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FRONTIER

Revolution in the diagnosis and management of hepatitis C virus infection in current era

Farina M Hanif, Zain Majid, Nasir Hassan Luck, Abbas Ali Tasneem, Syed Muddasir Laeeq, Muhammed Mubarak

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Abstract

Chronic hepatitis C virus (HCV) infection is a major global public health problem, particularly in developing part of the world. Significant advances have been made in the early diagnosis and treatment of the disease. Its management has been particularly revolutionized during the past two decades. In this review, we summarize the major advances in the diagnostic and management armamentarium for chronic HCV infection. The focus of the present review is on the newer directly acting anti-viral agents, which have revolutionized the management of chronic HCV infection. Management of uncomplicated chronic HCV infection and of specific complications and special at-risk populations of patients will be covered in detail. Despite the advent and approval of highly effective and well tolerable oral agents, still many challenges remain, particularly the affordability, the equitable distribution and access to later drugs. The World Health Organization aims to eliminate viral hepatitis including HCV by 2030 since its poses a major public health threat. There is an urgent need to ensure uniform and early access to diagnostic and therapeutic facilities throughout the world if the later goal has to be realized.

Key Words: Hepatitis C virus; Interferons; Diagnosis; Management; Directly acting anti-viral agents

Core Tip: Chronic hepatitis C virus (HCV) infection is a major public health threat worldwide, particularly in resource-constrained countries. Although significant advances have been made in the early diagnosis and treatment of the disease, many unmet challenges remain to be tackled, particularly the affordability, equitable distribution and access to these methods. The World Health Organization aims to eliminate viral hepatitis including HCV by 2030. This frontier article addresses the burden of chronic HCV infection, delineates the current therapeutic options, and identifies future strategies to tackle this highly prevalent disease.

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INTRODUCTION

Hepatitis C virus (HCV) accounts for majority of viral hepatitis-related mortality per annum worldwide [1]. Chronic HCV infection persisting for more than 20-30 years causes liver cirrhosis and/or hepatocellular carcinoma (HCC). World Health Organization (WHO) estimates that around 71 million people are suffering from chronic HCV infection with highest incidence in WHO Eastern Mediterranean and European Regions[2]. Globally, around 400000 people die annually due to chronic HCV-associated complications, predominantly as a result of decompensated hepatic cirrhosis or development of HCC. The economic impact of the disease is also enormous, particularly for emerging economies. HCV virus has greater genetic diversity than HBV or human immunodeficiency virus (HIV). HCV has seven genotypes with around 67 subtypes[3]. Spectacular advances have been made in the scientific field in the diagnosis and management of this infection in the past few years. Still, ironically, fewer than 20% of those living with HCV infection globally are aware of their disease, and the immediate challenge is to engage, screen, diagnose and treat everyone in whom treatment is warranted. These undiagnosed HCV cases worldwide represent an important hurdle to achieve the WHO goal of HCV elimination by 2030. Many social, access to healthcare and economic hurdles remain in the path to HCV elimination. However, many success stories are being reported from many high prevalence countries, including Egypt[4]. It is beyond the scope of this review article to cover all aspects of HCV infection; hence, the focus of this review is on the remarkable advances that have taken place in the diagnosis and management of chronic HCV infection, particularly in vulnerable and "difficult-to-treat" groups and on strategies being implemented to eliminate HCV infection.

DISCOVERY OF HEPATITIS C VIRUS

The story of discovery of HCV is unique in that it was identified by non-conventional means, i.e., molecular biologic techniques rather than direct visualization and cell culture. It is pertinent to briefly revisit this story here to better understand the advances that have taken place in the diagnostic and therapeutic aspects of HCV infection. It all started with the finding a new type of hepatitis in patients who received blood transfusion in early 1970s. As hepatitis A virus and hepatitis B virus (HBV) were not present in such patients, Alter and co-workers in 1975 coined the term "non-A, non-B (NANB) hepatitis" for this type of hepatitis. They collected plasma/serum samples from a blood donor with chronic hepatitis and four people who developed "NANB hepatitis" after receiving blood transfusion, and injected these samples into five chimpanzees. All five chimpanzees developed hepatitis, as evidenced by rise of serum alanine aminotransferase levels as well as liver pathological changes, confirming the presence of a yet unknown transmissible agent in the blood of patients with NANB hepatitis. In 1989, Houghton and co-workers constructed a random-primed complementary DNA (cDNA) library using plasma samples from patients with NANB hepatitis. One clone in this library was not derived from host DNA, and appeared to be from a novel RNA virus belonging to the *Flavivirus* family (at least 10000 nucleotides and positive-stranded). They named this novel virus as HCV. Later on, Rice and co-workers constructed a full-length clone of HCV cDNA that was able to be transcribed to an infectious RNA variant of HCV. Upon intrahepatic inoculation of this clone, chimpanzees developed chronic hepatitis, with production of antibodies against HCV and viral replication in the blood. Subsequently, Bartenschlager and co-workers developed an *in vitro* cell culture using a human hepatoma cell line to replicate HCV. This cell-based model was indispensable in highlighting the biological features of HCV as well as developing anti-HCV agents. The 2020 Nobel Prize in Physiology or Medicine was awarded to Drs. Harvey J. Alter, Michael Houghton and Charles M. Rice for the discovery of HCV[5].

HCV DIAGNOSTICS

An accurate diagnosis and linkage to care is the key to successful treatment and ultimately eradication of any infectious disease. HCV infection is no exception to this rule. Hence, simple, affordable, rapid and high quality diagnostic tests of active infection at the point-of-care (POC) are central to the achievement of HCV elimination goal. While the world has focused its attention over the last decade on the final stages within the cascade of care to develop and increase access to directly acting antiviral agents (DAAs), relatively less attention has been paid to ensure accurate and affordable diagnostic tools to make wide-scale global treatment a reality. The diagnostic armamentarium for HCV infection principally comprises of two approaches: detection of antibodies against the virus in the serum and HCV PCR, the later being the gold standard. More recently, HCV antigen test has also been introduced as an alternative to HCV PCR. Each of the tests has merits and demerits. Ironically, in many settings, prohibitively high costs of HCV diagnostics often now exceed the cost of curative therapy. Thus, improving access to rapid, simple, and affordable HCV diagnostics is critical to achieve global HCV elimination and should be considered a public health priority.

