]	59 ± 12	61 ± 14	0.710
Liver-specific treatment, Y/N	13/11	9/3	0.292
High-grade varices, Y/N	6/24	6/12	0.157
Platelet count as × 10^9/L	138 ± 54	105 ± 46	0.094
Albumin in g/dL	3.64 ± 0.53	4.13 ± 0.51	0.013
Spleen diameter in cm	13.1 ± 2.5	14.0 ± 2.3	0.325
Portal diameter in cm	1.29 ± 0.22	1.27 ± 0.21	0.768
Liver stiffness in kPa	19.7 ± 6.9	23.9 ± 9.2	0.139
Spleen stiffness in kPa	31.1 ± 7.4	36.1 ± 9.3	0.087
Serum zonulin levels in ng/mL	3.19 ± 1.02	4.15 ± 0.95	0.011

[1]Quantitative variables are expressed as mean±standard deviation.
F: Female; M: Male; N: No; Y: Yes.

Table 3 Univariate analysis of factors associated to transplant free survival

Examined parameter, baseline values	Patients alive/not transplanted at the end of the follow-up, *n* = 54	Patients transplanted or dead, *n* = 9	*P* value
Age in yr[1]	59 ± 15	66 ± 14	0.238
Liver-specific treatment, Y/N	28/26	4/5	0.474
CTP score	5.9 ± 1.0	7.4 ± 1.4	< 0.001
MELD score	11.3 ± 3.0	11.7 ± 3.6	0.772
High-risk varices, Y/N	19/54	5/9	0.241
Platelet count as × 10^9/L	120 ± 52	109 ± 40	0.602
Albumin in g/dL	3.87 ± 0.62	3.20 ± 0.62	0.010
Spleen diameter in cm	13.7 ± 2.8	15.2 ± 2.5	0.154
Portal diameter in cm	1.27 ± 0.21	1.55 ± 0.25	0.002
Liver stiffness in kPa	22.5 ± 9.2	27.9 ± 10.3	0.127
Spleen stiffness in kPa	35.0 ± 8.8	37.9 ± 6.9	0.383
Serum zonulin levels in ng/mL	3.70 ± 1.36	3.17 ± 1.21	0.300

[1]Quantitative variables are expressed as mean ± SD.
CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; N: No; Y: Yes.

DISCUSSION

BT is increased in cirrhosis and seems to play a pivotal pathophysiological role in the development of complications related to end-stage liver disease, such as hepatorenal syndrome, HE, spontaneous bacterial peritonitis and acute-on-chronic liver failure[2,14]. Although many factors have been implicated in the pathophysiology of BT, the exact pathogenic mechanisms leading to gut epithelial disfunction in liver cirrhosis remain unclear[1,15,16]. To date, the role of zonulin as a promoting factor of the intestinal barrier's disruption has been thoroughly investigated in several diseases, but in patients with cirrhosis there is only limited information.

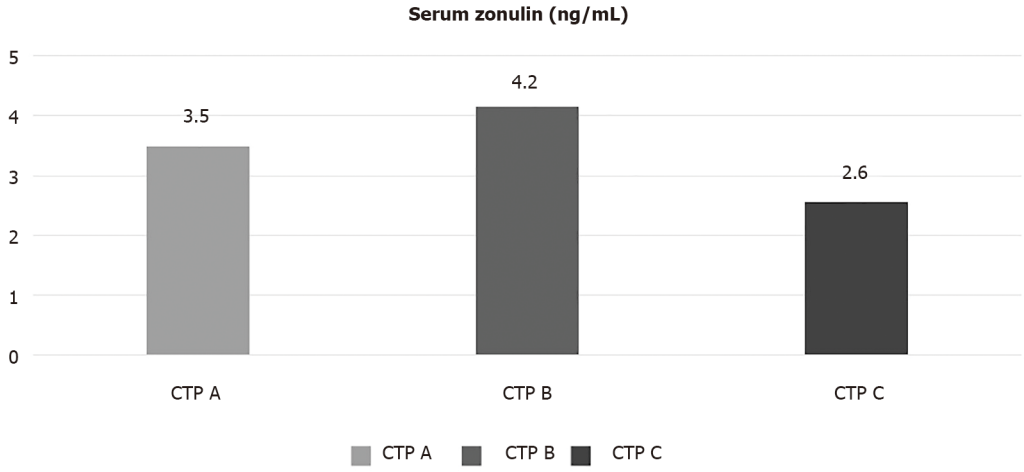

Figure 1 Mean serum zonulin levels (ng/mL) among different Child-Turcotte-Pugh classes. Serum zonulin levels (expressed in ng/mL as mean) according to Child-Turcotte-Pugh stage. CTP: Child-Turcotte-Pugh.

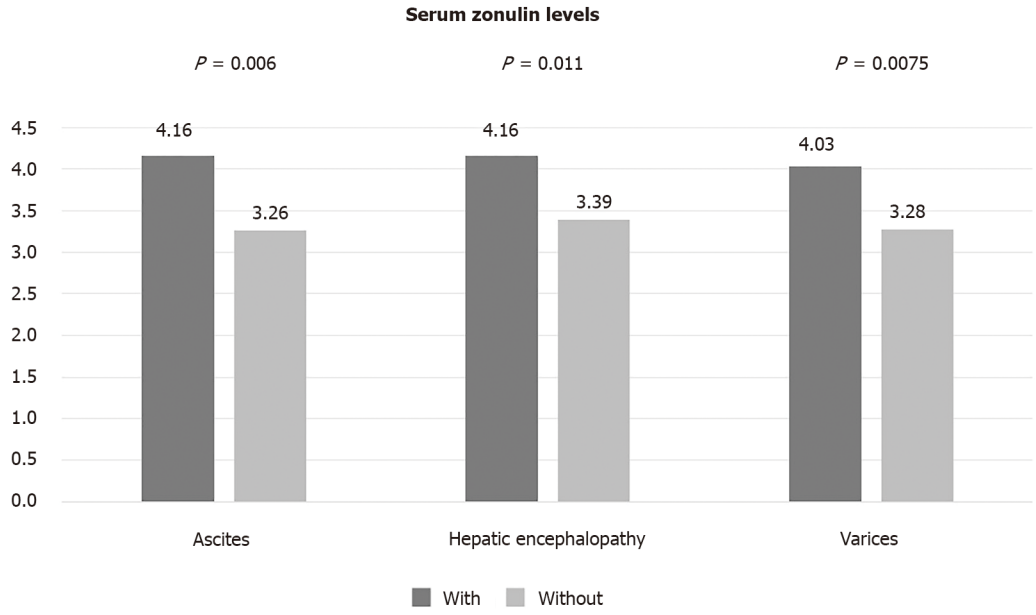

Figure 2 Mean serum zonulin levels (ng/mL) among different manifestations of advanced liver disease.

In our cohort, we investigated whether serum zonulin levels have any impact on the prognosis of patients with cirrhosis. Initially, we found that mean serum zonulin levels were higher in patients with CTP-B than CTP-A class cirrhosis, supporting its possible contribution in the development of decompensated liver disease (CTP-B stage). Interestingly, serum zonulin levels were lowest in our few cases with CTP-C cirrhosis; although, the small number of CTP-C patients in our study weakens the validity of such a finding, as any type of statistical errors cannot be excluded. The latter finding is in contrast to the results of a recently published study, which reported increasing serum zonulin levels from CTP-A to -B and to -C class. However, only chronic HBV patients were included in the abovementioned study and, more importantly, the study also included patients with HCC, a fact that could have affected the result[17]. The role of zonulin has also been previously investigated by others in small cohorts of patients with chronic liver disease. Serum zonulin levels were reported to decrease progressively, as liver function deteriorated in 9 patients with chronic viral hepatitis [18]. Obviously, such an under-powered study cannot lead to any valuable conclusion. In another study, serum zonulin levels were found to be lower in 40 patients with chronic HBV infection compared to 17 controls, but besides the small sample size of the study, no data for stage of liver disease were provided[19]. A pivotal study in children and adolescents reported increased serum zonulin levels in cases with rather

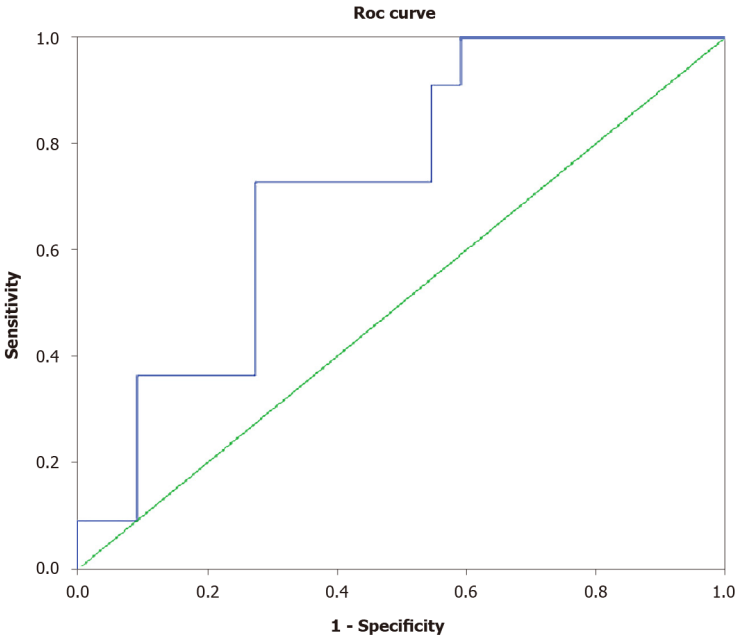

Figure 3 Receiver operating characteristic curve displacing baseline serum zonulin levels in predicting liver disease decompensation.
Area under the receiver operating characteristic curve: 0.723, *P* = 0.039. ROC: Receiver operating characteristic.

than without NASH[20]. In the latter study, zonulin levels were found to correlate with the severity of liver steatosis and not of liver fibrosis, but cases with cirrhosis were not included. Contrary to the previous studies, we recruited a larger number of cirrhotic patients, irrespective of liver disease etiology, while at the same time we excluded older patients or patients with HCC which could jeopardize our results.

Additionally, in our study, we found that patients with more advanced cirrhosis, as documented by the presence of ascites or history of HE, had higher serum zonulin levels compared to those without these complications. Unexpectedly, in our cohort, we found a numerical but not statistically significant difference in zonulin levels between patients with or without varices. Moreover, there was no correlation between serum zonulin and SSM, which by recent data is suggested to correlate wellwith hepatic venous pressure gradient levels and the presence of high-risk varices[21,22]. It could be argued that the secretion of zonulin is regulated by mechanisms acting locally in the gut and is not directly affected by changes in portal pressure. However, such a speculation, taking under consideration the complexity of mechanisms implicated in the regulation of gut permeability in liver cirrhosis carries a great level of uncertainty.

Finally, the potential association between serum zonulin levels and the development of liver decompensation is further supported by the predictive role of zonulin for such an outcome within 1year of follow-up. In particular, baseline serum zonulin levels in our patients with compensated cirrhosis were found to be independently associated with progression to decompensated liver disease within the next year. The predictability of serum zonulin levels to predict progression to decompensated liver disease was significant but suboptimal (AUROC: 0.723). In addition, serum zonulin levels < 3.65 ng/mL at baseline offered a NPV of 84% for progression to liver decompensation within the next year.

Our study has some limitations. A substantial proportion of patients did not participate in the follow-up study and we included a small number of patients with CTP-C stage disease. Furthermore, serum zonulin levels were measured in a single time frame. According to guidelines, in our department, no patient with compensated disease was under rifaximin treatment. Therefore, the effect of rifaximin or other antibiotic treatment (patients with acute infection were excluded from our study) in the transition from compensated towards decompensated disease and their correlation to zonulin levels were not assessed. Undoubtedly, serial measurements of zonulin levels and their fluctuations during the course of the liver disease would enforce its prognostic value.

CONCLUSION

In conclusion, we have clearly shown that serum zonulin levels are increased in patients with more advanced liver disease and are independently associated with the progression to decompensation. The results of our study may be of particular value as they reveal, for the first time, the adverse effect of a new agent, zonulin, on the deterioration of chronic liver disease. More studies are needed to confirm our findings and to further investigate the pathophysiological mechanisms by which zonulin is involved in alteration of intestinal barrier and gut permeability.Taking into consideration that zonulin antagonists are already being tested in phase IIb studies in diseases characterized by disrupted intestinal permeability, such as celiac disease, confirmation of our results may have significant clinical implications[23].

ARTICLE HIGHLIGHTS

Research background

Gut permeability is distorted in patients with liver cirrhosis and the observed deregulation of the intestinal integrity plays a crucial role in the development of bacterial translocation. Bacterial translocation contributes to the occurrence or aggravation of serious complications in patients with liver cirrhosis. Zonulin is a recently recognized protein, synthesized by the intestinal and liver cells, and thought to play an important role in the regulation of tight junctions between intestinal cells.

Research motivation

Increased zonulin levels have been observed in such diseases as celiac disease and inflammatory bowel disease and have shown correlation to the impairment of intestinal permeability. The exact mechanism that leads to the deregulation of the intestinal integrity in liver cirrhosis is not thoroughly investigated. Zonulin may have a role in the observed alterations of the gut barrier in advanced chronic liver disease.

Research objectives

We aimed to investigate if serum zonulin levels are altered in patients with different stages of liver cirrhosis and investigate their possible impact on patients' prognosis.

Research methods

We included 116 cirrhotic patients who attended our outpatient clinic during a 12-mo period. Serum zonulin levels were measured, as were epidemiological, laboratory and clinical data, and data from elastography and ultrasonography at baseline. Sixty-three patients were followed up for at least 1year and data from clinical events (death, liver transplantation and liver disease decompensation) were collected.

Research results

Our study included mainly Child-Turcotte-Pugh (CTP)-A (67%) and CTP-B patients (28%). We observed that serum zonulin levels are increased in patients with more advanced liver disease, such as patients with CTP–B stage, patients with ascites, or those with history of hepatic encephalopathy. What is more, serum zonulin levels were independently associated with the probability of decompensation within the next year.

Research conclusions

According to our study results, serum zonulin levels are increased in patients with advanced chronic liver disease. What is more, a new agent, zonulin, is found to be implicated in the progress towards advanced liver disease.

Research perspectives

Our findings highlight once more the significance of gut barrier deregulation in the setting of liver cirrhosis and emphasize the need of further studies in the field, aiming to reveal the complex pathophysiological interplay which leads to bacterial translocation. Especially, the role of zonulin should be further investigated, due to its possible therapeutic implications, as a zonulin antagonist alreadyexists and is being tested in studies of celiac disease.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Impact of biliary complications on quality of life in live-donor liver transplant recipients

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Abstract

BACKGROUND

Despite significant advancements in liver transplantation (LT) surgical procedures and perioperative care, post-LT biliary complications (BCs) remain a significant source of morbidity, mortality, and graft failure. In addition, data are conflicting regarding the health-related quality of life (HRQoL) of LT recipients. Thus, the success of LT should be considered in terms of both the survival and recovery of HRQoL.

AIM

To assess the impact of BCs on the HRQoL of live-donor LT recipients (LDLT-Rs).

METHODS

We retrospectively analysed data for 25 LDLT-Rs who developed BCs post-LT between January 2011 and December 2016 at our institution. The Short Form 12 version 2 (SF 12v2) health survey was used to assess their HRQoL. We also included 25 LDLT-Rs without any post-LT complications as a control group.

RESULTS

The scores for HRQoL of LDLT-Rs who developed BCs were significantly higher than the norm-based scores in the domains of physical functioning ($P = 0.003$), role-physical ($P < 0.001$), bodily pain ($P = 0.003$), general health ($P = 0.004$), social

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functioning (P = 0.005), role-emotional (P < 0.001), and mental health (P < 0.001). No significant difference between the two groups regarding vitality was detected (P = 1.000). The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains (P < 0.001) and the mental (P < 0.001) and physical (P = 0.0002) component summary scores.

CONCLUSION

The development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

Key Words: Live-donor liver transplantation; Quality of life; The Short Form 12 version 2; Cirrhosis; Biliary complications; Mental health

Core Tip: We retrospectively analysed data for 25 Live-donor liver transplantation recipients (LDLT-Rs) with biliary complications (BCs) and described their health-related quality of life (HRQoL) using the Short Form 12 version 2 health survey. All scores for HRQoL domains of LDLT-Rs with BCs were significantly higher than the norm-based scores except for vitality. The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains (P < 0.001) and in the mental (P < 0.001) and physical (P = 0.0002) component summary scores. We conclude that the development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

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INTRODUCTION

Health-related quality of life (HRQoL) is a multidimensional model reflecting the domains of social, mental, emotional, and physical health[1,2]. More than 50 different HRQoL tools have been used in liver transplant (LT) research[3], and no golden standard instrument has existed until now[4]. These tools can be classified into generic and disease-specific tools[3,5]. Generic HRQoL tools, of which the validated Short Form 36 (SF-36) health survey is the most frequently used for evaluating LT recipients, allow assessments across various medical conditions and health states[6,7].

Short Form 12 version 2 (SF-12v2) is a validated concise version of the SF-36 version 2 (SF-36v2) with only 12 questions[8,9]. Similar to the SF-36v2, it evaluates the same eight dimensions of HRQoL covering the previous 4 wk: General health, bodily pain, physical functioning, role physical, vitality, role emotional, mental health, and social functioning. Physical Component Summary (PCS) and Mental Component Summary (MCS) scores were created from patient responses[10]. The sum of scores ranges from 0 to 100, where 0 indicates the worst state of health and 100 indicates the best state of health[10,11].

The data are conflicting regarding the HRQoL of LT recipients. The heterogeneity between studies regarding the type of graft, diversity of included patients, and health survey precludes definitive conclusions[4,12]. In addition, an overlap exists between the primary liver disease and LT process with diverse events during peri- and postoperative management.

The global assessment of HRQoL after LT usually confirms improvement compared with pretransplant status[13]; however, it may remain suboptimal compared to the general population due to post-LT complications, recurrence of primary liver disease, or adverse effects of immunosuppressants[14-17]. In addition, cirrhosis leads to loss of muscle mass, sarcopenia, malnutrition, and physical impairment that manifest as

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physical frailty, increasing the risk of pretransplant mortality[18-20] and delayed improvement of physical functioning post-LT[21-23].

Fatigue affects up to 50% of patients with chronic liver disease; moreover, it demonstrates a significant association with poor HRQoL[24,25]. It also affects up to 60% of LT recipients[26]. It is a complex symptom that may be influenced by physical and mental states, including poor sleep quality, anxiety, and depression[27].

The LT candidates often have impaired HRQoL with a high prevalence of anxiety and depressive symptoms[28,29]. Moreover, LT was considered as post-traumatic stress disorder and was also found to be associated with anxiety and depression, which may further impair the HRQoL of LT recipients[30-33].

In the light of the above, HRQoL should be considered in terms of the outcome after LT[34,35]. Hence, we aimed to assess the impact of biliary complications (BCs) on the HRQoL of live-donor LT recipients (LDLT-Rs).

MATERIALS AND METHODS

Study design

We retrospectively analysed all LDLT-Rs at Ain Shams Centre for Organ Transplantation, Ain Shams Specialised Hospital, Cairo, Egypt, between January 2011 and December 2016. During this period, 215 adult patients underwent right-lobe LDLT at our centre. We included LDLT-Rs who developed BCs post-LT. We excluded LDLT-Rs with any of the following situations: cholestatic liver diseases (primary biliary cirrhosis or primary sclerosing cholangitis), vascular complications, acute or chronic rejection, recurrent hepatitis C virus (HCV) infection, graft failure, failure to follow up for at least one year post-LT, or patients who refused to participate in the research. As a result, 25 LDLT-Rs with BCs were included in the final analysis. We enrolled 25 LDLT-Rs who did not develop any post-LT complications as a control group. LT recipients were assessed at least 12 months post-LT, with median follow up duration of 5.5 years (range: 12 mo - 8 years).

This study was performed per the ethical principles of the declaration of Helsinki and was approved by the ethical committee of the Faculty of Medicine, Ain Shams University (No: FMASU MD 187/2016), which waived the requirement of informed consent due to the retrospective nature of the research.

Quality-of-life assessment

Eligible LDLT-Rs were invited to fulfil the SF-12v2 questionnaire during follow-up visits after obtaining verbal consent. We used anonymous questionnaires to ensure strict confidentiality. The SF-12v2 includes 12 questions: one question on general health perceptions, two questions concerning physical functioning, two questions on role limitations because of physical health problems, one question on bodily pain, one question on vitality, two questions on role limitations, one question on social functioning, and two questions on general mental health.

Statistical analysis

The data were analysed using IBM SPSS Statistics (v. 23; IBM Corp., Armonk, New York). Nonparametric numerical variables are presented as the median and interquartile range. Nominal variables are presented as the number and percentage. Ordinal data were analysed using the chi-squared test for trends. Two-sided P values < 0.05 were considered statistically significant.

RESULTS

This study included 25 adult right-lobe LDLT-Rs who experienced BCs. At the time of LT, the mean age of the recipients was 52 ± 7 years, and 19 (76%) recipients were male. Cirrhosis due to HCV was the most common indication for LT in 21 patients (84%; Tables 1 and 2).

Development and management of biliary complications

Among the 25 LDLT-Rs included in this study, minor biliary leakage occurred in 15 recipients (83.3%) and stopped spontaneously without further management. In only three (16.6%) recipients, pigtail insertion and further interventional management were needed. Moreover, 25 recipients developed a biliary infection, mainly occurring early

Table 1 Descriptive categorical data for live-donor liver transplant recipients with biliary complications

Variable		*n* (%)
Indication of liver transplantation	HCV	21 (84)
	HBV	1 (4)
	Combined HCV and HBV	1 (4)
	Hepatocellular carcinoma	2 (8)
Donors' gender	Male	17 (68)
	Female	8 (32)
Recipients' gender	Male	19 (76)
	Female	6 (24)
Immunosuppressant	Tacrolimus	22 (88)
	Cyclosporine	3 (12)
Biliary leakage	-	7 (28)
	+	18 (72)
Need of pigtail catheter for biloma (total = 18)	-	15 (83.3)
	+	3 (16.6)
Biliary infection	-	0 (0)
	+	25 (100)
Frequency of biliary infection (total = 25)	1-2 Episodes	16 (64)
	≥ 3 Episodes	9 (36)
Biliary stricture	-	5 (20)
	+	20 (80)
Frequency of biliary stricture (total = 20)	1-2 Episodes	13 (65)
	≥ 3 Episodes	7 (28)
Need for ERCP	-	5 (20)
	+	20 (80)
Frequency of ERCP	1-2 ERCP	13 (65)
	≥ 3 ERCP	7 (28)
Need for PTC	-	22 (88)
	+	3 (12)
Frequency of PTC	1 PTC	2 (66.6)
	2 PTC	1 (33.3)
Surgical intervention for stricture	-	19 (95)
	+	1 (5)
Admission related to biliary complications	-	0 (0)
	+	25 (100)
Early biliary infection (total = 25)	-	2 (8)
	+	23 (92)
Early biliary stricture (total = 20)	-	17 (68)
	+	8 (32)

Data presented in number (*n*) and percentage (%). ERCP: Endoscopic retrograde cholangiopancreatography; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PTC: Percutaneous transhepatic cholangiography.

(23; 92%) and in one to two episodes in 16 (64%) recipients (Table 1). Furth-

Table 2 Descriptive numerical data for live-donor liver transplant recipients with biliary complications

Variable	Data
MELD score	15 ± 3
Child score	9 ± 2
Donors' age (yr)	30 ± 4
Donors' BMI (kg/m^2)	25 ± 4
Recipient's age (yr)	52 ± 7
Recipient's BMI (kg/m^2)	27 ± 6
Total bilirubin (mg/dL)	2.9 (2-3.9)
Direct bilirubin (mg/dL)	1.6 (0.9-2.3)
Alkaline phosphatase (IU/L)	190 ± 49
Gamma-glutamyl transferase (IU/L)	100 (50-130)
Platelets (10^9/L)	75 ± 31
Cold ischemia time (min)	48 ± 25
Warm ischemia time (min)	47 ± 23
Graft arterialization time (min)	145 ± 53
Time to biliary infection (d)	13 (11-36)
Time to biliary stricture (d)	130 (120-190)

Data are presented as mean ± SD or median and range. BMI: Body mass index; MELD: Model for end stage liver disease.

er, 20 (80%) recipients developed biliary stricture, most of which presented in one to two episodes (13; 65%). The development of BCs caused a prolonged hospital stay (median = 46 days; range: 15 - 67 days), with nine (36%) patients needing ≥ three episodes of admission. Concerning the management of BCs, endoscopic retrograde cholangiopancreatography (ERCP) with stenting ± dilatation was done for 20 (80%) recipients, with seven (28%) recipients needing ≥ three ERCP sessions. Percutaneous transhepatic cholangiography was needed for only three (12%) recipients, with one recipient requiring another session. These methods only failed in one recipient who needed surgical reconstruction of the biliary stricture (Table 1).

Health-related quality of life

The scores of HRQoL of LDLT-Rs with BCs were significantly higher than the norm-based scores in the domains of physical functioning (P = 0.003), role-physical (P < 0.001), bodily pain (P = 0.003), general health (P = 0.004), social functioning (P = 0.005), role-emotional (P < 0.001), and mental health (P < 0.001). In contrast, no significant difference was found between the two groups regarding vitality (P = 1.000; Table 3 and Figure 1). The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains (P < 0.001) and in the mental (P < 0.001) and physical (P = 0.0002) component summary scores (Tables 4 and 5; Figures 1 and 2).

DISCUSSION

Despite the considerable advances in LT surgical techniques and perioperative care, post-LT BCs remain a significant source of morbidity, mortality, and graft failure[36]. To our knowledge, no previous study has specifically assessed the impact of BCs on the HRQoL of LDLT-Rs. In our study, LDLT-Rs with BCs had significantly higher HRQoL domain scores except for the vitality domain than norm-based scores; however, those patients gained a significantly lower range of improvement in HRQoL domains with lower MCS and PCS scores than those without BCs. This result can be attributed to more prolonged and frequent hospital admission and expectation reduction with anxiety, stress, and depression[37]. In agreement with the current results, the published literature has observed the positive effects of LT on the

Table 3 Comparison of the quality-of-life scores for live-donor liver transplant recipients with biliary complications and their corresponding norm-based scores

HRQoL score	LDLT-R with BC	NBS score	*P* value[1]
Physical functioning	50 (50-75)	41.3 (41.3-49.2)	0.003
Role physical	50 (31.3-75)	40.5 (34.2-49)	0.001
Bodily pain	50 (50-75)	39.7 (39.7-48.7)	0.003
General health	60 (60-85)	47.8 (47.8-57.7)	0.004
Vitality	50 (25-50)	49.1 (39.2-49.1)	1.000
Social functioning	50 (50-50)	39.1 (39.1-39.1)	0.005
Role emotion	50 (37.5-75)	35.5 (30.3-45.9)	< 0.001
Mental health	50 (50-62.5)	41.3 (41.3-47)	0.001

[1]Wilcoxon signed ranks test.

Data are shown as median and interquartile range. BC: Biliary complications; HRQoL: Health related quality of life; LDLT-R: Live donor liver transplant recipients; NBS: Norm based score.

Table 4 Comparison of health-related quality-of-life scores between patients and controls

HRQoL domain	Patients (*n* = 25)	Controls (*n* = 25)	*P* value[1]
Physical functioning	50 (50-75)	100 (100-100)	< 0.001
Role physical	50 (31.3-75)	100 (87.5-100)	< 0.001
Bodily pain	50 (50-75)	100 (100-100)	< 0.001
General health	60 (60-85)	85 (85-85)	< 0.001
Vitality	50 (25-50)	75 (75-87.5)	< 0.001
Social functioning	50 (50-50)	75 (75-100)	< 0.001
Role emotion	50.0 (37.5-75)	87.5 (75-100)	< 0.001
Mental health	50 (50-62.5)	87.5 (75-87.5)	< 0.001
PCS	44.8 (41.7-52.9)	57.8 (55.2-59)	< 0.001
MCS	42 (35.6-45.2)	52.9 (50.2-57.9)	< 0.001

[1]Wilcoxon signed ranks test.

Data are shown as median and interquartile range; Patients: Live donor liver transplant recipients with biliary complications; Controls: Live donor liver transplant recipients without biliary complications; HRQoL: Health-related quality-of-life; MCS: Mental component summary score; PCS: Physical component summary score.

recipients' HRQoL[12,37-40].

Similar to the present study[41], other authors have assessed the LT recipients' HRQoL using the WHOQOLBREF questionnaire[42] and Transplant Effects Questionnaire[43] and concluded that LT recipients, especially those who received LDLT, reported the highest level of HRQoL in all four dimensions of HRQoL in comparison to those with other organ transplantation.

In partial agreement with the current study, a review of 32 studies and 5402 patients found that the overall HRQoL scores of LT recipients remain improved and equivalent to the general population in the long term. However, physical functioning continues to be inferior to the general population despite a noticeable improvement from preoperative physical functioning[4]. Similarly, a review article of 31 publications reported improved overall HRQoL and physical functioning in deceased donor LT (DDLT) adult recipients during the first 2 years, which remains stable in the long term but does not reach the level of the general population[35]. Additionally, Sullivan *et al* [44] assessed the HRQoL two decades after DDLT using the SF-12 survey. In adult survivors, the MCS score (54.6) was equivalent to that of the general population; however, the PCS score (39.3) remained below average. This outcome can be explained by the presence of comorbidities, primary liver disease severity, postoperative

Table 5 Physical and mental component summary scores in patients and controls compared with norm-based scores

Variable	NBS	Patients (*n* = 25), %	Control (*n* = 25) , %	*P* value[1]
Physical component summary score	At or above	11 (44)	25 (100)	0.0002
	Below	8 (32)	0 (0)	
	Far below	6 (24)	0 (0)	
Mental component summary score	At or above	7 (28)	24 (96)	< 0.0001
	Below	7 (28)	1 (4)	
	Far below	11 (44)	0 (0)	

[1]Chi-squared test for trend.

Data are shown as number and percentage. Patients: Live donor liver transplant recipients with biliary complications; Controls: Live donor liver transplant recipients without biliary complications; NBS: Norm based score.

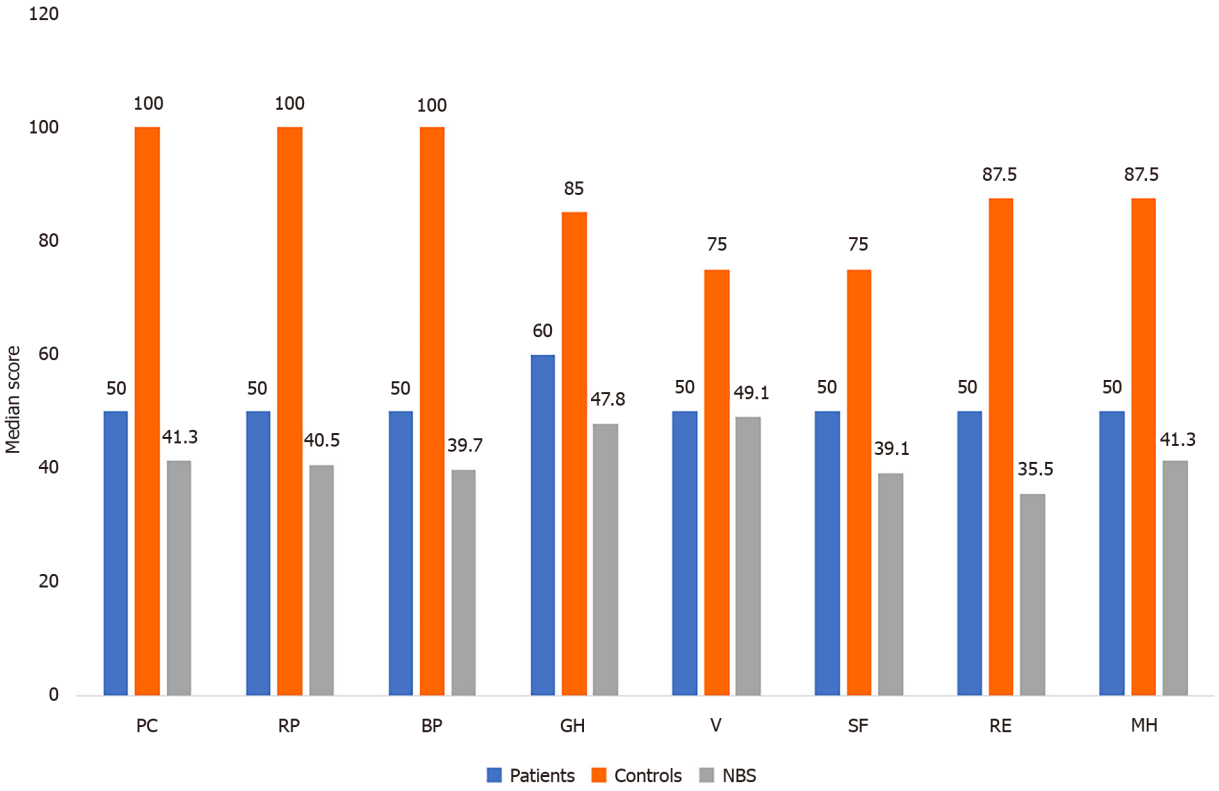

Figure 1 Short Form 12 (v. 2) domains in patients and controls compared to the norm-based score. BP: Bodily pain; GH: General health; MH: Mental health; NBS: Norm based score; PF: Physical functioning; RE: Role emotion; RP: Role physical; SF: Social functioning; V: Vitality.

morbidity, and graft type[20,33]. Additionally, Dunn *et al*[45] reported that group exercise activities were correlated with improved physical function, mental health, and HRQoL, independent of comorbidities, for up to 5 years after LT. Therefore, physical activity should be encouraged after LT[46].

In a study by Casanovas *et al*[47], the SF-36 scores of 156 LT candidates were assessed pre- and post-LT. They observed significantly lower patient baseline scores in all HRQoL domains than general population scores, especially in physical health. As early as 3 months till 1-year post-LT, they detected improvement in all SF-36 domains except vitality and social functioning, revealing no significant improvement. Moreover, sleeping problems were observed at the baseline and persisted post-LT. The poor sleep quality frequently noted in cirrhotic patients is known to cause fatigue and impair cognitive and physical functions[48].