Simplified diagnostic solutions

American Association for the Study of Liver Diseases (AASLD) recommends all individuals above 18 years to be screened for HCV for at least one-time owing to treatment benefits and reduction of morbidity and mortality. HCV-antibody tests approved by US Food and Drug Administration (FDA) should be utilized for screening of HCV infection. To detect active viremia and for treatment decisions, HCV RNA with a detection limit of ≤ 25 IU/mL is advised. However, immunocompromised populations or patients exposed to HCV within 6 mo should undergo HCV RNA testing despite negative HCV antibody test. A simple and updated HCV testing algorithm recommended by Center for Disease Control is shown in Figure 1. Such simplification of current hepatitis C diagnostic algorithms and the advent of digital diagnostic devices will play a pivotal role in achieving the WHO's target goals of hepatitis C elimination by 2030. Over the last decade or so, hepatitis C diagnostics have been revolutionized by the introduction and commissioning of state-of-the-art HCV diagnostic platforms which have been efficiently applied in high-risk HCV populations in developed countries as well as in some low-to-middle income countries (LMICs) to diagnose millions of undiagnosed hepatitis C-infected people. POC rapid diagnostic tests (POC-RDTs), reflexive RNA testing, dried blood spot sample analysis and hepatitis C self-test assays have demonstrated their diagnostic value in real-world clinical experiences, in mass hepatitis C screening campaigns, and disenfranchised native hepatitis C populations in remote areas[6].

HISTORY OF DISCOVERY OF HCV TREATMENT

The development of successful antiviral chemotherapeutic agents lagged behind the antibiotics and has primarily evolved in past 50 years[7]. This developmental delay has as its cause many hurdles like the delays in the advent of culture system, experimental animal models and a standard method for antiviral drug formulation. Moreover, the challenges encountered in developing a targeted therapy for a specific viral agent included drug toxicity, viral genetic variability and resistance profile, all these lengthened its developmental process[7].

HCV-specific antiviral agents met the same development delay. The discovery of HCV began from 1975 with the identification of new transfusion-related "NANB" hepatitis virus to the isolation of a single cDNA clone named HCV in 1989[5,8-10]. The successful pilot study on NANBH, by Hoofnagle *et al*[11], in 1986 formed the basis of two randomized trials. Both trials demonstrated on-treatment effectiveness of interferon (IFN) alpha-b in HCV eradication[12]. Thus, in 1991, US FDA approved IFN-alpha for the treatment of chronic HCV infection. Later on, ribavirin (RBV), an oral nucleoside analogue, was utilized as a monotherapy for HCV infection. Due to transient antiviral effect of RBV, the focus of clinical trials shifted to combination therapy[9,12].

By increasing the treatment duration and with the addition of IFN-alpha with RBV, the sustained virological response (SVR) rate escalated from 6% to 42%[13]. However, this treatment option had many caveats, mainly the IFN-associated side effects and intolerability, which were later improved with the advent of once weekly pegylated IFN (PegIFN). Moreover, with the encouraging response in HCV genotypes 2 and 3, having an SVR rate of 70% to 80%, the combination of PegIFN with RBV, thus became the standard of care[12,14]. Although the "golden era of IFN" persisted for over a decade but the large cohort of patients having decompensated chronic liver disease, hemoglobinopathies, pregnancy and organ transplants were deprived of its treatment benefit[6,15].

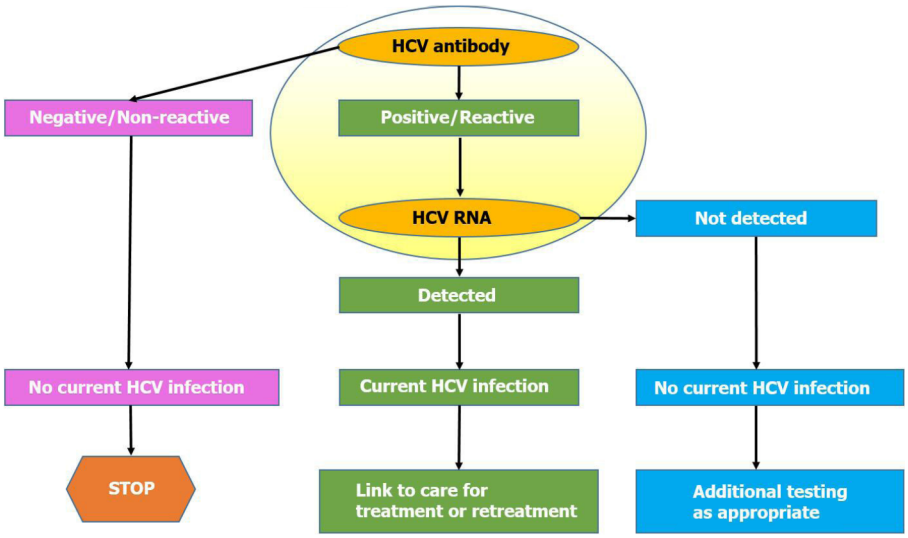

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Figure 1 Center for disease control recommended sequence of testing for the diagnosis of active hepatitis C virus infection with some conditions in certain situations (not detailed). HCV: Hepatitis C virus.