In contrast to our results, Domingos *et al*[37] retrospectively assessed the HRQoL of 93 DDLT recipients who survived 10 years post-LT using the SF-36 survey and observed that LT recipients had lower mental health scores than the general

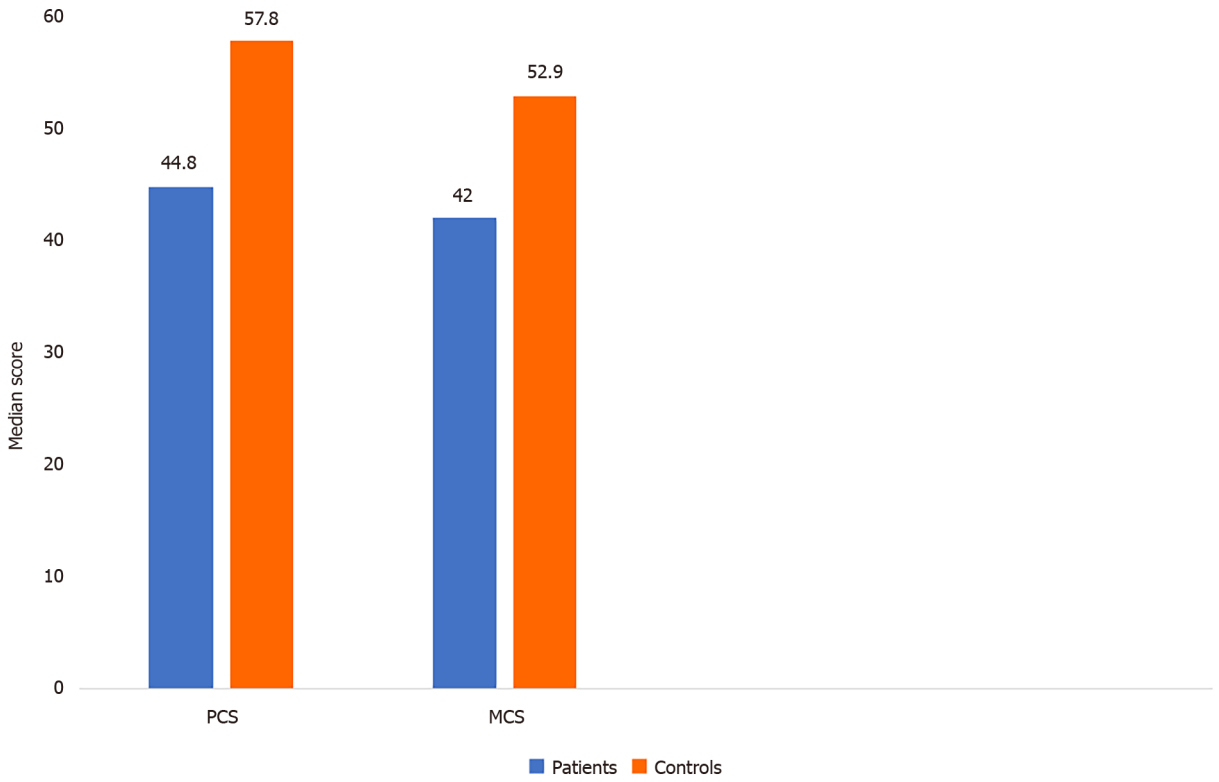

Figure 2 Physical and mental component summary scores of patients and controls. Patients: Live donor liver transplant recipients with biliary complications; Controls: Live donor liver transplant recipients without biliary complications; MCS: Mental component summary score; PCS: Physical component summary score.

population. In all other domains, LT recipients had similar (emotional limitations, pain, and general health status) or superior (physical limitations, social aspects, functional capacity, and vitality) scores than the general population. In addition, Dąbrowska-Bender *et al*[15] assessed the SF-36 health survey in 121 DDLT recipients and observed no change in mental health score, whereas significant physical impairment was reported by 18.18% of the recipients.

In a study by Annema *et al*[30], LT had a beneficial effect on the mental health of LT recipients by ameliorating anxiety and depression symptom severity. However, recipients with persistent symptoms of anxiety and depression experienced a negative effect on HRQoL and therapeutic adherence. They also observed that persistent anxiety and depression were correlated with the development of BCs and the duration of the hospital stay. Similarly, in another report[49], the HRQoL of 82 LT recipients was retrospectively assessed, finding 94% reported high mean scores on HRQoL, the McGill Quality of Life Questionnaire, and adherence to medications. Conversely, patients with a low HRQoL reported anxiety, depression, fatigue, slowing pace, and physical limitations, suggesting that LT recipients who fail to adapt to their post-LT state experienced a decreased ability to tolerate physical symptoms and post-LT complications[50]. Other causes for lower mental health scores post-LT are the worry regarding medication side effects, hepatic disease recurrence, and other potential complications[51].

Candidates for LT may have overly optimistic anticipations for post-LT improvement in their HRQoL. Unfulfillment of these expectations may negatively affect their HRQoL, highlighting the need to help patients expect and understand the outcomes of LT. Moreover, LT candidate education positively affects post-LT HRQoL[40]. Education is associated with better outcomes and higher patient adherence[52].

This study is limited by its retrospective nature and small sample size. More research is required to define the predictors of HRQoL and plan multidisciplinary strategies for HRQoL improvement in LT recipients. According to the current literature, HRQoL should be integrated into the clinical care of LT[53].

CONCLUSION

We conclude that the development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

ARTICLE HIGHLIGHTS

Research background

Despite the considerable advances in liver transplantation (LT) surgical techniques and perioperative care, post-LT biliary complications (BCs) remain a significant source of morbidity, mortality, and graft failure. Due to the current high survival rates of LT, the focus has shifted to improving the quality of life of LT recipients.

Research motivation

The data are conflicting regarding the health-related quality of life (HRQoL) of LT recipients.

Research objectives

To assess the impact of BCs on the HRQoL of live-donor LT recipients (LDLT-Rs).

Research methods

We retrospectively analysed data for 25 LDLT-Rs with BCs and described their HRQoL through the Short Form 12 version 2 (SF-12v2) health survey compared to 25 LDLT-Rs without post-LT complications.

Research results

The scores of HRQoL of LDLT-Rs with BCs were significantly higher than the norm-based scores in all HRQoL domains except vitality. The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains ($P < 0.001$) and in the mental ($P < 0.001$) and physical ($P = 0.0002$) component summary scores.

Research conclusions

The development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

Research perspectives

The assessment of HRQoL should be integrated into the clinical care of LT recipients. Identifying the determinants of HRQoL could improve the management plan of these patients through a multidisciplinary approach.

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ORIGINAL ARTICLE

Retrospective Study

Machine learning models for predicting non-alcoholic fatty liver disease in the general United States population: NHANES database

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, affecting over 30% of the United States population. Early patient identification using a simple method is highly desirable.

AIM

To create machine learning models for predicting NAFLD in the general United States population.

METHODS

Using the NHANES 1988-1994. Thirty NAFLD-related factors were included. The dataset was divided into the training (70%) and testing (30%) datasets. Twenty-four machine learning algorithms were applied to the training dataset. The best-performing models and another interpretable model (*i.e.*, coarse trees) were tested using the testing dataset.

RESULTS

There were 3235 participants (n = 3235) that met the inclusion criteria. In the training phase, the ensemble of random undersampling (RUS) boosted trees had the highest F1 (0.53). In the testing phase, we compared selective machine learning models and NAFLD indices. Based on F1, the ensemble of RUS boosted trees remained the top performer (accuracy 71.1% and F1 0.56) followed by the fatty liver index (accuracy 68.8% and F1 0.52). A simple model (coarse trees) had

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an accuracy of 74.9% and an F1 of 0.33.

CONCLUSION

Not every machine learning model is complex. Using a simpler model such as coarse trees, we can create an interpretable model for predicting NAFLD with only two predictors: fasting C-peptide and waist circumference. Although the simpler model does not have the best performance, its simplicity is useful in clinical practice.

Key Words: Artificial intelligence; Machine learning; Non-alcoholic fatty liver disease; Fatty liver; United States population; NHANES

Core Tip: A simple method with a good accuracy for identifying patients with non-alcoholic fatty liver disease is highly desirable. Among 24 machine learning models, the ensemble of random undersampling boosted trees was the top performer (accuracy 71.1% and F1 0.56). A simple model (coarse trees) with only two predictors (fasting C-peptide and waist circumference) had an accuracy of 74.9% and an F1 of 0.33. Not every machine learning model is complex. Using a simple model such as coarse trees, physicians can easily integrate machine learning model into their practice without any software implementation.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common chronic metabolic disease found in 25.5% of the United States population, and it is more common in patients with diabetes (55.5%), leading to a health and economic burden[1-3]. Non-alcoholic steatohepatitis (NASH) can lead to liver-related consequences, such as cirrhosis, hepatocellular carcinoma, and mortality. NASH is the second most common indication for liver transplantation in the United States and is likely to replace hepatitis C infection as the leading cause of liver transplantation in the future[4]. NAFLD is diagnosed primarily with imaging studies, transient elastography, magnetic resonance elastography, or liver biopsy[5]. Some of these diagnostic modalities are not available in every health care facility, require expert interpretation, and are invasive in case of biopsy[5,6]. To prevent adverse outcomes in these patients, early screening and detection based on risk factors are warranted. Healthcare providers and patients are aware of the risk factors of NAFLD, which include diabetes, obesity, dyslipidemia, and metabolic syndrome[5,7,8]. However, there is no well-performing tool for the early prediction of NAFLD; for example, liver enzyme levels can be normal in patients with NAFLD[9,10]. There are existing studies on the risk factors and prediction risk scores; however, their results are controversial[11-15]. Machine learning is a potential approach for the identification of the best predictive model[16].

Machine learning can be used to construct a predictive model by teaching computer algorithms to learn from data without being explicitly programmed. Applications of machine learning in gastroenterology field are steadily increasing[17]. However, there is no machine learning model for predicting NAFLD in the United States. The published models in China, Germany, and Canada focus on NAFLD prediction scores using laboratory parameters and demographic data[11,13-15]. Therefore, we aimed to evaluate the applications of machine learning in NAFLD diagnosis for easy use at clinical setting.

MATERIALS AND METHODS

Study population and study design

The Third National Health and Nutrition Examination Survey (NHANES III) was a nationwide probability sample of 39695 persons aged 2 mo and older, conducted from 1988-1994 by the National Center for Health Statistics (NCHS). It aimed to evaluate the health and nutritional status of the general United States population[18]. Multiple datasets were collected in this survey, including demographics, interviews, physical examinations, and laboratory testing of biologic samples. The NHANES protocol was approved by the NCHS Research Ethics Review Board.

Definitions

Participants aged 20 years or older in NHANES III with gradable ultrasound results were included in this study. The exclusion criteria included: (1) Excessive alcohol consumption; (2) Hepatitis B or C infection; (3) Fasting period outside of 8-24 h; and (4) Incomplete or missing data on physical examination and laboratory testing. The participants were divided into two groups: The NAFLD participants and non-NAFLD participants. Since participants aged above 74 years were not eligible for ultrasonography in NHANES III, participants aged above 74 years were excluded from this study.

'NAFLD participants' was defined based on: (1) Moderate to severe hepatic steatosis on ultrasound; (2) No history of alcohol drinking more than 2 drinks per day for men or 1 drink per day for women in the last 12 mo; and (3) No history of hepatitis B or C infection.

Thirty factors associated with NAFLD were included in this study: demographic (*i.e.*, age, gender, and race/ethnicity), body measurement [*i.e.*, body mass index (BMI) and waist circumference], general biochemistry tests [*i.e.*, iron, total iron-binding capacity, transferrin saturation, ferritin, cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, C-reactive protein, and uric acid], liver chemistry (aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase, total bilirubin, total protein, albumin, and serum globulin), diabetes testing profile [*i.e.*, glycated hemoglobin, fasting plasma glucose, fasting C-peptide, and fasting insulin], and the use of diabetes medication.

Statistical analysis

Categorical and ordinal factors are presented as frequencies (%). Continuous factors are presented as medians (interquartile ranges). The dataset was divided into the training (70%) and testing (30%) datasets using stratified sampling. Differences between the two datasets were tested using the Mann-Whitney U test. Twenty-four machine learning algorithms were applied to the training dataset. Then, we selected the best performing models determined by accuracy and the F1 score and compared the out-of-sample performance with another interpretable model (coarse trees, a decision tree model with a maximum of four splits) and three NAFLD indices on the testing dataset. The selected NAFLD indices included fatty liver index (FLI), hepatic steatosis index (HSI), and triglyceride and glucose index (TyG)[19-21]. The cut-off levels for NAFLD were ≥ 60 for FLI, > 36 for HSI, and ≥ 8.5 for TyG. The performance metrics include accuracy, sensitivity or recall, specificity, precision, area under the receiver operating characteristic curve (AUC), and the F1 score. It is worth noting that the F1 score is the harmonic mean of precision and recall. All statistical analyses were performed using MATLAB R2020a (MathWorks, MA, United States).

RESULTS

The study had 3235 participants (n = 3235). The participant selection process is shown in Figure 1. Based on ultrasound findings, 817 (25.26%) participants had NAFLD. The data of 2265 (70%) and 970 (30%) participants made up the training and testing groups, respectively. The baseline characteristics of participants in the training and testing groups are summarized in Table 1. There were no significant differences between the datasets for all factors.

The performances of 24 machine learning algorithms that were applied to the training dataset are illustrated in Table 2. The ensemble of subspace discriminant and ensemble of random undersampling (RUS) boosted trees had the highest accuracy (78.3%) and highest F1 score (0.53), respectively; both models had an AUC of 0.76. The coarse trees, decision trees with a few leaves, had an accuracy of 76%, AUC of 0.68,

Table 1 Baseline characteristics of participants in training and testing data

	Training data (*n* = 2265)	Testing data (*n* = 970)	*P* value
Demographic			
Age (yr)	43 (29)	43.5 (28)	0.328
Gender (male) (%)	944 (41.68)	428 (44.12)	0.197
Race/ethnicity			
White (non-Hispanic) (%)	959 (42.34)	392 (40.41)	0.308
Black (non-Hispanic) (%)	627 (27.68)	271 (27.94)	0.882
Mexican American (%)	576 (25.43)	254 (26.19)	0.652
Others (%)	103 (4.55)	53 (5.46)	0.265
Body measurement			
Body mass index (kg/m^2)	26.4 (7.2)	26.7 (7.4)	0.120
Waist circumference (cm)	93 (20.5)	93.5 (20.8)	0.182
Biochemistry tests			
Iron (ug/dL)	73 (39)	74 (39)	0.098
Total iron-binding capacity (ug/dL)	355 (72)	356 (72)	0.450
Transferrin saturation (%)	20.5 (11.1)	20.8 (11.8)	0.329
Ferritin (ng/mL)	87 (125)	84.5 (124)	0.508
Cholesterol (mg/dL)	201 (57)	204 (59)	0.155
Triglyceride (mg/dL)	120 (100.25)	122.5 (102)	0.562
HDL cholesterol (mg/dL)	48 (18)	48.5 (18)	0.585
C-reactive protein (mg/dL)	0.21 (0.29)	0.21 (0.23)	0.686
Uric acid (mg/dL)	5 (1.9)	5.1 (2)	0.427
Liver chemistry			
Aspartate aminotransferase (U/L)	19 (8)	19 (7)	0.908
Alanine aminotransferase (U/L)	14 (10)	14 (10)	0.581
Gamma glutamyl transferase (U/L)	21 (18)	21 (18)	0.787
Alkaline phosphatase (U/L)	83 (33)	81 (32)	0.524
Total bilirubin (mg/dL)	0.5 (0.2)	0.5 (0.2)	0.855
Total protein (g/dL)	7.4 (0.6)	7.4 (0.6)	0.559
Albumin (g/dL)	4.1 (0.5)	4.1 (0.4)	0.543
Serum globulin (g/dL)	3.3 (0.6)	3.3 (0.7)	0.941
Diabetes testing profile			
Glycated hemoglobin (%)	5.4 (0.8)	5.4 (0.7)	0.075
Fasting plasma glucose (mg/dL)	91.6 (12.52)	92.05 (12.2)	0.726
Fasting C-peptide (pmol/mL)	0.65 (0.68)	0.66 (0.69)	0.746
Fasting insulin (uU/mL)	9.36 (9.51)	9.73 (10.04)	0.378
Diabetes medication	165 (7.28%)	68 (7.01%)	0.782

and F1 score of 0.36.

As shown in the first half of Table 3, the ensemble of subspace discriminant, coarse trees, and ensemble of RUS-boosted trees models were selected for testing the process on the testing data. When tested on the testing data, ensemble of subspace discriminant and ensemble of RUS-boosted trees still had a high accuracy (77.7%) and high F1 (0.56), respectively. The coarse tree had an accuracy of 74.9% and an F1 of 0.33. All the machine learning models and datasets are available for public access in the File

Table 2 The performance comparison of machine learning models on training data

No.	Description	Accuracy (%)	AUC	PPV/precision (%)	NPV (%)	Sensitivity/recall (%)	Specificity (%)	F1
1	Fine tree	71.6	0.64	42.9	79.8	37.8	83.0	0.40
2	Medium tree	74.4	0.70	48.9	79.1	30.1	89.4	0.37
3	Coarse tree	76.0	0.68	55.1	78.9	26.4	92.7	0.36
4	Linear discriminant	78.0	0.75	61.1	80.9	35.5	92.4	0.45
5	Logistic regression	78.1	0.75	62.2	80.6	33.9	93.0	0.44
6	Gaussian naïve Bayes	75.1	0.74	50.8	81.1	40.2	86.8	0.45
7	Kernel naïve Bayes	72.7	0.73	46.8	85.1	60.1	76.9	0.53
8	Linear SVM	77.0	0.74	64.4	78.1	19.9	96.3	0.30
9	Quadratic SVM	77.4	0.70	59.9	80.1	31.8	92.8	0.42
10	Cubic SVM	72.8	0.64	45.1	79.6	35.3	85.5	0.40
11	Fine Gaussian SVM	74.7	0.67		74.7		100.0	
12	Medium Gaussian SVM	77.5	0.74	63.9	79.0	25.3	95.2	0.36
13	Coarse Gaussian SVM	75.7	0.74	66.2	76.0	7.9	98.6	0.14
14	Fine KNN	68.9	0.58	38.0	78.9	36.9	79.7	0.37
15	Medium KNN	76.5	0.71	59.7	78.1	21.0	95.2	0.31
16	Coarse KNN	76.6	0.75	78.1	76.5	10.0	99.1	0.18
17	Cosine KNN	76.6	0.72	57.9	79.2	27.6	93.2	0.37
18	Cubic KNN	77.0	0.72	62.0	78.5	22.6	95.3	0.33
19	Weighted KNN	76.5	0.71	56.7	79.4	28.8	92.6	0.38
20	Ensemble of boosted trees	76.9	0.74	57.3	80.3	33.6	91.6	0.42
21	Ensemble of bagged trees	77.2	0.74	58.9	80.2	32.5	92.3	0.42
22	Ensemble of subspace discriminant	78.3	0.76	66.7	79.7	28.3	95.2	0.40
23	Ensemble of subspace KNN	75.5	0.69	54.7	77.2	16.4	95.4	0.25
24	Ensemble of RUS boosted trees	70.4	0.76	44.2	86.3	66.4	71.7	0.53

AUC: Area under the curve; KNN: K-nearest neighbors; NPV: Negative predictive value; PPV: Positive predictive value; RUS: Random undersampling; SVM: Support vector machine.

Exchange portal of the MATLAB Central File Exchange[22]. The performance of three NAFLD on the testing data are also displayed in the second half of Table 3. FLI was the best performer among the NAFLD indices with the accuracy of 68.6% and F1 score of 0.52. However, the ensemble of RUS boosted trees was superior to FLI in all metrics.

DISCUSSION

Our study compared 24 different machine learning techniques to determine the optimal clinical predictive model for NAFLD. The accuracy of these models on the training data did not show much variation (range 9.4%), with an average of 75.5% (Table 2). The top two models were ensemble of subspace discriminant and ensemble of RUS boosted trees. The ensemble of subspace discriminant model had a higher accuracy while the ensemble of RUS boosted trees model had a better performance in classifying positive NAFLD, as indicated by the F1 score. Both models were ensemble type, which use multiple diverse models in combination to produce an optimal prediction. They are more complex machine learning models that apparently yield better predictions. Compared to accuracy, the F1 score is regarded as a superior performance metric for a class imbalance problem (often a large number of actual

Table 3 The performance of machine learning models and other non-alcoholic fatty liver disease indices on testing data

No.	Description	Accuracy (%)	AUC	PPV/precision (%)	NPV (%)	Sensitivity/recall (%)	Specificity (%)	F1
Machine learning models								
1	Ensemble of subspace discriminant	77.7	0.78	66.7	78.8	23.7	96	0.35
2	Coarse trees	74.9	0.72	50.8	78.3	24.5	92	0.33
3	Ensemble of RUS boosted trees	71.1	0.79	45.5	88.4	72.7	70.6	0.56
NAFLD indices								
4	Fatty liver index	68.6	0.74	42.4	86.6	68.6	68.6	0.52
5	Hepatic steatosis index	65.1	0.70	37.9	83.3	60.4	66.6	0.47
6	Triglyceride and glucose index	56.9	0.69	34.8	88.3	80.8	48.8	0.49

AUC: Area under the curve; NAFLD: Non-alcoholic fatty liver disease; NPV: Negative predictive value; PPV: Positive predictive value; RUS: Random undersampling.

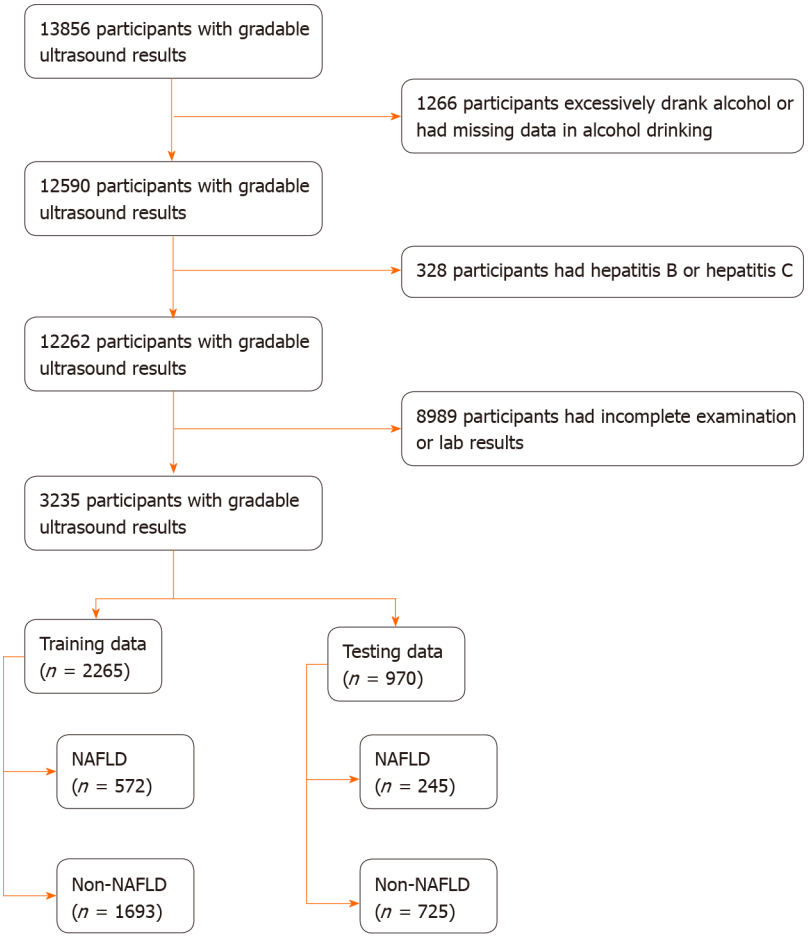

Figure 1 Study design and data partitioning flow chart. NAFLD: Non-alcoholic fatty liver disease.

negatives). In our opinion, the ensemble of RUS boosted trees model was the best performing machine learning model in this study.

Technically, the final prediction of the ensemble method was derived from a combination of multiple predictions from different algorithms. In our case, the predicted outcome of the ensemble of RUS boosted trees model was derived from a weighted average outcome of 30 RUS boosted trees; the sample visualization of these RUS boosted trees can be found in the file uploaded to the MATLAB Central File Exchange[22].

On the other hand, we compared the performance of the previous model with the coarse trees model, simple decision trees with several leaves and splits (Figure 2). The decision logic of the coarse trees model consisted of only two factors: Waist circumference and serum C-peptide. In terms of testing performance, it had a reasonable accuracy (AUC, 0.72; accuracy, 74.9%; and F1 score, 0.33). Since it is simple-to-use and easily interpretable, the coarse trees model can be more practically used in clinical practice.

Waist circumference is directly associated with obesity and metabolic syndrome[23,24]. They are also the established risk factors of NAFLD. The cut-off of 109.35 cm seems to be slightly higher than the general cut off value for metabolic syndrome (men, 102 cm and women, 80 cm)[25]. It is used to calculate the visceral adiposity index, which provides a good predictive capability[26]. The advantage of incorporating waist circumference into the model is its retrieval ability.

Our results are similar to those of a previous study identifying the risk factors of NAFLD[27]. C-peptide is an indicator of insulin resistance[28,29]. Serum C-peptide is associated with NAFLD, NASH, and fibrosis progression[28-30]. Additionally, serum C-peptide levels increase with NAFLD severity[29,31,32]. In our study, serum C-peptide is more significantly associated with NAFLD prediction than liver function test. This can be explained by the fact that liver enzymes are possibly not specific to NAFLD. They can also be elevated in other liver diseases. On the contrary, serum C-peptide is related to metabolic alterations, which play a direct role in NAFLD development.

We compared the performance of three NAFLD indices (FLI, HSI, and TyG) on the testing data. Among these three NAFLD indices, FLI had the highest performance in terms of accuracy (68.6%) and F1 (0.52). However, performance-wise, the ensemble of RUS boosted trees was superior to FLI in all aspects. In terms of simplicity, FLI is not complex, but it might be impossible for physicians to use it without spreadsheets or computers because it involves many mathematical operations, such as multiplication, logarithm function, and exponential function. Therefore, coarse trees remained the simplest model.

Previously developed machine learning models for NAFLD prediction have used more complex parameters, including laboratory and noninvasive scores. A population-based study in Italy developed a score for NAFLD diagnosis with a moderate accuracy of 68% in the model development phase, but extremely high performance in the testing (prediction) phase using the small sample size of 50. The predictors used in the model were of abdominal volume index, glucose, gamma glutamyl transferase, age, and sex[33]. A Chinese study incorporated three demographic factors and 15 Laboratory tests as predictors for Bayesian network model[8]. The inclusion of simple constituents, liver enzymes, lipid panels, and complete blood count resulting in an accuracy of up to 80% in a 10-fold cross validation; there was no separate data set for external validation or testing. A Taiwanese study revealed that waist circumference was the most influential factor in the model resulting in a high performance with an AUC of 0.925[13]. Similarly, such performance was based on a 10-fold cross validation, not on a separate data set for external validation or testing. In addition, the ethnic Chinese population generally has a lower alcohol consumption; it might not be generalized to other ethnic groups[12,15]. A Canadian study revealed that HDL, BMI, sex, plasma glucose, blood pressure, and age were factors used in the decision criteria of decision trees with an AUC of 0.73[14]. These reports showed different significant factors in their models. This might be explained by the different populations in terms of ethnicity, alcohol consumption, and obesity prevalence. Compared to prior reports, our study involved a general population of the United States, which has less selection bias and contains diverse races. Therefore, the derived models in this study can be applied to diverse ethnic and racial backgrounds. A detailed comparison of the proposed machine learning models in prior reports is summarized in Table 4.

The application of machine learning in regarding NAFLD has evolved from the diagnosis with the noninvasive screening methods to liver biopsy. The new score achieves the reasonable performance with AUC of 0.70, in terms of differentiating between NAFL and NASH[11]. Deep learning model was evaluated for diagnosis NAFLD based on ultrasound images and had a good predictive ability (AUC > 0.7)[34]. Given the advancement in this field, it can also be used to quantify steatosis, inflammation, ballooning, and fibrosis in biopsy histology of patients with NAFLD having excellent results[35].

This study had strengths. First, this is the first United States population-based study with more than 3000 individuals from NHANES III. Secondly, we aimed to propose the simple model with a reasonable predictive power for NAFLD. This model will be potentially applied in clinical practice, especially by primary care providers, prior to

Table 4 The performance comparison of published machine learning models on non-alcoholic fatty liver disease prediction

Ref.	Type of data/country or territory of data	Number of train/ external testing data	Model	Accuracy (%)	AUC	Sensitivity (%)	Specificity (%)	F1
Sorino *et al* [33], 2020	Population/Italy	2920/50	Support vector machine	68[1]	N/A	98.5	100	N/A
Wu *et al*[13], 2019	Hospital/Taiwan	577/NA	Random forest	86.5[1]	0.925[1]	87.2[1]	85.9[1]	N/A
Islam *et al* [36], 2018	Hospital/Taiwan	994/NA	Logistic regression	70[1]	0.763[1]	74.1[1]	64.9[1]	N/A
Ma *et al*[12], 2018	Hospital/China	10508/NA	Bayesian network	82.92[1]	N/A	67.5[1]	87.8[1]	0.655[1]
Perveen *et al* [14], 2018	Primary care network/Canada	64%/34% of 40637	Decision trees	N/A	0.73	73	N/A	0.67
Yip *et al*[15], 2017	Hospital/Hong Kong	500/442	Ridge regression	87	0.87	92	90	N/A
Birjandi *et al* [37], 2016	Hospital/Iran	359/1241	Decision trees	75	0.75	73	77	N/A
Our study	Population based/United States	2265/970	Ensemble of RUS boosted trees	71.1	0.79	72.7	70.6	0.56
			Coarse trees	74.9%	0.72	24.5%	92%	0.33

[1]Cross-validation performance (no separate dataset designated for testing the performance).
RUS: Random undersampling; AUC: area under the receiver operating characteristic curve; N/A: Not applicable; NA: Not available.

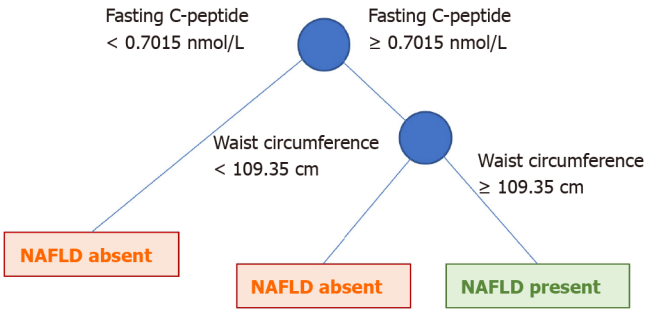

Figure 2 The decision logic of coarse trees. NAFLD: Non-alcoholic fatty liver disease.

referring patients to hepatologists. This study had some limitations. (1) Missing data were inherited from the nature of population dataset from NHANES III; (2) NAFLD was diagnosed with ultrasonography, which is not the gold standard; however, it is the primary imaging modality for NAFLD diagnosis in population-based studies and available in primary care medical facilities; (3) At the time of writing this article, there was no external dataset available that like that of NHANES III for validating the models; and (4) It may be impossible to completely reproduce the machine learning algorithms in this study since randomization was used in the modeling process, such as data partitioning, cross validation, and creation of some machine learning models. This explains why we made the trained models available to the public so that anyone can use the models directly and/or validate our results.

CONCLUSION

Machine learning algorithms can summarize a large dataset into predictive models. The best performing model measured by the F1 score from our study is the ensemble of RUS boosted trees, which is a complex model that uses all 30 factors and behaves more like a black box to physicians. In contrast, the coarse trees model, which is composed of serum C-peptide and waist circumference, can generate a reasonable predictive performance, and most importantly is the simplest to use. To facilitate

clinical decision-making, complex models should be incorporated into the electronic medical record system. This will lead to proper investigation and treatment selection for specific individuals at risk, helping to maximize healthcare resource utilization. If software deployment is not achievable, a simple model be used directly by physicians. Therefore, the model choice depends on the user objectives and resources. Therefore, the more complex model required more resources and was likely to outperform. The less complex model may not be the most accurate model but can be easily implemented and interpreted in clinical practice.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease that can progress to more severe liver disease.

Research motivation

Early patient identification using a simple method is highly desirable for preventing the progression of NAFLD.

Research objectives

To create machine learning models for predicting NAFLD in the general United States population.

Research methods

This study was designed as a retrospective cohort by using the NHANES 1988-1994. Adults (20 years and above in age) with gradable ultrasound results were included in this study.

Research results

Based on F1, the ensemble of ensemble of random undersampling boosted trees was the top performer (accuracy 71.1% and F1 0.56) while a simple model (coarse trees) had an accuracy of 74.9% and an F1 of 0.33.

Research conclusions

Although a simpler model such as coarse trees was not the top performer, it consisted of only two predictors: fasting C-peptide and waist circumference. Its simplicity is useful in clinical practice.

Research perspectives

The findings from this study can facilitate clinical decision-making for clinicians and also allow researchers to investigate the developed machine learning models. This will lead to proper investigation and treatment selection for specific individuals at risk, helping to maximize healthcare resource utilization.

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ORIGINAL ARTICLE

Retrospective Study

Acute liver failure with hemolytic anemia in children with Wilson's disease: Genotype-phenotype correlations?

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Abstract

BACKGROUND

Wilson's disease (WD) is a rare autosomal recessive inherited disorder of copper metabolism. Acute liver failure (ALF) and hemolytic anemia represent the most severe presentation of WD in children. No clear genotype-phenotype correlations exist in WD. Protein-truncating nonsense, frame-shift, or splice-site variants may be associated with more severe disease. In contrast, missense variants may be associated with late-onset, less severe disease, and more neurological manifestations. Recently, a gene variant (HSD17B13:TA, rs72613567) with a possible hepatic protective role against toxins was associated with a less severe hepatic phenotype in WD.

AIM

To analyze the possible genotype-phenotype correlations in children with WD presented with ALF and non-immune hemolytic anemia.

METHODS

The medical records of children with WD diagnosed and treated in our hospital from January 2006 to December 2020 were retrospectively analyzed. The clinical manifestations (ALF with non-immune hemolytic anemia or other less severe forms), laboratory parameters, copper metabolism, *ATP7B* variants, and the HSD17B13:TA (rs72613567) variant were reviewed to analyze the possible genotype-phenotype correlations.

RESULTS

We analyzed the data of 51 patients with WD, 26 females (50.98%), with the mean age at the diagnosis of 12.36 ± 3.74 years. ALF and Coombs-negative hemolytic anemia was present in 8 children (15.67%), all adolescent girls. The Kayser-Fleisher ring was present in 9 children (17.65%). The most frequent variants of the *ATP7B* gene were p.His1069Gln (c.3207A>G) in 38.24% of all alleles, p.Gly1341Asp (c.4021G>A) in 26.47%, p.Trp939Cys (c.2817G>T) in 9.80%, and p.Lys844Ter (c.2530A>T) in 4.90%. In ALF with hemolytic anemia, p.Trp939Cys (c.2817G>T) and p.Lys844Ter (c.2530A>T) variants were more frequent than in other less severe forms, in which p.His1069Gln (c.3207A>G) was more frequent. p.Gly1341Asp (c.4021G>A) has a similar frequency in all hepatic forms. For 33 of the patients, the HSD17B13 genotype was evaluated. The overall HSD17B13:TA allele frequency was 24.24%. Its frequency was higher in patients with less severe liver disease (26.92%) than those with ALF and hemolytic anemia (14.28%).

CONCLUSION

It remains challenging to prove a genotype-phenotype correlation in WD patients. In children with ALF and hemolytic anemia, the missense variants other than p.His1069Gln (c.3207A>G) and frame-shift variants were the most frequently present in homozygous status or compound heterozygous status with site splice variants. As genetic analysis is usually time-consuming and the results are late, the importance at the onset of the ALF is questionable. If variants proved to be associated with severe forms are found in the pre-symptomatic phase of the disease, this could be essential to predict a possible severe evolution.

Key Words: Wilson's disease; Children; Acute liver failure; Hemolytic anemia; *ATP7B* variant; Genotype-phenotype correlation

Core Tip: Acute liver failure (ALF) and hemolytic anemia represent the most severe presentation of Wilson's disease (WD) in children, with a possible fatal evolution. There is no definite genotype-phenotype correlation in WD, but many studies try to solve this puzzle. Our research reports a higher presence of a missense [p.Trp939Cys (c.2817G>T)] and frame-shift variant [p.Lys844Ter (c.2530A>T)] in children with ALF and hemolytic anemia, while in less severe form, p.His1069Gln (c.3207A>G) was more frequent. HSD17B13:TA variant may be associated with less severe liver disease, as it was proved to have a protective role against liver toxins.

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INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive inherited disorder of copper metabolism caused by homozygous or compound heterozygous variants of the *ATP7B* gene. The prevalence of WD is estimated as 1/30000[1]. The *ATP7B* gene encodes transmembrane copper-transporting ATPase (*ATP7B*) and is located on chromosome 13q14.3, containing 20 introns and 21 exons[2]. According to the Human Gene Mutation Database, more than 800 variants of the *ATP7B* gene have been described. More than half of these variants are single nucleotide missense and nonsense, and the others are insertions/deletions and splice-site variants[3,4].

The clinical forms of WD are very variable due to the copper accumulation in different organs. The age of onset has been reported to be between 2 and 70 years[5,6]. Liver disease is the first clinical manifestation in 40%-60% of WD patients, more often

details that might disclose the identity of the subjects under study were omitted or anonymized.