DAAs

Later on, with the advancement in the molecular virology of HCV and the unraveling of HCV life cycle, three dimensional structure of HCV and its enzymes, led to development of the first generation of NS3-4A protease inhibitors: namely telaprevir and boceprevir[6]. These agents were only approved for treatment of chronic HCV genotype 1 patients in combination with PegIFN and RBV[12]. Due to their unfavorable pharmokinetics, drug- drug interactions, adverse effects and efficacy only for genotype 1, these agents were eventually replaced by second generation directly acting antiviral agents (DAAs); the early agent, simeprevir (SMV), in combination with PegIFN and RBV was effective against only genotypes 1, 2 and 4[16]. It was sofosbuvir (SOF), NS5A polymerase inhibitor, which led to a paradigm shift in the treatment cascade of HCV[6,9]. The discovery of SOF was not only a breakthrough in the advent of all-oral DAAs but was a beacon of light for dealing with the HCV infection in cirrhotics. Figure 2 depicts a simplified classification and major sites of action of various DAAs.

Initially, SOF was used in combination with PegIFN and RBV and was approved for genotypes 1 to 4. Cumulative SVR of various trials documented 87.6%, 95.6%, 91.3% and 92.3% in genotypes 1, 2, 3 and 4, respectively. Phase 4 TARGET trial documented effectiveness of SOF and RBV combination for 12 wk. The trial reported 91.9% and 75% SVR12 in non-cirrhotic population while 71.9% and 55.3% in cirrhotics treatment-naïve population with genotype 2 and genotype 3, respectively[17]. Moreover, ASTRAL 2 and ASTRAL 3 trials reported 80.4% and 73% SVR for HCV infection amongst the non-cirrhotic and cirrhotic populations treated with SOF and RBV combination for 24 wk[18].

In 2014, SOF with ledipasvir (LDV), a single pill combination, was approved for genotypes 1, 4, 5 and 6. ION-1 and ION-2 trials documented high efficacy of SOF/LDV combination in naïve and treatment-experienced population. Additionally, the use of RBV or treatment extension to 24 wk did not provide any significant benefit[19,20]. The pooled SVR12 of various trials was 92.2% and 96.1% in genotype 1 infected patients with and without cirrhosis[17].

Later in the same year, triple DAAs fixed dose combination was approved for genotype 1. The paritaprevir, ombitasvir, ritonavir combination with dasabuvir (PrOD) was evaluated in various trials with or without RBV for 12 wk. The combination showed more than 90% SVR12 in both cirrhotic and non-cirrhotic genotype 1 patients[17]. However, the risk of hepatotoxicity precluded its use in decompensated cirrhosis[4].

The usage of these initial DAAs made the least responsive genotype, genotype 1, safely and effectively treatable as opposed to what was seen with IFN, while genotype 3 became the "difficult to treat" genotype[21]. This scenario was dealt with by NS-5A replication complex inhibitor, daclatasvir (DAC). ALLY-3, a phase III trial included treatment naïve and experienced HCV populations with genotype 3. The trial documented overall 96% SVR12 with 12-week therapy of SOF and DAC combination but with sub-optimum response in cirrhotic patients[22]. However, ALLY-3+ study evaluated response of SOF, DAC and RBV combination for 12 and 16 wk in HCV genotype 3 patients with fibrosis stage > 3. The authors reported 100% SVR 12 in patients with fibrosis stage 4, while 86% in patients with compensated cirrhosis. Moreover, the trial also concluded comparable SVR 12 in 12- and 16-wk groups[23].

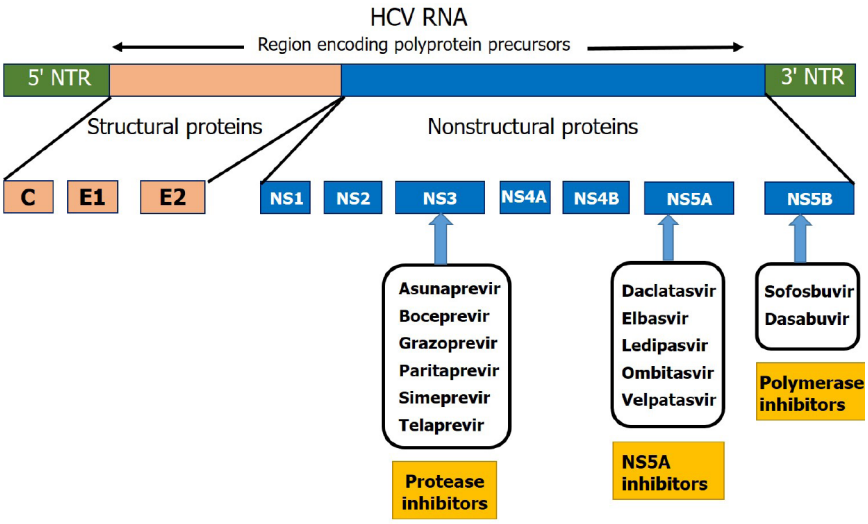

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Figure 2 Mechanisms of action and main classes of direct-acting antivirals.

ASTRAL 3 study compared the response of 12-wk SOF/Velpatasvir (VEL) with 24-wk SOF with RBV combination in genotype 3 HCV infected patients. In this study population, overall SVR12 rate was statistically significantly higher in SOF/ VEL group (95%) than SOF RBV group (80%) [$P < 0.001$]. Moreover, in the SOF/VEL group with cirrhosis, 93% SVR12 was achieved in treatment-naïve group and 89% in experienced group with no discontinuation of treatment due to adverse effects[18].

Both the EASL and AASLD have recommended a shorter duration of therapy with the DAAs (8-12 wk) for non-cirrhotics and 12 wk for the cirrhotic patients[24,25].

The POLARIS 2 and 3 trials, which were phase III trials, compared 8 wk of SOF-VEL-voxilaprevir (VOX) with 12 wk SOF-VEL combination in cirrhotic and non-cirrhotic HCV population. POLARIS-2 study group excluded genotype 3 and documented non-inferior 95% SVR12 with SOF-VEL-VOX while SOF-VEL combination showed SVR 12 of 98%. However, the lower efficacy was attributed to higher relapse rate in genotype 1a population. POLARIS 3 trial documented similar efficacy of both regimens in HCV genotype 3 patients with compensated cirrhosis[25].

Thus, it has become possible to treat HCV infection even in the presence of decompensated cirrhosis, which was otherwise quite cumbersome during the IFN era. ASTRAL-4 trial documented response to SOF/VEL with or without RBV in decompensated liver disease. Therapy was associated with improved disease severity as documented by Model for End-stage Liver Disease (MELD) and Child Turcotte Pugh (CTP) scores. The study documented 83% and 86% overall SVR 12 with SOF/VEL for 12 and 24 wk, respectively. Moreover, 94% SVR12 was observed with SOF/VEL for 12 wk with RBV combination. However, study was not powered to detect significant difference between regimens[26]. Moreover, since the HCV protease inhibitors undergo hepatic elimination, many regimens that contain these agents are not recommended in patients with decompensated cirrhosis. FDA has received rare reports of worsening liver function or liver failure when these patients are treated with the following drugs: Elbasvir/grazoprevir (ELB-GRA); glecaprevir/pibrentasvir (GLE-PIB); SOF-VEL-VOX; and PrOD. Hence, these drugs should not be prescribed in patients with a history of prior hepatic decompensation [27].

The treatment of decompensated HCV-associated cirrhosis with DAAs has shown to cause an improvement in CTP as well as MELD scores. The TOSCAR study in which patients with MELD 15 or more were treated with SOF/DAC for 24 wk, showed that three fourth of the patients who achieved SVR had their median MELD and CTP scores decreased by two points[28]. Furthermore, an Italian multicenter study, showed a significant increase in the rate of switch to CTP A, at 24 wk post-SVR[29]. The improvement in the CTP and MELD scores has also been shown to result in the delisting of patients who were earlier candidates for liver transplantation[30,31].

Table 1 depicts the summary of the main trials of various DAAs in the treatment of chronic HCV infection.

HCV IN CHILDREN

It is estimated that 3.26 to 5.0 million children and adolescents worldwide have chronic HCV infection. To date, the global response has focused mainly on the adult population, but DAA regimens are now approved for children aged ≥ 3 years. Transmission routes, disease progression and treatment

Table 1 Summary of the main trials of various directly acting antivirals in the treatment of chronic hepatitis C virus infection

Trial Names	Regimens	Treatment experienced /naive	Genotype	Duration	Cirrhotics/non-cirrhotics	SVR12, %
TARGET	Sofosbuvir-Ribavirin	Naïve	2	12 wk	Cirrhotics	91.9
					Non-cirrhotics	71.9
			3		Cirrhotics	75
					Non-cirrhotics	55.3
ASTRAL 2 and ASTRAL 3	Sofosbuvir-Ribavirin	Naïve	3	24 wk	Cirrhotics	73.3
					Non-cirrhotics	90.4
ION 1	Sofosbuvir-Ledipasvir	Naïve	1	24 wk	Cirrhotics	96.9
					Non-cirrhotics	99.5
TURQUOISE-III	Ombitasvir-Paritasprevir-Ritonavir	Naïve	1b	12 wk	Cirrhotics	100
ASTRAL 3	Sofosbuvir- Velpatasvir	Naïve	3	12 wk	Cirrhotics	93
					Non-cirrhotics	98.2
		Treatment Experienced		12 wk	Cirrhotics	89.2
					Non-cirrhotics	91.2
ASTRAL 4	Sofosbuvir- Velpatasvir-Ribavarin	Naïve	1	12 wk	Cirrhotics	94.4
		Treatment Experienced				90
ASTRAL 4	Sofosbuvir- Velpatasvir-Ribavarin	Naïve	3	12 wk	Cirrhotics	84.6
		Treatment Experienced				96.2
POLARIS 2	Sofosbuvir-Velpatasvir-Voxilaprevir	Naïve	1-6	8 wk	Cirrhotics	91
					Non-cirrhotics	96
POLARIS 3	Sofosbuvir-Velpatasvir-Voxilaprevir	Naïve	3	8 wk	Cirrhotics	96.3
		Treatment Experienced				97

indications in children differ from those in adults. Globally, vertical transmission accounts for most HCV infections in the pediatric population, but transmission also occurs through unsafe medical interventions, especially in LMICs. Adolescents may acquire infection through injection drug use (IDU), and high-risk sexual practices especially among men who have sex with men (MSM). Although the occurrence of severe disease or cirrhosis in children is low at 2%, progression of liver disease can occur in childhood, and can impact quality of life. Early diagnosis can help timely access to treatment and prevention of long-term morbidity.

There are significant gaps in policies for HCV-infected children and adolescents. Many countries have no national guidance on HCV testing and treatment in children and adolescents. There is an urgent need for advocacy and updated policies and guidelines specific for children and adolescents. According to the joint recommendation by the AASLD and IDSA, children born to HCV infective mothers should be first checked with anti-HCV antibody at 18 mo followed by checking of HCV RNA at 3 years of age to confirm the diagnosis. DAAS are recommended in children aged 3 years and above[25].

HCV in oncology patients

Chronic HCV infection is a significant problem in patients with various types of cancer. The prevalence of chronic HCV infection among patients with cancer in the United States has been estimated to range from 1.5% to 10.6%, but this range may be an underestimate because many cancer centers do not routinely screen patients for HCV. The impact of chronic HCV infection on cancer management can be profound but can be mitigated through early diagnosis and treatment. Early diagnosis of HCV infection and virologic cure improve liver and cancer outcomes and survival of patients with various cancers. Chronic HCV is not a contraindication to any cancer regimens but can disrupt liver functions and eventually lead to fibrosis in those on such regimens. Increased HCV replication in cancer patients on immunosuppressive therapy is less common than in HBV. In the DAAs era, it is not acceptable to exclude patients with cancer and chronic HCV infection from oncology trials because of HCV alone; HCV-infected patients facing life-threatening malignancies should have access to investigational

chemotherapy. In summary, overall benefits of DAAs in terms of virologic, hepatic, and oncologic outcomes far outweigh the risks of not treating this curable infection[25].