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in the first decade[1,5,7,8]. In children, WD patients present with an incidental finding of high levels of transaminases in an asymptomatic child, acute or chronic hepatitis, or decompensation of cirrhosis (in older children and adolescents)[5,9]. As an initial form of presentation, neurological disease is described in 18%-68% of patients, mainly in young adults (20-30 years). WD's most common neurological features are tremor, dystonia, parkinsonism, associated with dysarthria, gait and posture disturbances, drooling, and dysphagia[1]. Also, psychiatric disease (mainly mood disturbances, depression, or bipolar disease) may be present, mainly in adulthood. A decline in school performances, impulsiveness, and inappropriate behavior was reported in adolescents[1]. Hematologic disease, renal disease, skeletal and cardiac disease may be described in WD patients[1,10,11].

Acute liver failure (ALF) may be the initial presentation or a complication during WD evolution in children and young adults[12]. Approximately 2%-6% of ALF cases may be caused by WD[13,14]. A rare presentation of WD (5%[15]), ALF accompanied by a hemolytic crisis may have a severe evolution, with coagulopathy, encephalopathy, and progressive renal failure, resulting in death without an emergency liver transplantation[1,10,11,16]. This clinical form of WD occurred in 30% of children with ALF requesting liver transplantation and 60% of those with fatal evolution before transplantation[5]. Therefore, early diagnosis and referral to specialized centers are determinants for the prognosis in these patients[12].

There is a continuous interest in genotype-phenotype correlations in WD. Based on the phenotypic classification, studies tried to find a link between the genetic variants and clinical forms or severity of WD disease, important for the prognosis of the disease [2,17]. The *ATP7B* gene variants may have different effects on the presence and function of the ATPase encoded with various consequences on the clinical presentation. Many studies have tried to analyze these correlations regarding the age of the onset, neurological or hepatic form, ceruloplasmin activity, hepatic copper level, or the presence of Kayser-Fleischer (KF) ring[18-20]. Still, there is no definite genotype-phenotype correlation so far, which may be due to the disease's high genetic heterogeneity and rareness[2,21]. Some authors suggest that the severe hepatic phenotype and earlier onset are more likely associated with the nonsense or frame-shift variants. A less severe hepatic or a neurologic phenotype is linked to missense variants [2,22-26]. The clinical presentation in WD may also be influenced by environmental and epigenetic factors or modifier genes[4]. Recently, a gene variant (HSD17B13:TA, rs72613567) with a possible hepatic protective role against toxins was associated with a less severe hepatic phenotype in WD[27].

Our study aimed to analyze the possible genotype-phenotype correlations in children with WD presented with ALF and non-immune hemolytic anemia and to investigate the most common *ATP7B* variants in our patients with this severe form of the disease.

MATERIALS AND METHODS

The medical records of children with WD diagnosed and treated in our hospital from January 2006 to December 2020 were retrospectively analyzed. The clinical manifestations (acute or chronic liver disease, neurologic disease, ALF with non-immune hemolytic anemia), laboratory parameters, copper metabolism, and *ATP7B* variants were reviewed.

Diagnostic of WD was based on positive family history, clinical symptoms (including the presence of KF ring), and laboratory tests (low serum ceruloplasmin, < 20 mg/dL, elevated 24-h urinary copper excretion, baseline or stimulated by penicillamine) following the current diagnostic and management guidelines[28,29]. ALF with hemolytic anemia was diagnosed on the coagulopathy (prolonged prothrombin time, increased international normalized ratio (INR) > 2 without hepatic encephalopathy or > 1.5 in the presence of encephalopathy), low hemoglobin level, and negative Coombs test. Laboratory tests were performed using standard methods. None of our patients had a liver biopsy to assess the histology, as we could not measure the copper content in our service. The severity of the fibrosis was evaluated at diagnosis and during the follow-up using a non-invasive assessment of liver stiffness by transient elastography (FibroScan, Echosense, France)[9].

The molecular analysis of the *ATP7B* gene was performed using a semi-nested polymerase chain reaction-based restriction fragment length polymorphism assay for p.His1069Gln (c.3207A>G) variant detection as previously described. If negative or heterozygous for p.His1069Gln (c.3207A>G) variant, samples were Sanger sequenced

by the ABI Prism 310 Genetic Analyzer (Perkin Elmer; Norwalk, CT, United States) until 2012, followed by the 3500 Genetic Analyzer (Applied Biosystems; Foster City, CA, United States) using published primers.

The HSD17B13:TA (rs72613567) variant was determined using allelic discrimination real-time polymerase chain reaction and validated by Sanger sequencing in normal controls having different HSD17B13 genotypes. Unfortunately, this evaluation was technically possible only for the second half of the study, and only 33 patients were assessed. We included only the children with WD confirmed by molecular analysis, and we excluded all suspected WD patients without genetic confirmation or with incomplete data.

We analyzed the clinical and laboratory features, including the most frequent variants in children with ALF and hemolytic anemia compared to those with other clinical forms.

Statistical analysis of the data collected was performed using Statistica 13.5 (Tibco Software; Palo Alto, CA, United States). The variables with normal distribution were presented as mean and standard. Comparison of continuous variables was performed using the Student *t*-test. Categorical variables were presented as numbers and percentages; they were compared using the Chi-square test. Two-sided *P* values were analyzed, and the *P* value < 0.05 was considered statistically significant.

RESULTS

During the last 15 years, 67 patients with WD were diagnosed and treated in our clinic. After reviewing genetic data, we included 51 patients, 26 females (50.98%), with the mean age at WD diagnosis of 12.36 ± 3.74 years (between 5 and 23 years).

Almost all patients included in our study presented liver diseases; only one was with a neurological form, and one was diagnosed following the screening due to WD in the family. Our clinic is the main pediatric hepatology service and center for expertise in pediatric liver rare disorders in Transylvania, Romania. Therefore, the selection of the patients referred to our center would be biased regarding the clinical presentation in our WD patients. ALF and Coombs-negative hemolytic anemia was present in 8 children (15.67%), all adolescent girls. The KF ring was present in 9 children (17.65%). The clinical and laboratory characteristics of the WD patients included in this study, based on the clinical onset, are presented in Table 1.

In two girls, ALF with hemolytic anemia was not the initial presentation that led to the WD diagnostic. Initially, they had only increased transaminases but progressed shortly to this severe clinical evolution.

In our patients, the most frequent variant of the *ATP7B* gene was p.His1069Gln (c.3207A>G), present in 12 children in homozygous status and 17 children in compound heterozygous status (38.24% of all alleles). p.Gly1341Asp (c.4021G>A) variant was present in 10 children in homozygous status and 7 patients in compound heterozygous status (26.47%). The other two frequent variants (mainly in patients with ALF and hemolytic anemia) were p.Trp939Cys (c.2817G>T), in 5 children in homozygous status (9.80%) and p.Lys844Ter (c.2530A>T), in one child as homozygous status and three children as a part of compound heterozygous status (4.90%). In Table 2, we present the most frequent variants grouped by the clinical form of presentation. In ALF with hemolytic anemia, p.Trp939Cys (c.2817G>T) and p.Lys844Ter (c.2530A>T) were more frequent than in other less severe forms, in which p.His1069Gln (c.3207A>G) was more frequent. p.Gly1341Asp (c.4021G>A) variant has a similar frequency in all hepatic forms.

For 33 of the patients included in our study, the HSD17B13 genotype was evaluated. The overall HSD17B13:TA allele frequency in our study was 24.24%. HSD17B13:TA allele frequency was higher in patients with less severe liver disease (26.92%) than ALF and hemolytic anemia (14.28%). Table 3 presents the demographic, clinical, and genotype association in patients investigated for the HSD17B13:TA variant.

Two patients with ALF were transplanted, five survived with the native liver following supportive intensive care, and one girl had a fatal evolution on the second day after admission. Also, another child with cirrhosis died due to severe complications before liver transplantation was possible.

Table 1 Clinical and laboratory characteristics in Wilson's disease children with acute liver failure and hemolytic anemia

Parameter	All (*n* = 51)	ALF and hemolytic anemia (8 pts)	Other clinical forms (43 pts)	*P* value
Females, *n* (%)	26 (50.98)	8 (100)	18 (41.86)	0.00252
Mean age (yr)	12.36 ± 3.74	14.59 ± 2.21	11.59 ± 3.79	0.03598
ALT (IU/L)	131.54 ± 119.69	87.38 ±154.07	142.24 ± 110. 04	0.24969
AST (IU/L)	125.05 ± 88.73	78.62 ± 47.44	136.30 ± 93.16	0.09950
TB (mg/dL)	5.41 ± 12.93	22.55 ± 21.95	0.99 ± 0.95	0.000002
DB (mg/dL)	4.20 ± 11.56	18.84 ± 20.40	0.42 ± 0.51	0.000007
GGT (IU/L)	83.54 ± 43.44	97.71 ± 57.14	80.44 ± 40.33	0.34731
WBC (mm^3)	8568 ± 7360	14838 ± 14185	6666 ± 1799	0.00415
Hb (g/dL)	12.15 ±2.96	7.4 ± 2.39	13.51 ± 1.22	0.000000
PLT (mm^3)	253306 ±125421	164875 ± 74043	278571 ± 126455	0.02142
INR	2.94 ± 6.37	7.53 ± 11.34	1.18 ± 0.27	0.01359
KF ring, *n* (%)	9 (17.65)	3 (37.50)	6 (13.95)	0.10868
Ceruloplasmin (mg/dL)	9.81 ± 6.118	7.98 ± 5.77	10.11 ± 6.19	0.39976
Urinary copper (μg/24 h)	648.94 ± 1093.90	2 236.33 ± 2 174.46	384.38 ± 455.04	0.000006
Outcome, *n* (%)				0.00121
Survivors	47 (92.16)	5 (62.50)	42 (97.67)	
Transplanted	2 (3.92)	2 (25.00)	0	
Deceased	2 (3.92)	1 (12.50)	1 (2.33)	

ALF: Acute liver failure; ALT: Alanine-aminotransferase; AST: Aspartate-aminotransferase; TB: Total bilirubin; DB: Direct bilirubin; GGT: Gamma-glutamyl transferase; WBC: White blood cells; Hb: Hemoglobin; PLT: Platelets; INR: International normalized ratio; KF ring: Kayser-Fleisher ring.

Table 2 Variants of *ATP7B* gene in children with hemolytic anemia and acute liver failure

Variants		Hemolytic Anemia + ALF (8 patients)	Other clinical forms (44 patients)	*P* value
p.Trp939Cys (c.2817G>T)	5 homozygotes	6 (42.86%)	4 (4.55%)	0.0000
p.Lys844Ter (c.2530A>T)	1 homozygotes	4 (28.57%)	1 (1.14%)	0.0000
	3 heterozygotes			
p.Gly1341Asp (c.4021G>A)	10 homozygotes	4 (28.57%)	23 (26.14%)	0.8482
	7 heterozygotes			
p.His1069Gln (c.3207A>G)	12 homozygotes	0	39 (44.32%)	0.0015
	15 heterozygotes			
Other variants	1 homozygotes	2 (14.29%)	19 (21.59%)	0.5304
	19 heterozygotes			
Total alleles		14 (100%)	88 (100%)	

ALF: Acute liver failure.

DISCUSSION

Our study aimed to assess the possible genotype-phenotype correlations regarding WD's most severe clinical form in children and adolescents. This endeavor in patients with WD is challenging, as was proved by many studies already published. So far, the research failed to conclude this issue due to the high heterogeneity of *ATP7B* variants

Table 3 Data regarding the patients evaluated for the HSD17B13:TA variant

	All	HSD17B13 genotype		
		T/T	TA/T	TA/TA
n (%)	33	19 (57.58)	12 (36.36)	2 (6.06)
Females, *n* (%)	16	11	5	0
Mean age (yr)	11.67 ± 3.12	11.09 ± 3.45	12.14 ± 2.25	14.70 ± 3.24
Clinical presentation, *n* (%)				
ALF and/or hemolytic anemia	7 (21.21)	5 (71.43)	2 (28.57)	0
Less severe hepatic forms	26 (78.79)	14 (53.85)	10 (38.46)	2 (7.69)
ATP7B variant				
p.His1069Gln (c.3206A>G)/p.His1069Gln (c.3206A>G)	5	2	3	0
p.His1069Gln (c.3206A>G)/p.Gly1341Asp (c.4021G>A)	6	3	3	0
p.His1069Gln (c.3206A>G)/other	7	4	3	0
p.Gly1341Asp (c.4021G>A)/p.Gly1341Asp (c.4021G>A)	7	3	2	2
p.Trp939Cys (c.2817G>T)/p.Trp939Cys (c.2817G>T)	4	4	0	0
p.Trp939Cys (c.2817G>T)/other	1	1	0	0
p.Lys844Ter (c.2530A>T)/p.Lys844Ter (c.2530A>T)	1	1	0	0
p.Lys844Ter (c.2530A>T)/ other	2	1	1	0

ALF: Acute liver failure.

(more than 1300 described) and the rareness of the disease (small series of patients). Furthermore, the increased number of compound heterozygotes involving different kinds of variants makes this analysis more difficult[18,19,24,30,31]. As phenotypic differences were reported in siblings with the same genotype or monozygotic twins, the involvement of other factors is possible[2,30,32-35]. Environmental factors (nutritional copper intake, infections, drugs, or other toxins), modifier genes, and epigenetic factors' interaction with the genetic variants may explain the different clinical presentations in WD[4,8,18,22].

The introduction of a phenotype classification tried to ease analyzing the clinical forms in WD[17]. Our patients mainly have the hepatic form (the most frequent one in children and adolescents). Some of our older patients also had neurological and psychiatric manifestations. Not all our patients suspected of WD had a genetic confirmation of the *ATP7B* variants. Therefore, only patients with two WD variants in cis were included in our study to analyze the possible genotype-phenotype correlations.

ALF with hemolytic anemia was present in 8 children, all girls. The age of onset was higher than other hepatic presentations (acute or chronic hepatitis, autoimmune features, or cirrhosis, data not shown). The increased frequency of ALF described in females is not fully understood, but it may be explained by hormonal differences or the intervention of epigenetic factors (methylome and transcriptome differences)[4,13,18,19].

The KF ring was described in 9 children with liver disease (17.65%), three of them with ALF, and 6 in the other forms. Four of those six children with KF ring in other forms of liver disease presented neurological manifestations in their evolution. Other studies proved that ocular involvement is less frequent in hepatic than in neurological involvement[31]. The presence of the KF ring was reported lower in children. In Greek children with WD, the KF ring was present in 48.7% of those with liver disease and 16% of those diagnosed through family screening[7], while in the Italian children, only in 8.6% of those with liver disease[8]. The KF ring was present in more than half of the ALF patients, compared to 37.5% in our small series[10].

Regarding the laboratory results, the differences in children with ALF and hemolytic anemia and the other forms are expected for the bilirubin, hemoglobin, and INR. The number of white blood cells (WBC) is higher in children with ALF, and platelets are lower. There are no significant differences in the serum level of transam-

inases (even lower in ALF patients) and gamma-glutamyl transferase. The number of WBCs is an important risk factor as it was included in the prognostic score to predict mortality and evaluate the need for liver transplantation[5]. Also, the low level of transaminases in children with the ALF form of WD is a well-known feature and would help the diagnostic. High aspartate-aminotransferase (AST) to ALT ratio and low alkaline phosphatase to serum bilirubin ratio may be used to differentiate the WD patients in ALF[13,15]. In our cohort, two children with ALF had an AST/ALT ratio higher than 4 and only one alkaline phosphatase to bilirubin level ratio lower than 2. In children, the ratio between serum alkaline phosphatase and total bilirubin level may not always be helpful due to bone-derived alkaline phosphatase[5]. The transaminase level was lower than in children with acute hepatitis. As the age is higher in patients presenting with ALF, the evolution of the disease without any clinical sign for years explains the severe fibrosis or cirrhosis in these patients. In a large study that aims to analyze the genotype-phenotype correlation, fulminant liver failure or hemolysis were associated with liver cirrhosis in 93.4% and 66.7% of patients, higher than in the other milder presentations of WD[19].

In our children with ALF and hemolytic anemia, the serum ceruloplasmin level was lower than in the other patients but not significantly. In the meantime, the urinary copper excretion was higher, as can be expected, due to the severe necrosis associated with ALF.

No clear genotype-phenotype correlations exist in WD. Protein-truncating nonsense, frame-shift, or splice-site variants have a significant functional and structural impact on the ATP7B protein and may be associated with more severe disease (early-onset, low ceruloplasmin level, high copper content in liver). In contrast, missense variants are associated with late-onset, less severe disease and more neurological manifestations[18,25,26,36]. There are also reports of some missense variants associated with the early onset of disease with various severity in the same family[30]. Previous reports proposed the association of exon 18-20 variants with hepatic and hematological onset but not with neurological disease[37].

The most frequent variants in Central Europe, p.His1069Gln (c.3207A>G), was also the most frequent one in our cohort. It was found in homozygous or heterozygous status in 38.24% of all alleles in our study, compared with 72% in Poland, 35% in Greece, and 38% in a previous study from Romania[17,21,23,26,38]. This variant is more frequent in older patients with the neurological form of WD[3,7,23]. In our cohort, there was no child with ALF and hemolytic anemia with p.His1069Gln (c.3207A>G) variant. This is a missense variant and is probably associated with protein misfolding, abnormal phosphorylation of the P-domain, and altered ATP binding orientation and affinity[13]. R969Q, another missense variant present in our children, is almost exclusively associated with late-onset liver disease[3,23].

Another missense variant, p.Gly1341Asp (c.4021G>A), was the second most frequent one in our children. p.Gly1341Asp (c.4021G>A) is a variant of the transmembrane domain of the *ATP7B* gene and, in homozygous status, was proved to be associated with more severe and early onset of WD[39]. This variant was associated in homozygous status with ALF and/or hemolytic anemia in two children. In one girl, hemolytic anemia developed after treatment with zinc for a chronic increase of transaminases with questionable compliance. The second girl with this genotype-phenotype association has a younger sister with the same genetic status presenting only an increase of transaminases. The most frequent variant in our patients with ALF and/or hemolytic anemia was p.Trp939Cys (c.2817G>T), described previously in early-onset hepatic disease and with a high risk for liver failure in homozygotes[24]. Three adolescents (girls) with ALF presented this variant in homozygous status; the other two children (males) had the same status but did not have a severe form. The p.Lys844Ter (c.2530A>T) variant is the fourth most frequent in our cohort; it was present more in children with a severe form of WD. One girl was homozygote, and in another two girls, the variant was associated with splice-site variants in a compound heterozygous status. The p.Lys844Ter (c.2530A>T) variant is a frame-shift variant presumed to be associated with severe clinical evolution, as are also splice-site variants. It was previously described in WD patients of Hungarian origin[40] and few patients with late-onset of WD[41].