HCV in chronically transfused patients

Transfusion-dependent patients (*e.g.* Thalassemia) are at a higher risk of acquiring blood borne infections even under conditions of safe transfusion. Since, HCV is one of the most common blood borne pathogen, HCV infection is highly prevalent in children with β-thalassemia major in many countries despite strict pre-transfusion blood testing. This should raise the attention to environmental and community acquired factors. Quality management to insure infection control in minor operative procedures and adding more sensitive tests for blood screening are recommended. Patients with acute HCV and thalassemia have low rates of spontaneous resolution of HCV infection, and the majority develop chronic HCV infection. DAAs combinations are associated with high SVR rates and low adverse events in treatment naïve and experienced patients with chronic HCV and thalassemia. Liver fibrosis is accelerated in thalassemia patients with chronic HCV; therefore, early diagnosis, treatment with DAAs, adequate iron chelation, and non-invasive monitoring of liver status are recommended to prevent development of cirrhosis and HCC[25].

People who inject drugs (PWID)

Currently, the most common mode of transmission for HCV infection in the United States is through IDU; approximately 54% to 77% of new HCV diagnoses are among people who inject drugs (PWID). According to an estimate, 3.5 million people have injected drugs in the United States during their lifetime, with the prevalence of HCV infection in this population projected to be 73% (range 70%-77%). Because of the high probability of contracting HCV infection through needle-sharing, treating PWID infected with HCV, particularly in early stages of the disease, may reduce transmission. Treatment of people who inject drugs (PWIDs) is a top priority because of both the high burden of infection and the potential to transmit to others. The success of treating PWIDs is well established. In the recent SIMPLIFY trial, 103 persons with recent injection drug use (74% injected in the past month) received treatment with SOF-VEL for 12 wk and 94% achieved HCV cure with no virologic failures. Those with prior and current drug use, those on opiate substitution therapy (OST), and those not on OST had similar rates of cure with DAA therapy. Modeling of treatment in populations of PWIDs highlights the need for prevention measures concurrent with HCV treatment[25].

Men who have sex with men (MSM)

Global HCV prevalence in MSM varies by region and HIV status. Behavior counseling and regular HCV monitoring are needed in HIV-positive subgroups and high-risk regions. Given the upward trend of HCV incidence and sexual risk behaviors, there is also a continued need to reinforce risk-reduction intervention. Antiviral therapy along with counselling regarding the disease process regarding a high risk of disease recurrence in these patients is advised. Furthermore, these patients should also be told to incooperate measures that reduce the recurrence of HCV infection. Annual checking with HCV RNA is recommended in these patients who are sexually active[25].

HCV resistance

According to the EASL guidelines of 2020, those patients who were treated with DAAs and had failed to achieve clearance, are advised to be treated *via* a multidisciplinary team and should undergo HCV RNA resistance testing before retreatment. EASL HCV guidelines 2020 also recommend that those patients without cirrhosis or with compensated cirrhosis who failed DAAs regimen should be retreated with SOF/VEL/VOX for a duration of 3 mo. Those who fail to achieve SVR even after treatment with SOF/VEL/VOX, should be administered therapy containing the SOF/GLE/PIB for a period of 24 wk with RBV. Those with decompensated CLD who fail DAAs and with contraindications to the use of DAAs are advised to be treated with SOF/VEL/RBV for 24 wk[25].

HCC

Patients with decompensated cirrhosis who have achieved an SVR after treatment with DAAs are still at high risk of developing HCC due to the advanced stage of cirrhosis. This is due to the oncogenic property of virus itself along with the interaction of viral with the host factors that cause liver cirrhosis to progress towards HCC[32]. This risk increases in obese patients, those co-infected with hepatitis B virus (HBV) and/or human immunodeficiency virus (HIV), type 2 diabetes mellitus and HCV genotype [4,32,33]. Kanwal *et al*[34] evaluated Veteran American HCV Clinical Case Registry and documented 80% higher risk of HCC with genotype 3 as compared to genotype 1. Lee *et al*[35] showed that genotype 6 has higher risk association in South Asian population.

On the contrary, various studies have documented that successful viral eradication, established by SVR, is associated with a decreased risk of HCC and decompensation events; hence, reduced HCV-related morbidity and mortality[36,37]. However, in the past, as PegIFN was contraindicated in

decompensated cirrhosis, large cohorts of patients were deprived of treatment benefits[38,39].

Although, treatment with IFN was associated with a low cure rate and higher adverse effects, studies reported that achieving SVR reduced the risk of HCC to 0.5%-1% per annum[40]. The IHIT Study Group also documented decrease in the incidence of HCC in patients with chronic HCV infection treated with IFN and who achieved SVR and biochemical response[41]. Similar response was also documented by Hsu *et al*[42] in Taiwanese population. Moreover, a few studies also demonstrated beneficial response of IFN therapy in patients with cirrhosis and advanced fibrosis but with a lower response[37,43,44].

Although, the favorable response to oral DAAs revolutionized the treatment armamentarium, this development was marred by reports of higher incidence of HCC, HBV reactivation and drug-drug interactions[45-47]. Kanwal *et al*[34] reported cumulative incidence of HCC at 2.8% while Tani *et al*[48] documented 6% at 3 years following treatment with DAAs. However, other studies negated these results. A large prospective observational French cohort documented association of DAAs therapy with decreased all-cause mortality and HCC[49]. Delgado Martínez *et al*[50] reported lower incidence of HCC with DAAs as compared to untreated patients (2.90 and 4.48 per 100 person-years, respectively). Moreover, Romano *et al*[51] reported declining trend in the incidence of HCC among HCV-related cirrhotics treated with DAAs.

A meta-analysis of 41 studies compared recurrence of HCC in patients treated with either DAAs or IFN therapy. The authors demonstrated similar rate of HCC recurrence in patients treated with IFN and DAAs' studies (9.21/100 per year *vs* 12.16/100 per year), respectively[52]. However, Reig *et al*[53] reported higher recurrence of HCC (27.6%) in 5.7 mo of follow-up in 103 DAAs treated patients. Even though, Cabibbo *et al*[54] in a multicenter Italian study reported higher incidence of HCC recurrence in DAAs treated population but concluded that risk is comparable to untreated population. In a recent review by Muzica *et al*[40] the author concluded that incidence of both HCC occurrence and recurrence is significantly reduced by achieving SVR with DAAs.

To summarize, a vast majority of studies support the use of DAAs therapy in patients with advanced liver disease and successfully treated HCC. Whether or not to treat patients with uncured HCC with DAAs is an issue, which still needs to be studied.

RISK OF HBV REACTIVATION

The encouraging response of DAAs in decompensated cirrhosis was refuted by detrimental reports of HBV reactivation. In 2016, FDA had issued black box warning of HBV reactivation based on multiple reports including liver failure and deaths[55]. In HBV-HCV co-infected patients, both HBV and HCV have reciprocal inhibition on each other[56]. In the IFN era, HBV reactivation was a rare occurrence due to its dual antiviral effects on both viruses[57]. In the DAAs era, the reported prevalence of HBV reactivation ranges from 2%-57% in HBsAg-positive while 0%-3% in HBsAg-negative or anti-HBc-positive patients when treated with DAAs[58]. Thus, the pharmacological suppression of HCV with DAAs curbs the inhibitory effect on HBV genome and thus may lead to HBV reactivation[56].

Recently, Mücke *et al*[59] in a meta-analysis reported 24% and 1.4% risk of HBV reactivation in patients with chronic HBV and HBc-total positive patients, respectively. The authors also highlighted that the risk of reactivation was not related to nadir HBV DNA levels or severity of liver disease. Thus, frequent testing and monitoring is required in this population.

AASLD recommends that patients fulfilling treatment criteria for HBV should be treated in patients with co-infection. On the other hand, patients with a low or undetectable HBV DNA levels, can either be treated prophylactically or monitored regularly. Thus, all HCV patients to be treated with DAAs should be tested for HBsAg, anti-HBc total and anti-HBs titer prior to start of DAAs[4].

KIDNEY INVOLVEMENT IN CHRONIC HCV INFECTION

The kidney is involved in chronic HCV infection due to immune complex deposition. Epidemiological studies have documented a high prevalence of chronic kidney disease (CKD) in HCV positive patients [60].

Fabrizi *et al*[61] in a systematic review and meta-analysis documented increased risk of proteinuria and CKD in patients with positive HCV serology. Mendizabal *et al*[62] retrospectively evaluated large database of HCV infected and non-infected population. The authors documented 27% increased risk of CKD in HCV infected compared to non-infected population. Moreover, the risk of CKD reduced by 30% with effective antiviral therapy. Studies have documented rapid renal deterioration from CKD to end-stage renal disease (ESRD). Thus, there is a high rate of morbidity and mortality in HCV-infected CKD patients[63-65].

On the other hand, dialysis patients are at increased risk of acquiring the virus due to poor hygiene, increased risk of nosocomial infection and lack of proper sterilization techniques along with improper handling of equipment. Hence, testing all dialysis patients at entry and periodically thereafter is recommended[66,67]. However, there is some controversy on the type of testing (serology or NAT) in

these patients[68]. These patients have higher prevalence of HCV infection, higher risk of HCC and cirrhosis, and lower survival than the general population[69]. Therefore, withholding HCV treatment till renal transplantation would be detrimental.

During the early era of DAAs, SOF was not recommended in patients with eGFR < 30 mL/min due to the fear that these patients accumulate SOF and its metabolites[70,71].To address this issue, various authors have experimented with SOF dosage in these patients[72,73] Bhamidimarri *et al*[74] documented virological response with daily 200 mg *vs* alternate 400 mg SOF dose in patients with ≤ 30 mL/min per 1.73 m^2 of eGFR. However, we documented 96.9% SVR12 in 133 hemodialysis patients treated with SOF-based regimen at daily dose of 400 mg[75]. Moreover, Shehadeh *et al*[76] in a systemic review and meta-analysis reported no statistically significant differences in SVR12 rates amongst dialysis patients treated with 400 mg daily, 400 mg on alternate days or 200 mg daily SOF dose.

A meta-analysis of patients with CKD stage 4 and 5 documented cumulative SVR12 of 89.4% on treatment with SOF-based regimen[77-79]. Borgia *et al*[80] reported SVR12 of 95% among 59 dialysis-dependent patients treated with SOF-VEL combination. In 2019, considering the published safety and efficacy of SOF in advanced CKD patients, Food and Drug Administration (FDA) permitted the use of SOF-based therapy in patients with eGFR ≤ 30 mL/min including dialysis. AASLD also recommended that no dose adjustment is required in this population[4].

Elbasvir/grazoprevir (EBR-GZR) combination was the first to be approved for hemodialysis patients with HCV infection[81]. The efficacy of EBR-GZR for 12 wk was demonstrated in C-SURFER trial in genotype 1 with CKD stage 4 or 5 (eGFR < 30 mL/min). In the immediate treatment group, SVR12 was achieved in 99.1%[82]. The deferred group population were prescribed EBR/GZR combination after 16 wk of trial inclusion (n = 99). The authors documented 98% SVR 12 in this group[82]. The study by Bruchfeld *et al*[83] re-inforced the safety and efficacy of EBR/GZR combination in stage 4-5 CKD with HCV genotype 1 infection. AASLD recommends EBR/GZR in genotype 4 infection in stage 4/5 CKD considering encouraging response in general population[4].