The early diagnosis of WD in children would probably prevent the evolution and sometimes the onset of the disease with a severe form. As mentioned in other studies, gender would modify the disease presentation due to different hormone balance[18,19]. If we analyze the possible influence of the sex of the patients, the severe form of the disease was present in two of the four girls and none of the boys with p.Gly1341Asp (c.4021G>A) homozygous status. All children with p.Gly1341Asp (c.4021G>A) variant in compound heterozygous status associated with p.His1069Gln

(c.3207A>G) variant experienced a less severe form of WD.

HSD17B13 encodes a protein involved in regulating the biosynthesis of lipids, and by its enzymatic roles, is implicated in lipid-mediated inflammation. Recently, a protein-truncation variant (HSD17B13:TA, rs72613567) was shown to have a protective role against liver toxins, including copper toxicity in WD[27]. In our cohort, the allele frequency of HSD17B13:TA was similar to other results for the Caucasian population, higher in patients with less severe liver disease than those presented with ALF and hemolytic anemia. The age of diagnosis was higher in patients homozygous for this variant than in heterozygous status or without this variant. Even without statistical significance, these results suggest the possible role of the HSD17B13:TA variant in the modulation of the WD severity together with factors, including sex, age, *ATP7B* variant, and other gene variants.

ALF was fatal only in one of our cases included in this study. Two girls underwent emergency liver transplantation on the fourth day after their presentation in our service. The liver transplantation was performed at the Fundeni Institute in Bucharest, Romania. This clinical presentation should be regarded as an emergency[5,42]. The patients should be referred as soon as possible to a center that could provide intensive care, including extrahepatic liver support, until liver transplantation would be possible for severe cases. Unfortunately, one girl died the second day after her admission to our center.

Strengths and limitations. This study presents the largest cohort of children with genetically confirmed WD from our country and the neighboring region. It represents the first description of the possible correlation of ALF and hemolytic anemia with p.Trp939Cys (c.2817G>T) and p.Lys844Ter (c.2530A>T) variants in Eastern European children with WD. However, there are some limitations of our study. Firstly, the small number of children with this severe form made the statistical analysis of our findings difficult. Another issue is represented by the selection of patients, as our pediatric hepatology service admits mainly children and adolescents with hepatic disease. A significant limitation was the difficulty of considering and analyzing other possible factors that would lead to an acute, severe clinical form compared to children with the same genotype [p.Gly1341Asp (c.4021G>A)].

In the future, with the onset of a National Registry for patients with WD, including the genetic analyzes, more data on WD patients from Romania would be available. In the severe clinical form of WD, the genetic background would be less critical from the point of view of immediate medical care. The result of the genetic analysis would arrive with the clinician late, after the evolution of the patient would be clear. With the recent progress in screening for WD[43], the genetic analysis in children with an early suspected disease would help predict future evolution. When nonsense, frame-shift, or splicing-site variants are identified in a pre-symptomatic period, the importance of this genotype-phenotype correlation for the prognostic is evident.

CONCLUSION

It remains challenging to prove a genotype-phenotype correlation in WD patients due to the small number of patients in the reported series and the increased genetic heterogeneity. In children with ALF and non-immune hemolytic anemia, the nonsense variants other than p.His1069Gln [as p.Trp939Cys (c.2817G>T)] and frame-shift variants [p.Lys844Ter (c.2530A>T)] were the most frequently present in homozygous status or compound heterozygous status with site splice variants. As genetic analysis is usually time-consuming and the results are late (except in the screening of the relative of an index patient), the importance for the prognosis at the onset of the ALF is questionable. However, if variants proved to be associated with severe forms are found early in the evolution of the disease, this could be essential to predict a possible severe evolution if the patients would not follow treatment.

ARTICLE HIGHLIGHTS

Research background

There is a continuous interest in genotype-phenotype correlations in Wilson's disease (WD).

Research motivation

The aim is to study the possible genotype-phenotype correlations in children with acute liver failure (ALF) and hemolytic anemia in WD.

Research objectives

The objectives include the analysis of *ATP7B* variants in children with ALF and hemolytic anemia in WD compared to the other clinical presentations and the possible role of the HSD17B13:TA variant in the modulation of the WD severity.

Research methods

The retrospective study included 63 children with WD diagnosed and follow-up during 2006-2020. The clinical manifestations (acute or chronic liver disease, neurologic disease, ALF with non-immune hemolytic anemia), laboratory parameters, copper metabolism, *ATP7B* variants, and the HSD17B13:TA (rs72613567) variant were reviewed.

Research results

In our cohort, in children with ALF and non-immune hemolytic anemia, the nonsense variants other than p.His1069Gln (c.3206A>G), as p.Trp939Cys (c.2817G>T), and frame-shift variants, as p.Lys844Ter (c.2530A>T), were the most frequently present. The allele frequency of HSD17B13:TA was similar to other results for the Caucasian population, higher in patients with the less severe liver disease than those presented with ALF and hemolytic anemia.

Research conclusions

It remains challenging to prove a genotype-phenotype correlation in WD patients due to the small number of patients in the reported series and the increased genetic heterogeneity. When nonsense, frame-shift, or splicing-site variants are identified in a pre-symptomatic period, the importance of this genotype-phenotype correlation for the prognostic is evident.

Research perspectives

A more extensive study involving children and adolescents with ALF and hemolytic anemia form of WD should be provided to confirm the findings. New studies are needed to evaluate the role of protective variant, HSD17B13:TA (rs72613567), in association with other factors, in less severe forms of WD in children.

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ORIGINAL ARTICLE

Observational Study

Clinical outcomes of patients with two small hepatocellular carcinomas

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Abstract

BACKGROUND

Management of single small hepatocellular carcinoma (HCC) is straightforward with curative outcomes achieved by locoregional therapy or resection. Liver transplantation is often considered for multiple small or single large HCC. Management of two small HCC whether presenting synchronously or sequentially is less clear.

AIM

To define the outcomes of patients presenting with two small HCC.

METHODS

Retrospective review of HCC databases from multiple institutions of patients with either two synchronous or sequential HCC ≤ 3 cm between January 2000 and March 2018. Primary outcomes were overall survival (OS) and transplant-free survival (TFS).

RESULTS

104 patients were identified (male n = 89). Median age was 63 years (interquartile range 58-67.75) and the most common aetiology of liver disease was hepatitis C (40.4%). 59 (56.7%) had synchronous HCC and 45 (43.3%) had sequential. 36 patients died (34.6%) and 25 were transplanted (24.0%). 1, 3 and 5-year OS was 93.0%, 66.1% and 62.3% and 5-year post-transplant survival was 95.8%. 1, 3 and 5-year TFS was 82.1%, 45.85% and 37.8%. When synchronous and sequential groups were compared, OS (1,3 and 5 year synchronous 91.3%, 63.8%, 61.1%, sequential 95.3%, 69.5%, 64.6%, P = 0.41) was similar but TFS was higher in the sequential group (1,3 and 5 year synchronous 68.5%, 37.3% and 29.7%, sequential 93.2%, 56.6%, 48.5%, P = 0.02) though this difference did not remain during multivariate analysis.

CONCLUSION

TFS in patients presenting with two HCC ≤ 3 cm is poor regardless of the timing of the second tumor. All patients presenting with two small HCC should be considered for transplantation.

Key Words: Hepatocellular carcinoma; Liver cancer; Prognosis; Transplantation; Transplant-free survival

Core Tip: Transplant-free survival in patients with 2 small hepatocellular carcinomas is poor, whether presenting synchronously or sequentially, and so should be considered for transplantation.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and is the fourth leading cause of cancer-related mortality globally[1]. With uptake of standardized HCC surveillance programs, a greater number of patients are being diagnosed at earlier stages of disease when curative treatment is still possible[2-5]. In patients presenting with small tumors the probability of survival has progressively

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improved over recent decades with 5-year survival rates greater than 50% now frequently reported[6,7].

Curative therapies for HCC include surgical resection, percutaneous thermal ablation and liver transplantation. Within widely adopted eligibility criteria, transplantation may be considered when up to three individual HCC are present[8,9]. For solitary HCC, selection of therapy is based upon tumor size and location, in addition to severity of underlying hepatic dysfunction and portal hypertension. Surgical resection and ablative therapies have comparable survival rates in patients with solitary HCC less than 3 cm in diameter[10-13].

Whilst the guidelines are relatively clear for management of patients presenting with a single HCC ≤ 3 cm or three small HCC, there is little data to guide decision-making in patients who present with two small HCC, particularly when a second lesion appears sequentially after the index lesion. In this present study we sought to define the outcome of patients presenting with two HCC each up to 3 cm, in addition to exploring whether outcomes vary depending on whether tumors present either synchronously or sequentially (metachronously).

MATERIALS AND METHODS

Study design

Retrospective data of all HCC diagnosed between 1st of January 2000 to 31st of March 2018 from four tertiary referral centres in Melbourne, Victoria were reviewed. Data were retrieved from site-specific prospectively collected electronic health records. Institutional ethics committee approval was obtained from participating sites prior to commencement at each centre.

Inclusion criteria

Patients ≥ 18 years old with either two synchronous or two sequential HCC each up to 3 cm in size were identified. Patients with and without cirrhosis were included. Cirrhosis was established on standardized clinical, biochemical and radiologic grounds with or without histologic confirmation. In non-cirrhotic patients, HCC diagnosis was established histologically in all cases. HCC diagnoses between 2001 and 2012 were made according to 2001 European Association for the Study of the Liver (EASL) guidelines; all other lesions outside of these criteria required biopsy for diagnosis[14]. Diagnoses made beyond 2012 were in accordance with revised EASL criteria[2].

Exclusion criteria

Patients who only ever had a single HCC or more than two tumors at diagnosis were excluded. Patients were also excluded if either of their first two HCC exceeded 3 cm or if they had radiologic evidence of vascular invasion or distant metastasis. Patients managed at more than one centre were only included once. After inclusion and exclusion criteria were applied, 104 patients were included in the study for analysis.

Data collection

Data was collated from patient records into a central database and included demographics (age, gender), aetiology of chronic liver disease, the presence of or absence of cirrhosis, Child-Turcotte-Pugh (CTP) and model for end-stage liver disease (MELD)[15] scores, α-feto protein (AFP) level and radiologic tumor characteristics (total diameter of both lesions and diameter of largest individual lesion). Date of disease progression, the nature of progression (local recurrence, new disease, portal vein invasion or metastases) and date of death were recorded.

Treatment

Treatment modalities and number of treatments were recorded. Treatment was administered according to multidisciplinary consensus at each institution. Locoregional therapies included percutaneous ablation (inclusive of microwave and radiofrequency ablation), percutaneous ethanol injection (PEI), transarterial chemoembolization (TACE) and irreversible electroporation. All cases being considered for transplantation were referred to the Victorian Liver Transplantation Unit at Austin Health. Patients with HCC waitlisted for transplantation in Victoria are not granted MELD exception points, with decisions on timing of transplant made at twice-weekly multidisciplinary meetings and priority given to patients with active tumor rather

than cumulative time on the waitlist.

Outcome measures

For the synchronous group, follow-up time began at the date two HCC were confirmed radiologically (Figure 1). For the sequential group, records of patients presenting with a single lesion were reviewed for occurrence of a second lesion. Follow-up time in the sequential group began at the time the second HCC was diagnosed (the first lesion may have received treatment; response to treatment whether it be partial or complete was not a requirement for inclusion). The primary outcome was overall survival (OS) which was calculated from the date of meeting inclusion criteria until death. Transplant-free survival (TFS) was calculated from the date of meeting inclusion criteria until liver transplantation or death without transplantation. Progression-free survival was from date of meeting inclusion criteria until either disease progression according to mRECIST[16] criteria or death without confirmed radiologic progression.

Statistical analysis

Demographic and continuous variables were assessed for normality and were accordingly presented as mean ± SD or median and interquartile range (IQR). Categorical variables were presented as frequencies with percentages. Baseline characteristics were compared between groups using one-way ANOVA and Mann-Whitney *U* test for normally-distributed and non-normally-distributed continuous variables, respectively. Pearson chi square test was used to compare categorical variables.

Survival was calculated by Kaplan-Meier analysis with all patients alive at the end of the follow-up period or transplanted before confirmed radiological progression being censored from survival analysis. Univariate analysis of prognostic factors was performed by log-rank testing; group comparisons included age ≤ 70 *vs* > 70 years, male *vs* female, aetiology of underlying liver disease, CTP class, MELD ≤ 14 *vs* > 14, AFP at diagnosis <10 or ≥ 10 μg/L, presentation with synchronous or sequential lesions both ≤ 3 cm and transplanted *vs* non-transplanted. Multivariate Cox proportional hazard analysis of univariate variables with a P value < 0.10 was performed and reported as hazard ratios (HR) with 95%CI. Significance tests were two-tailed with a P value < 0.05 considered statistically significant. All analyses were performed using SPSS version 22 (Armonk, NY: IBM Corp).

RESULTS

Patient characteristics

One hundred and four patients were identified as having two HCC and were followed up for a median of 2.54 years (IQR 2.73 years, range 0.08-13.67); only six patients (5.8%) had less than six months follow-up. Eighty-nine (85.6%) were male and the median age was 63 years (IQR 58-68). The most common cause of liver disease was chronic hepatitis C (n = 42, 40.4%) followed by chronic hepatitis B (n = 15, 14.4%). The majority were CTP score A (n = 66, 63.7%) and median MELD at diagnosis was 9.5 (IQR 7-13).

Baseline characteristics comparing synchronous *vs* sequential tumors are shown in Table 1. Fifty-nine patients (56.7%) had two synchronous HCC at inclusion, whilst forty-five (43.3%) had sequential lesions with the median time between index and sequential lesions 14 mo (IQR 7.5-29.5). There was no difference in follow-up time between the two groups (P = 0.54). Mean MELD score at diagnosis was the only statistically significant difference between the two groups, higher in the synchronous cohort (11 ± 7 *vs* 8 ± 5, P = 0.01). The median combined diameter of the two tumors in the synchronous group was not significantly different from the sequential group (3.8 cm *vs* 3.4 cm, P = 0.28).

Treatment

The most common single treatment for patients with synchronous HCC was TACE (32.2%) followed by percutaneous ablation (20.3%), whilst two patients (3.4%) had unsuccessful locoregional therapy due to technical limitations and received transplantation as their primary treatment modality (Supplementary Table 1). Percutaneous ablation was the commonest single treatment for index lesions in the sequential group (57.8%) followed by surgical resection (17.8%). As first line treatment, TACE was more commonly utilized in the synchronous group (32.2% *vs* 8.9%, P < 0.01), whilst percutaneous ablation was more common in the sequential group (57.8%

Table 1 Baseline characteristics of 104 patients with two hepatocellular carcinomas according to synchronous or sequential tumor

	All (*n* = 104)	Synchronous group (*n* = 59)	Sequential group (*n* = 45)	*P* value
Age, yr, median (IQR)	63 (10)	63 (10)	63 (9)	0.41
Gender, *n* (%)				0.08
Male	89 (85.6)	51 (86.4)	38 (84.4)	
Female	15 (14.4)	8 (13.6)	7 (15.6)	
Aetiology, *n* (%)				0.73
Alcohol	12 (11.5)	8 (13.6)	4 (8.9)	
HCV	42 (40.4)	22 (37.3)	20 (44.4)	
HBV	15 (14.4)	8 (13.6)	7 (15.6)	
NASH	5 (4.8)	4 (6.8)	1 (2.2)	
Alcohol and HCV	18 (17.3)	9 (15.3)	9 (20.0)	
Other[1]	12 (11.5)	8 (13.6)	4 (8.9)	
Cirrhosis status, *n* (%)				0.07
Non-cirrhotic	10 (9.6)	3 (5.1)	7 (15.6)	
Cirrhotic	94 (90.4)	56 (94.9)	38 (84.4)	
CTP class, *n* (%)				0.1
A	66 (63.5)	35 (59.3)	31 (68.9)	
B	25 (24.0)	13 (22.0)	12 (26.7)	
C	13 (12.5)	11 (18.6)	2 (4.4)	
MELD, median (IQR)	9.6 (6)	11 (7)	8 (5)	0.01
AFP (μg/L), median (IQR)	9.6 (24.0)	8.6 (26.0)	10.4 (22.8)	0.61
Combined tumour diameter (cm), median (IQR)	3.5 (1.7)	3.8 (1.7)	3.4 (1.2)	0.28
Transplanted, *n* (%)	25 (24)	18 (30.5)	7 (15.6)	0.08
Death, *n* (%)	36 (34.6)	23 (39.0)	13 (28.9)	0.28

[1]Other refers to aetiologies not listed here and is inclusive of: Mixed aetiologies, autoimmune hepatitis, hereditary haemochromatosis, α-1-antitrypsin deficiency and cryptogenic liver disease. IQR: Interquartile range; AFP: α-feto protein; CTP: Child-Turcotte-Pugh; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MELD: Model for End-Stage Liver Disease score; NASH: Non-alcoholic steatohepatitis.

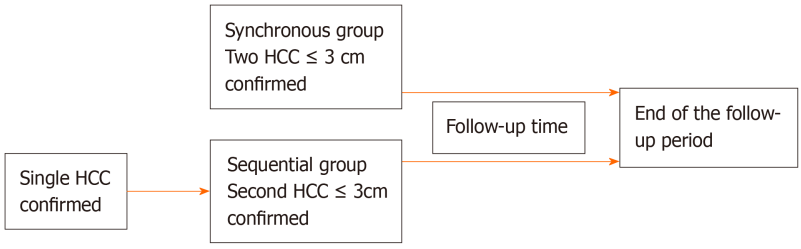

Figure 1 Study flow diagram. HCC: Hepatocellular carcinoma.

vs 20.3%, $P < 0.01$). There was no significant difference in the rate of PEI or resection between the two groups ($P = 0.25$ and $P = 0.16$, respectively). Synchronous lesions were more frequently treated with two modalities upfront (30.5% *vs* 13.3%, $P = 0.04$). The second lesion in the sequential group was most frequently treated by percutaneous ablation (31.1%) followed by TACE (28.9%), with only three patients (6.67%) undergoing transplantation (Supplementary Table 2).