EXPEDITION-4 trial assessed pan-genotypic fixed dose combination of GLE-PIB (100/20 mg) in non-cirrhotic stage 4/5 CKD patients. The trial included HCV genotypes 1 to 4 and also treatment-naïve and experienced population. In a total of 104 patients, SVR 12 was achieved in 98% with no virological failures[84]. Subsequently, EXPEDITION-5, a phase 3 trial, evaluated the same fixed dose combination in stage 3b, 4, or 5 CKD in compensated cirrhotic and non-cirrhotic populations. The overall SVR12 was achieved in 97% of the study population[85]. Although trial reported 5% non-serious side effects, Harrison *et al*[86] reported a case of drug- drug interaction with colchicine. Despite 50% reduction of colchicine dose, patient with stage 4 CKD developed rhabdomyolysis and acute kidney injury (AKI) with GLE-PIB combination. The authors recommended withholding colchicine during treatment with NS5A inhibitor containing DAAs, specifically with renal dysfunction. Similarly, Patel *et al*[87] also reported rhabdomyolysis in stage 3 CKD patients secondary to interaction with SOF/LDV, atorvastatin and colchicine use.

In summary, various novel DAAs are highly effective and safe in CKD population. However, drug-drug interactions should be considered in case of use of NS5A inhibitor containing DAAs with P-glycoprotein (P-gp) inducers.

CRYOGLOBULINEMIC GLOMERULONEPHRITIS

Although, HCV can lead to tubulointerstitial nephritis, it is the HCV-associated glomerular disease that is more frequently encountered[81,88]. Nonetheless, its incidence remains fairly low. Other than immune complex deposition in glomeruli, Toll-like receptor 3 has also been postulated to cause renal injury in HCV-infected population[60,89].

Various histological types of HCV-associated renal diseases include cryoglobulinemic membranoproliferative glomerulonephritis (MPGN), mesangial proliferative GN, focal segmental glomerulosclerosis, membranous nephropathy, *etc*[88]. However, the most frequent glomerulopathy is Type I MPGN associated with type II mixed cryoglobulinemia (MC)[60,89]. Around 20% to 56% of patients with HCV-associated MC type II may develop renal involvement[60]. The clinical presentation may vary and nephrotic syndrome, acute nephritic syndrome and oliguric acute renal failure have been reported in 20%, 30% and 5% of patients, respectively[90,91]. In pre-DAAs era, HCV-associated glomerulopathies were treated with IFN. A systematic review and meta-analysis reviewed response of conventional or PegIFN for HCV-associated MC. The kidney involvement was documented in 11%-74% in analyzed 11 studies. The authors reported excellent association of virological response with clinical remission in majority of patients[92]. Similarly, another meta-analysis documented association of virological and clinical response in patients treated with combination of PegIFN and RBV therapy in HCV-infected MC. The kidney involvement in study population ranged from 4% to 39%[93]. Other studies have reported lesser efficacy and more side effects with IFN-based treatment in HCV-MC as compared to HCV-infected general population[94,95]. Moreover, even with > 70% remission with IFN-based therapy in MC-induced vasculitis, the associated adverse effects discourage it use in this population[96].

Although limited, a few studies have documented good response with DAAs. It has also been observed that clinical and immunological response may not correspond to SVR[97-99]. Fabrizi *et al*[88], reviewed 9 clinical studies (n = 67) and documented 92% SVR with DAAs though cumulative complete clinical response was low *i.e.*, 38.5%. Furthermore, few case reports have documented new-onset or relapsing glomerular diseases even in patients who achieved SVR with DAAs[100,101].

In view of satisfactory efficacy and lesser side effects, DAAs are advised for viral eradication in patients with HCV-associated MC[88]. Treatment is based on severity of disease involvement. In patients with mild to moderate form of disease (GFR > 30 mL/min/1.73 m^2 (with or without non-nephrotic proteinuria), DAAs are the first line of treatment. However, immunosuppressive agents (IS) are advised for non-responsive cases or drug intolerance. In patients with cryoglobulinemic flare or severe glomerular injury, IS agents (rituximab) are in the initial treatment algorithm with or without plasma exchange. The resolution of acute phase is followed by HCV treatment with DAAs. However, IS agents and DAAs can be prescribed as per clinicians' discretion[88].

In summary, HCV can be found in 85%-95% of patients with MC[102]. However, only 10%-15% will have clinical manifestations including glomerulopathy[103]. Thus, KDIGO Clinical Practice Guidelines recommend annual evaluation for proteinuria, hematuria and eGFR in HCV-infected population with or without renal dysfunction especially with cryoglobulinemia[104]. The mainstay treatment still remains HCV eradication. Studies have documented encouraging response with DAAs, which, albeit, may lead to partial response[88].

LIVER TRANSPLANT RECIPIENTS

Apart from having a favorable response in the decompensated liver disease, the novel DAAs have led to a paradigm shift in the management of HCV-related disease in the post-transplant setting. In this section, we will highlight the important landmark studies and trials for the treatment of HCV in the solid organ transplant recipients.

During the IFN era, majority of patients with end-stage liver disease were deprived of therapy due to its deleterious side effects or contraindications[105,106]. Hence, HCV positive liver transplant recipients experienced universal liver graft reinfection; consequently leading to poor outcome[107]. However, attainment of SVR post liver transplant was associated with improved survival[108,109].

This scenario has been altered with the advent of DAAs. Cholankeril *et al*[110] in a retrospective study documented 91.9% and 89.8%, one year survival in HCV positive liver recipient transplanted in DAA era *vs* pre-DAA era, respectively. Similarly , Cotter *et al*[111] in a prospectively collected cohort of 18,746 documented statistically significant improved 1 and 3 year post transplant survival in HCV positive recipients in DAA era as compared to past. Among various factors, viral genotype is an important determinant of SVR in post liver transplant recipients[112,113]. Campos-Varela *et al*[112] reported higher risk of advanced fibrosis and lower rate of SVR with PegIFN-based treatment in genotype 1 infected liver transplant recipients. Moreover, the authors also reported statistically significant association of HCV genotypes 2 and 3 with SVR as compared to genotype 1. Similarly, Chen *et al*[114] concurred that HCV genotype 1 was less likely to achieve SVR than non- genotype 1 infection. Zanaga *et al*[115] reported higher SVR with genotype 3 and statistically significant association with SVR on a univariate analysis in post-liver transplant population. A systematic review and a meta-analysis reported pooled SVR12 of 90% with simeprevir (SMV) and SOF combination with or without RBV in recurrent genotype 1 in post liver transplant population. However, interaction with Cyclosporine immunosuppression was also documented[116].