During the follow-up period, 25 patients (24%) were transplanted with median time to transplantation 12 mo (IQR 2.83). The only significant differences between transplanted and non-transplanted patients were CTP and MELD score ($P < 0.01$ for both) (Supplementary Table 3). Although a higher proportion of patients with synchronous HCC were transplanted compared to the sequential group (30.5% *vs* 15.6%), this did not reach statistical significance ($P = 0.08$).

Survival analysis

Overall survival: Thirty-six (34.6%) patients died during the study period with median time to death 1.45 years (IQR 1.17-2.63) (Supplementary Figure 1). OS at 1-, 3- and 5-years was 93%, 66.1% and 62.3%, respectively (Table 2). There was no difference in OS between the synchronous and sequential groups ($P = 0.41$, Figure 2A). On univariate analysis (Supplementary Table 4), only age ≥ 70 years was associated with increased risk of mortality (HR 2.19, 95%CI: 1.08-4.45, $P = 0.03$), whilst only transplantation was associated with reduced mortality (HR 0.19, 95%CI: 0.07-0.55, $P < 0.01$). On multivariate analysis, only transplantation remained significant with HR 0.20, 95%CI: 0.07-0.61, $P < 0.01$ (Supplementary Table 5).

TFS: TFS was 77.1%, 45.4% and 37.8% at 1-, 3 and 5-years, respectively (Table 2, Supplementary Figure 2). TFS was significantly different between the synchronous and sequential groups, with five-year transplant-free survival of 29.7% in the synchronous group and 48.5% in the sequential group ($P = 0.02$, Figure 2B). Univariate analysis identified CTP C status (HR 5.17, 95%CI: 2.59-10.29, $P < 0.01$) and MELD > 14 (HR 4.07 95%CI: 2.27-7.32, $P < 0.01$) as predictors of mortality (Supplementary Table 6), whilst the sequential tumor was associated with survival (HR 0.53, 95%CI: 0.31-0.92, $P = 0.03$). After multivariate analysis (Table 3), the difference between the sequential and synchronous groups did not remain significant (HR 0.70, 95%CI: 0.38-1.27, $P = 0.24$) and only MELD > 14 remained a significant predictor of death (HR 2.51, 95%CI: 1.15-5.46 $P = 0.02$).

Transplanted patients: 1-, 3- and 5-year survival in transplanted patients was 100%, 95.8% and 95.8% (Table 2) with median time to death after transplant 6.42 years (IQR 1.33-6.67 years). Four transplanted patients (16%) died; three from recurrent HCC and the fourth from complications of motor neurone disease. All three transplanted patients with recurrent HCC had initially presented with synchronous lesions.

Disease progression

Progressive disease in the entire cohort was seen in 71 patients (68%) by five years. Median time to progression was 1.58 years (IQR 1-3). Amongst those with disease progression, recurrence with new lesions was the commonest form of progression, occurring in 30 patients (42.2%). Progression-free survival was not significantly different between the synchronous and sequential groups ($P = 0.19$). Subgroup analysis showed that the sequential group had longer progression-free survival without local recurrence ($P < 0.01$, Supplementary Figure 3) and without new lesions ($P < 0.01$, Supplementary Figure 4). No differences were seen in survival without progression, survival without failure of primary treatment or survival without metastatic spread (data not shown).

DISCUSSION

This study provides novel data on the clinical outcome of patients who develop two HCC up to 3 cm in diameter and explores the question of whether small HCC behave differently when presenting synchronously compared to sequentially. We found that regardless of whether HCC are diagnosed synchronously or sequentially, transplant-free survival is poor, with 5-year transplant free survival being only 37.8%. This suggests that liver transplantation should be considered earlier amongst the treatment options for patients with two HCC regardless of the timing of the second HCC. This is supported by the excellent five-year survival of transplanted patients in our cohort of 95.8%.

Our five-year OS of 62.3% was similar to that reported elsewhere. A retrospective survival analysis of an international, multi-institution HCC cohort of 814 patients that underwent hepatectomy with curative intent identified a five-year OS of 69% in patients with BCLC stage A disease[17]. Whilst this encompasses patients with two small HCC ≤ 3cm, the target group in our study, their cohort also included patients

Table 2 One-, three-, and five-year survival analysis of patients with two hepatocellular carcinomas

	Overall survival (*n* = 104)	Overall survival	Overall survival	Transplanted survival (*n* = 25)	Transplant-free survival (*n* = 104)	Transplant-free survival	Transplant-free survival
		Synchronous group (*n* = 59)	Sequential group (*n* = 45)			Synchronous group (*n* = 59)	Sequential group (*n* = 45)
1-yr survival (%)	93	91.3	95.3	100	77.1	68.5	93.2
3-yr survival (%)	66.1	63.8	69.5	95.8	45.4	37.3	56.6
5-yr survival (%)	62.3	61.1	64.6	95.8	37.8	29.7	48.5

Table 3 Multivariate analysis of factors impacting transplant-free survival

	n (%)	HR	95%CI	*P* value
CTP class				
A	66 (63.5)	-	-	-
B	25 (24.0)	1.51	0.81-2.82	0.19
C	13 (12.5)	2.26	0.90-5.65	0.8
MELD at diagnosis				
≤ 14	85 (81.7)	-	-	-
> 14	19 (18.3)	2.51	1.15-5.46	0.02
Lesion group				
Synchronous	59 (56.7)	-	-	-
Sequential	45 (43.3)	0.7	0.38-1.27	0.24

HR: Hazard ratios; CI: Confidence interval; CTP: Child-Turcotte-Pugh; MELD: Model for End-Stage Liver Disease score.

with single lesions ranging 2-5 cm in size or 3 lesions ≤ 3 cm each and therefore represented a broader range of patients. Additionally, we included patients that received a heterogeneous array of therapies in contrast to this study that looked only at surgical outcomes. The authors identified AFP > 400 ng/mL as being associated with poorer survival, in line with data elsewhere on surgical outcomes in low volume disease[18], yet our study did not find this association at AFP thresholds of 10 μg/L nor 400 μg/L (latter data not shown). Rather, we identified transplantation as the single independent variable that influenced survival.

We had excellent outcomes in patients who underwent transplantation for two small HCC, with 5-year survival 95.8%. The reported five year survival for transplantation with HCC is in the order of 70%[7]. For early HCC, a recent meta-analysis of low volume disease showed post-transplant survival to be 61.26% at 5 years[19]. Our higher post-transplant survival is likely due to the selection criteria for inclusion in this study, with patients only included if they had two small HCC. Despite excellent survival data, we note that in four deaths amongst transplanted patients, three were from recurrent HCC and all three of these patients had synchronous HCC.

The only independent factor impacting TFS in this study was MELD score. This suggests that in patients with two HCC, the severity of liver disease is an important factor in defining outcome, rather than lesion synchronous or sequential presentation, a similar finding to other series that examined the prognostic value of MELD scores in non-transplant HCC survival[20]. It is noteworthy that the non-transplant outcomes in patients with MELD ≤ 14 remained poor in our cohort, with five-year survival of only 45.9%. This indicates that many patients with two small HCC would benefit from

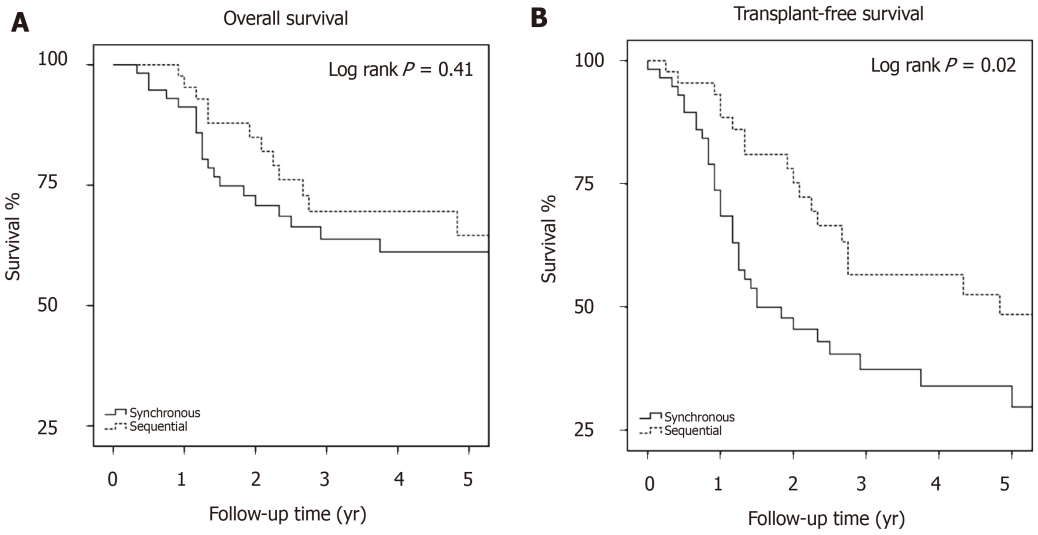

Figure 2 Kaplan-Meier survival curve for synchronous *vs* sequential groups. A: Overall survival; B: Transplant-free survival.

consideration of transplantation.

Strengths of this study include robust and comprehensive follow-up data, with only 5.8% of patients having less than 6 mo follow-up, and real-world data from four large tertiary centres. The data in this series was prospectively collected onto HCC databases at treating institutions. Given that all transplants occur in a single centre, we are confident that all transplant records are complete with accurate data and outcome of transplantation. The primary methodological limitation of this study is that it was not randomized, which can lead to inherent biases in the groups transplanted and not transplanted that may have influenced outcomes. Some patients who were deemed not appropriate for transplantation may have had other co-factors that influenced survival, such as severe non-liver comorbidities or ongoing substance abuse. There are also differing treatment algorithms and techniques between institutions involved in our study. The index presentation of a single small HCC tends to be treated by thermal ablative techniques, rather than transarterial chemoembolization, which was the treatment of choice for unresectable synchronous tumors[21].

Our study was also limited by being focused on tumor number and size as surrogate markers for tumor biology. We were not able to evaluate the impact of histology on outcomes as the majority of diagnoses were made according to radiological criteria, in line with international guidelines[2,14]. As reported previously, transplantation according histological tumor grade leads to improved outcomes beyond selection by Milan criteria alone[22]. However, a single-centre series found that pre-transplant liver biopsy did not affect outcomes when selecting patients that are within Milan criteria, as our patients were[23]. Additionally, we recognize that amongst both groups it is not possible to determine which patients experienced intrahepatic metastasis compared to multi-centric hepatocarcinogenesis as both scenarios may lead to presentation with 'two' lesions. However, our study was focused purely on the number of lesions and whether this clinical determinant could guide our multidisciplinary meeting treatment decisions.

Choice of curative *vs* non-curative locoregional therapies may also have affected survival time between the two groups. The synchronous group had a higher rate of TACE as initial therapy compared to the sequential group, which more frequently received ablative therapies as first line treatment. This in part may explain the difference seen in TFS between the two groups.

Our data collection period spanned almost two decades and it is recognized that survival of patients diagnosed at the beginning of the observation period may not be directly comparable to patients diagnosed towards the latter portion. In an analysis of HCC cases from the Australian Cancer Registry, a national database that began in 1982, the median OS of patients doubled from 6.15 mo in those diagnosed between 2000-2004 to 12.07 mo for those diagnosed 2010-2014[6]. These data represent all patients and due to this heterogeneity, identification of the causes of improved survival are difficult but potentially attributable to better patient selection, earlier detection through HCC screening, widespread adoption of multidisciplinary decision-making, evolving locoregional treatments along with emergence of palliative therapies for advanced disease, such as oral multi tyrosine kinase inhibitors.

CONCLUSION

In conclusion we report for the first-time data specifically pertaining to patients presenting with two small HCC 3 cm in size or smaller. Our results demonstrate that the non-transplant survival of patients presenting with two small HCC is poor. Survival was similarly poor in patients presenting with two synchronous HCC as compared to sequential HCC. We therefore recommend that patients that develop a second small HCC after their first should be considered for early liver transplantation. Further larger-scale studies are required to validate these results in other populations and determine broader implications for liver transplantation waitlist management.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, and is a growing cause for cancer-related mortality globally. Curative therapies include ablation for small tumors, surgical resection, and liver transplantation.

Research motivation

At present, there is clear evidence underpinning the guidelines for management of small tumors (≤ 3 cm in maximal diameter) and three small tumors (*i.e.*, all ≤ 3 cm), however a scarcity of literature surrounding the optimal management of two small tumors. In addition, it is unclear if synchronous (*i.e.*, occurring at the same time) and sequential (*i.e.*, occurring at different points in time) tumors have differing prognoses.

Research objectives

This study aimed to assess the outcome of two small tumors (*i.e.*, ≤ 3 cm in maximal diameter), and whether there was a difference in prognosis between those occurring synchronously and sequentially. This is to help guide future guidelines for management of two small HCCs.

Research methods

This was a retrospective multicenter study conducted in Victoria, Australia, including all patients diagnosed with two small HCCs between 1st January 2000 and 31st March 2018. Review of the medical record for patient demographics, liver disease, tumor-specific details, treatment and outcome was collected. Diagnosis of HCC was based on accepted radiographic and/or histologic criteria. Primary outcomes were overall survival (OS) and transplant-free survival (TFS).

Research results

One-hundred and four patients, majority male (n = 89, 86%), with a median age of 63 years-old (interquartile range 58-67.75), and predominantly suffering from viral chronic liver disease (n = 57, 55%) were included in the final analysis and followed up for a median of 2.54 years. There was a slight majority in those presenting synchronously (n = 59, 57%) compared with those diagnosed sequentially (n = 45, 43%), with the only difference between these two groups being more severe liver disease on the basis of model for end stage liver disease (MELD) (11 *vs* 8, P = 0.01). 1-, 3-, and 5-year OS was similar between the two groups (P = 0.41), however TFS was higher in the sequential group (1-, 3- and 5-year TFS 93.2%, 56.6% and 48.5%, compared with 68.5%, 37.3% and 29.7% in the synchronous group, P = 0.02). This difference did not persist in multivariate analysis (P = 0.24), with only MELD > 14 being predictive of mortality in the model (hazard ratio 2.51, 95%CI: 1.15-5.46, P = 0.02).

Research conclusions

Transplant-free survival in patients with two HCCs ≤ 3 cm is poor irrespective if diagnosed synchronously or sequentially, and so all patients with two small tumors should be assessed and considered for liver transplantation.

Research perspectives

Given limited availability of liver transplantation, future research should aim to define the molecular carcinogenetic signature in multifocal tumors, which can occur from

multi-centric hepatocarcinogenesis or intrahepatic metastases, and whether this impacts recurrence, prognosis, and response to curative therapy.

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CASE REPORT

Focal nodular hyperplasia associated with a giant hepatocellular adenoma: A case report and review of literature

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Author contributions: Gaspar-Figueiredo S and Kefleyesus A performed the literature review, collected all the data related to the case report, and recorded/edited the video-vignette related to the case report; Sempoux C did the anatomopathological examination/appraisal; Halkic N and Uldry E did the surgical appraisal; all authors have read and approved the final manuscript.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

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Abstract

BACKGROUND

Focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) are well-known benign liver lesions. Surgical treatment is usually chosen for symptomatic patients, lesions more than 5 cm, and uncertainty of diagnosis.

CASE SUMMARY

We described the case of a large liver composite tumor in an asymptomatic 34-year-old female under oral contraceptive for 17-years. The imaging work-out described two components in this liver tumor; measuring 6 cm × 6 cm and 14 cm × 12 cm × 6 cm. The multidisciplinary team suggested surgery for this young woman with an unclear HCA diagnosis. She underwent a laparoscopic left liver lobectomy, with an uneventful postoperative course. Final pathological examination confirmed FNH associated with a large HCA. This manuscript aimed to make a literature review of the current management in this particular situation of large simultaneous benign liver tumors.

CONCLUSION

The simultaneous presence of benign composite liver tumors is rare. This case highlights the management in a multidisciplinary team setting.

Key Words: Liver; Focal nodular hyperplasia; Hepatocellular adenoma; Composite tumor; Video vignette; Case report

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Core Tip: Focal nodular hyperplasia and hepatocellular adenoma (HCA) are frequent but non-malignant tumors. There is rarely indication for surgery. Combination of these two masses is a very unusual situation. Their diagnosis is mainly based on radiology. Oral contraception is a risk factor for HCA. Malignant transformation of HCA is the predominant argument for surgery. All these cases, especially composite tumors, must be discussed in a multidisciplinary team.

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INTRODUCTION

Focal nodular hyperplasia (FNH) has become a pretty well-known disease in the past two decades. It is defined by a benign hyperplasic nodule with a central scar, appearing in the normal liver parenchyma, and is composed of normal hepatocytes in a multinodular structure[1]. Its incidence is between 0.6%-3%, predominantly affecting females patients (80%-90%) in their third or fourth decade. The pathophysiology is thought to be due to an increased arterial flow that leads to secondary hepatocellular hyperplasia[2,3]. The correlation with oral contraceptives (OCs) is unproven but very likely, given that OCs are taken almost exclusively by women (sex ratio 9:1) and the proven correlation between OCs and change in lesion size[4,5].

Hepatocellular adenoma (HCA) is a benign lesion with a malignant potential between 4% and 8%, according to recent works of Farges *et al*[6] and Sempoux *et al*[7]. It classically arises in a noncirrhotic liver, in young females with an OC background. However, the understanding of HCA has evolved dramatically and we now know that it can also develop in patients with non-alcoholic steatohepatitis, certain vascular malformations, or alcoholic cirrhosis. Moreover, there are a wide variety of subtypes of this complex disease, making it very difficult to establish treatment guidelines[8-10].

In this present article, we aimed to describe the detailed management of a rare simultaneous case of FNH and HCA and a brief review of the literature.

CASE PRESENTATION

Chief complaints

A 34-year-old woman in general good health, with a medical history of oral contraceptives (desogestrel, ethinylestradiol) for 17 years consulted her general practitioner (GP) for a check-up.

History of present illness

She was completely asymptomatic.

History of past illness

She had no past illness.

Personal and family history

The patient had no past medical history except a knee orthopedic surgery 1 year before, had a stable weight with normal body mass index (21.1 kg/m^2) and no familial medical history.

Physical examination

During the examination, her GP found a mobile and palpable abdominal mass of more than 10 cm in diameter, with no skin bulging at the Valsalva's maneuver (Figure 1).

Figure 1 Pre-operative patient's supine and stand-up picture – no external signs of tumor.

Laboratory examinations

The blood exams were normal, except for an elevation in alkaline phosphate level of 519 U/L (normal range = 36-108). Tumoral markers were normal.

Imaging examinations

Abdominal ultrasound revealed an aspecific giant mass next to the left hepatic lobe. A computed tomography (CT scan) revealed a double mass attached to the left lobe of the liver. The first one had the typical characteristics of FNH and the second one of uncertain dignity. Further magnetic resonance imaging (MRI) confirmed a 6 cm x 6 cm mass suggestive of FNH in the inferior part of segment III. This 6 cm lesion was right next to a second one measuring 14 cm × 12 cm × 6 cm which dignity was unclear. The differential diagnosis was between an HCA, a hepatocellular carcinoma (fibrolamellar variant), or an atypical FNH (Figures 2-5).

FINAL DIAGNOSIS

The pathologist's report confirmed the diagnosis of 6 cm FNH resected with good margin and showed a non-beta-catenin–mutated HCA (inflammatory subtype with more risk of malignant transformation) (Figure 6).

TREATMENT

Indication for surgery was retained during a multidisciplinary team (MDT) meeting as the first option for definitive diagnosis and treatment.

The surgery was completed without complication. We summarize hereafter the key points of the minimally invasive procedure. After inserting 4 trocars for the laparoscopy (para-umbilical, right and left flank, subxiphoid) and staying away from the large dual mass which limited the range movements, we performed an ultrasound confirming a pedunculated mass (FNH) highly vascularized attached to segment III and a second component pedunculated between segment II and III. The mass showed no adhesion with the segment IV and the gallbladder allowing a left lobectomy. Dissection was performed with ultrasonic shears (Ultracision Harmonic, Ethicon Inc., NJ, United States) and transsection was completed with a 60mm stapler (tri-staple vascular cartridge, Endo-GIA, Medtronic, Minneapolis, MN, United States). We extracted the specimen with both lesions through a suprapubic (Pfannenstiel) incision. The operative time was 122 min. Blood loss was minimal (50 mL) (Video 1).

The postoperative course was uneventful and the patient was discharged on postoperative day 3.

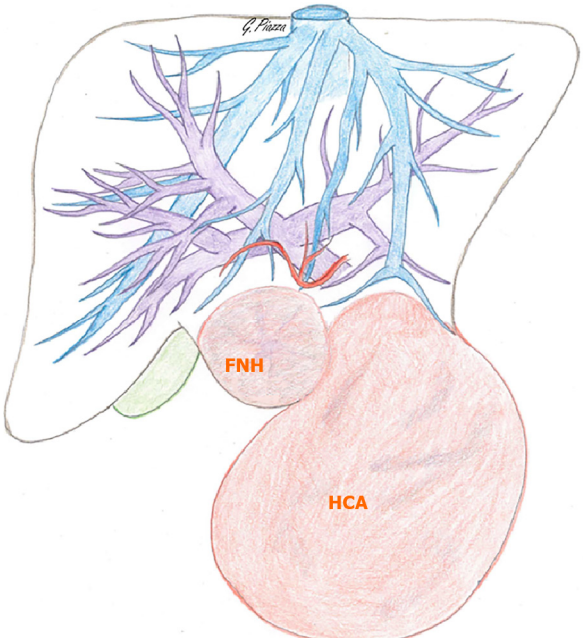

Figure 2 Preoperative drawing – tumor and liver major vessels' relationship (credits: Dr. Giulia Piazza). FNH: Focal nodular hyperplasia; HCA: Hepatocellular adenoma.

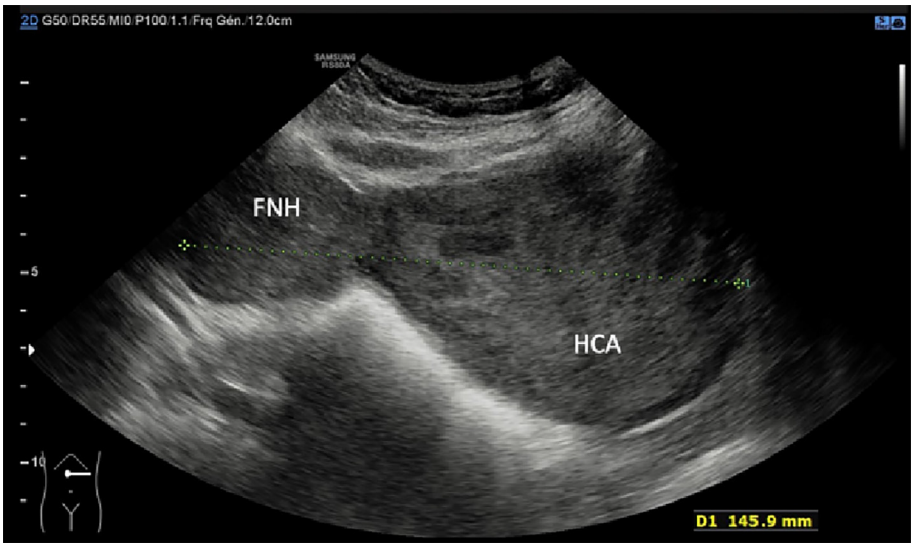

Figure 3 Ultrasonography with a sagittal view of focal nodular hyperplasia and hepatocellular adenoma. D1: Greater axis length. FNH: Focal nodular hyperplasia; HCA: Hepatocellular adenoma.

OUTCOME AND FOLLOW-UP

The MDT meeting proposed a 1-year MRI follow-up with oral contraceptive discontinuation.

One month after surgery, the patient was good without any complaint, her scar evolution was satisfactory and there was no sign of an early incisional hernia.

DISCUSSION

The interest of this case lies in the simultaneous discovery of 2 adjacent but pathologically different benign liver lesions: the first one (FNH) without a strong indication for surgery and the second one requiring surgery because of its uncertain diagnosis.

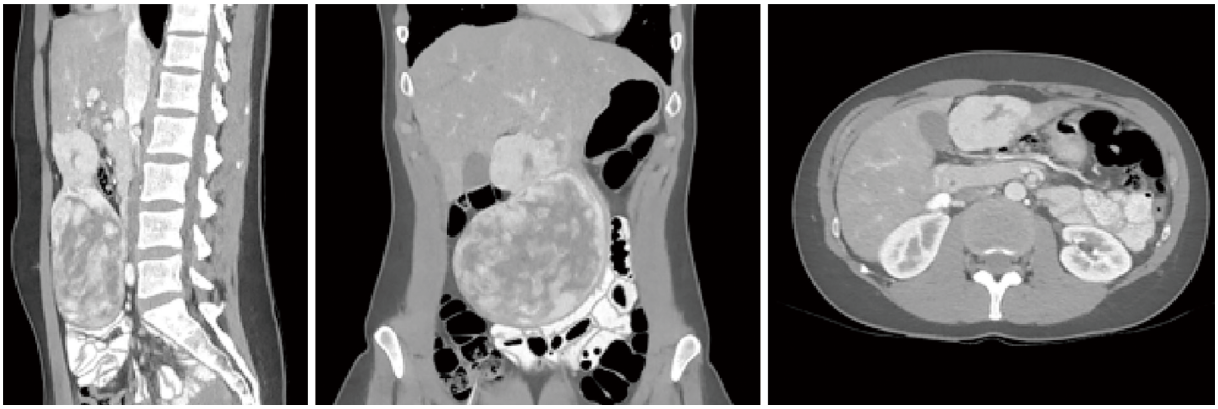

Figure 4 Computed tomography late portal phase, with a multiplanar reconstruction of focal nodular hyperplasia and hepatocellular adenoma.

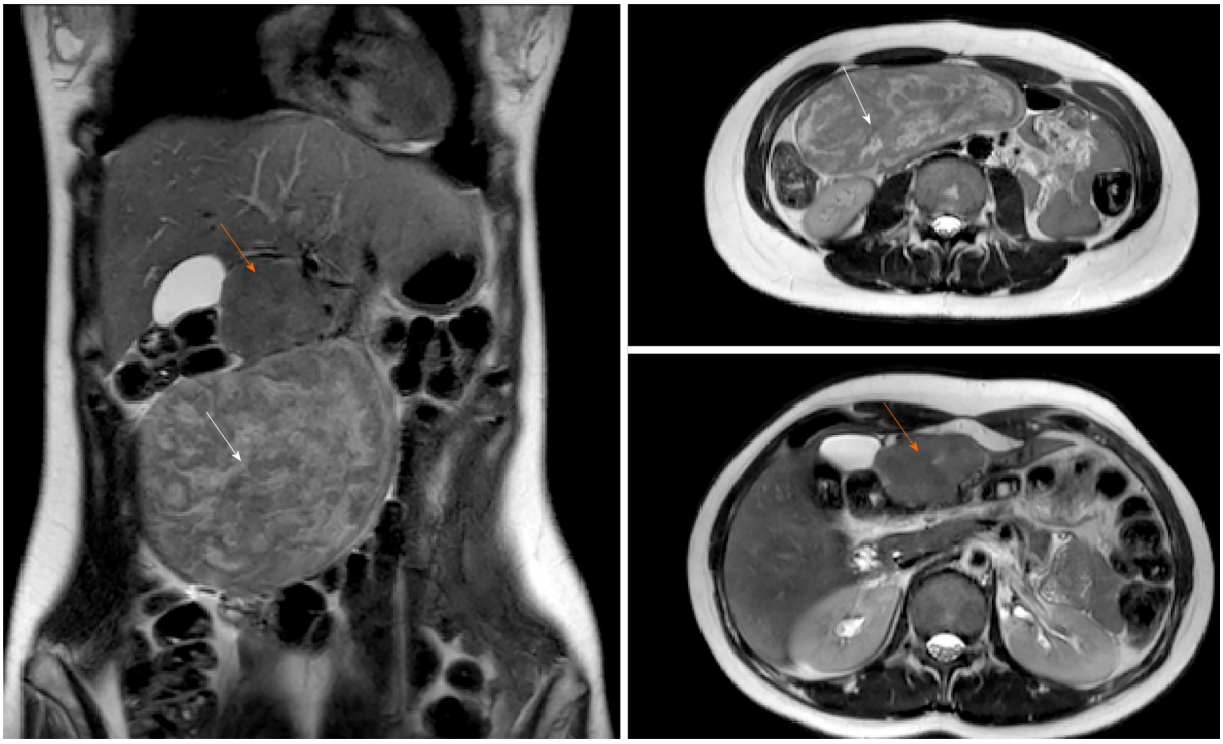

Figure 5 Magnetic resonance imaging – T2 sequence. Orange arrow: Focal nodular hyperplasia; White arrow: Hepatocellular adenoma.

FNH has no recognized risk of malignant transformation or bleeding and usually has an uneventful course. Therapeutic abstention is usually recommended for asymptomatic patients with a definitive diagnosis[11]. Surgical management is reserved for symptomatic patients or with diagnosis uncertainty despite a complete workup[12,13]. Twelve cases of spontaneous rupture of FNH are described and considering these extremely rare events, conservative treatment is the actual well-established standard of care [English-language literature until 2019; NCBI.gov with terms "spontaneous; rupture; FNH]. Close follow-up is however recommended for FNH more than 5 cm. Some authors advocate for upfront surgery with FNH larger than 5 cm[14-16]. However, we do not recommend a surgical resection in our daily practice but advocate for a close follow-up strategy. In the present case report, the diagnosis of FNH of the segment III lesion was radiologically typical and in the absence of the HCA component, a 1-year MRI follow-up would have been recommended.

On the contrary, the risk of malignant transformation of HCA is 4%-5%. As reported by Sempoux *et al*[7], risk factors for complications of HCA (bleeding or malignant transformation) are the size (> 5 cm), male gender, activating mutation in β-catenin,

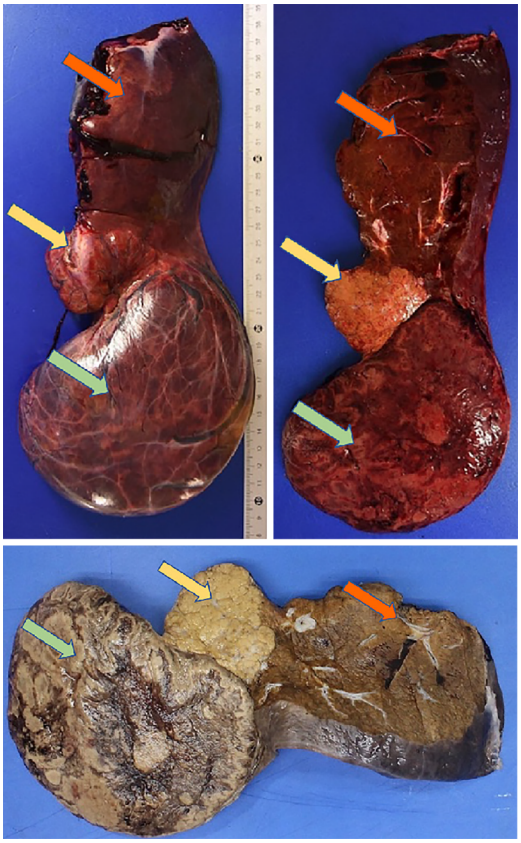

Figure 6 Anatomopathological pictures (top: fresh sample; bottom: formalin-fixed sample), sagittal section plane. Yellow arrow: Focal nodular hyperplasia; Green arrow: Hepatocellular adenoma; Orange arrow: Left liver (segment II).

and specific clinical background (glycogen storage disease, androgens, vascular diseases). The resulting recommendations for surgery are based on initial size (> 5 cm), imaging or histological signs of malignancy, size progression after OC discontinuation, and male patients. Selected patients and those who are not fit for surgery can benefit from embolization[17-19]. When the diagnosis cannot be achieved with imaging, a percutaneous biopsy or resection may be required[20].

Moreover, Bröker *et al*[21] 2012 advocated the surgery for adenoma greater than 5 cm with patients who had planned a pregnancy. Our patient didn't have a pregnancy plan but size and uncertainty of diagnosis were our principal arguments for surgery.

We made a literature review of the simultaneous cases of FNH and HA. Although there is some case reports in the eighties, the article was not available for consulting [22-25]. Table 1 summarizes the other cases with enough data.

Case 1 was operated on because of the lack of obvious radiological evidence[26]. The authors of case 2 don't clearly explain the indication for the operative procedure but they interestingly explain the possible same pathophysiological etiology for 4 different simultaneous hepatic masses[27].

Shih *et al*[28] made a left hepatectomy for a case with common features between FNH and HA and operate for the uncertainty of diagnosis.

The French group of Laurent *et al*[29] found in their records 5 over 30 patients operated for "benign hepatocytic nodules" with simultaneous HNF and adenoma. All of them went under surgery when the radiology reports an HA or unidentified mass. The diagnosis of FNH was already known at the time of the surgical procedure except for one case where the FNH was too small[29].

Concerning the surgical technique, the laparoscopic approach is relatively recent. Unfortunately, Shih *et al*[28] didn't report this in their paper although they did the same procedure for a similar patient. Despite the lack of high-level evidence data (randomized control trials, meta-analysis), current literature about laparoscopic *vs* open liver surgery for benign tumors suggests an advantage for the minimal-invasive technique[30,31]. On the other hand, evidence for laparoscopic malign liver resection is much more consistent. Furthermore, safety, feasibility, and long-term results confirmed the advantages of laparoscopy for malign liver tumors[32-34].

Table 1 Summary of current literature review

No.	Ref.	Sex, age	OC	Pathology - Size (cm)	Location (segment)	Symptoms	Treatment
#1	Dimitroulis *et al* [26], 2012	F, 18 yr	No	FNH – 2.5	S3	RUQ pain	Wedge resection
				HA – 6	S5-6		Lt S5-6
#2	Di Carlo *et al*[27], 2003	F, 25 yr	No	FNH – < 5	S4	RUQ pain	*En bloc* (+ gallbladder)
				HA – NA	S4		Enucleation
				HH – > 4	S2		Enucleation
				HCy – NA	S5		*En bloc* (+ gallbladder)
#3	Shih *et al*[28], 2015	F, 40 yr	Yes	FNH – 6	III	Abdominal pain	LH
				HA – 9.5 & small ones (max 1.5 cm)	III for the largest, small ones on both lobes		
#4	Laurent *et al*[29], 2003	F, 45 yr	Yes	FNH – 1	S3	Fatigue	Lt S3 segmentectomy + wedge
				HA – NA	S7		Lt RH
		F, 40 yr	Yes	FNH – 5	S6	None	Biopsy
				FNH – 4	S7		Biopsy
				NA – 3	Left lobe		Lt LH
				HA – 3	Left lobe		Lt LH
		F, 38 yr	Yes	HA surrounded by FNH –13	Right lobe	None	Lt RH
		F, 29 yr	Yes	HA – 5 × 1	S1 (bleeding), S2, 3, 7, 8	Abdominal pain + shock	Lt LH + S1
				FNH – 1	S6		
		F, 41 yr	Yes	HA – 1	RL	Abdominal pain	Lt RH
				FNH – 1	RL		
#5	Our case-report	F, 38 yr	Yes	6 × 614 × 12 × 6	S3	None	Ls LL

FNH: Focal nodular hyperplasia; HA: Hepatic adenoma; HCy: Hepatic hydatid cyst; HH: Hepatic hemangioma; RL: Right lobectomy; LH: Left hepatectomy; LH: Left hepatectomy; LL Left lobectomy; RUQ: Right upper quadrant; Lt: Laparotomy; Ls: Laparoscopic; F: Female; OC: Oral contraception.

CONCLUSION

We hereby report a laparoscopic resection of a macro-adenoma associated with focal nodular hyperplasia. The review of the literature shows that the simultaneous presence of these two masses is rare and that every case must be discussed in a multidisciplinary board. Factors like age, pregnancy wish, size, and uncertainty of diagnosis must be considered for shared decision in the setting of a multidisciplinary team. The laparoscopic approach should be preferred as much as possible.

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