SOF, combined with RBV, was used in 40 liver transplant recipients of all genotypes, and achieved an SVR12 rate of 70% in the study population with no graft loss or rejection. However, no genotype-specific response was documented[117]. Subsequently, the phase 3 ALLY-1 trial documented response of SOF and DAC combination with RBV for 12 wk in liver transplant population with recurrent HCV. Although the study population included treatment-experienced recipients, trial documented 95% and 91% SVR12 in patients with genotype 1 and 3 infections, respectively[118].

SOLAR 1, a phase 2 open label study that was conducted in USA, evaluated the response of LDV and SOF with RBV in 223 liver transplant recipients with HCV genotypes 1 and 4 infections. The study participants were randomly assigned 12 and 24 wk of treatment and achieved SVR12 in 96% and 98%, respectively, without cirrhosis. Moreover, lower rate of SVR12 was achieved in participants with CTP B and CTP C cirrhosis[119]. Similarly, SOLAR 2 trial conducted in Europe, Australia, Canada, and New Zealand also reported higher SVR rate of 93% and 100% in post liver transplant non-cirrhotic recipients treated with LDV/SOF combination with RBV for 12 and 24 wk, respectively. However, amongst recipients with CTP class C cirrhosis, SVR was higher with 24-wk treatment[120].

The use of the first pan-genotypic oral agent, SOF/VEL combination for 12 wk was evaluated in liver transplant recipients with genotypes 1 to 4[121-123]. Considering the beneficial effect of SOF/VEL in decompensated cirrhosis, AASLD, recommends SOF/VEL with RBV combination in liver transplant recipient with decompensated cirrhosis for 12 or 24 wk. Extended treatment is considered for recipients with treatment-experienced genotype 3 infection and presence of HCC[4].

Another pan-genotypic fixed dose single pill combination of GLE-PIB (300/120 mg) is also recommended in transplant recipients[4]. MAGELLAN-2 trial evaluated 100 non-cirrhotic post-transplant patients with or without treatment experience. In intention-to-treat analysis, liver and kidney transplant recipients achieved 97.5% and 100% SVR12, respectively. Although, minor reduction in tacrolimus was required in 1st week but the median dose of cyclosporine, everolimus or sirolimus, remained unchanged[124]. Similarly, SVR12 of 98% with 8 wk or 12 wk of GLE/PIB combination was observed in a multicenter trial of 24 liver transplant patients. Study population also included prior DAAs experience, severe renal impairment, hemodialysis and post-liver transplant jaundice[125].

Although, DAAs are safe and highly efficacious in treatment-naïve and experienced recipients, but in general 5% of the population fails to achieve SVR. This is mostly encountered in recipients with associated decompensated cirrhosis or HCC[126]. Despite lack of published data, on the basis of expert consensus, AASLD recommends SOF/VEL/VOX in patients with DAAs experienced post liver transplant patients[4]. Cardona-Gonzalez *et al*[127] reported successful treatment of recurrent genotype 3 in liver transplant recipients with SOF/VEL/VOX combination in DAAs experienced individuals. Similarly, Higley *et al*[126] recently published a case series of six HCV liver transplant recipients with DAAs failure. The authors documented successful HCV eradication with 12 wk of treatment with no adverse effect or virological relapse during study period.

EASL recommends to initiate DAAs as early as possible after liver transplant once the recipient's clinical condition is stabilized. Generally, it is advised to start treatment after 3 mo of transplant. However, exact time frame for starting DAAs in non-hepatic solid organ transplants has not being recommended. We believe that with widespread availability of new DAAs in pre-transplant period, there will be an increase in the number of DAAs-experienced and/or treatment failure patients among transplant recipients. Thus, pan-genotypic, efficacious and safe salvage therapy is warranted in these special scenarios.

With the increasing availability of liver transplant facilities, the growing demand of donor organs has yet to be met worldwide. Historically, HCV positive donors were only accepted for transplantation in recipients with dire complications like fulminant hepatic failure[128]. However, the recurrence of HCV infection and associated morbidity and mortality were added risks. With the advancements in DAAs, the question of utilizing HCV positive donors was addressed by multiple studies[129]. As compared to renal transplants, data on PCR positive donors to PCR negative liver recipients is limited. However, Cholankeril *et al*[130] and Cotter *et al*[131] reviewed the OPTN registry from 2015 to 2020 and reported comparable 1 and 2-year post transplant survival of patients transplanted with HCV viremic organs, in NAT negative recipients. Bethea *et al*[132] reported 100% SVR in 10 liver recipients who received NAT positive donors treated with 12 wk of GLE/PIB combination. Nonetheless, one recipient developed acute cellular rejection. In a real world experience, Jandovitz *et al*[133] also reported beneficial response of GLE/PIB combination in three HCV negative liver transplant recipients.

RENAL TRANSPLANT RECIPIENTS

HCV-infected renal transplant recipients (RTRs) have a higher survival as compared to being on waiting list despite the complications[134-136]. However, HCV-infected RTRs have reduced graft and patient survival compared to non-infected counterparts. Fabrizi *et al*[137] documented the presence of anti-HCV antibody as a prognostic factor for patient and allograft survival in RTRs with relative risk of 1.79 (95%CI: 1.57-2.03) and 1.56 (95%CI: 1.35-1.80), respectively. An observational meta-analysis reported higher rate of liver- and cardiovascular-related mortality[138]. Although HCV in RTRs leads to slow progression to chronic liver disease (CLD), the increased risk of HCC cannot be disregarded. Long-term immunosuppression is possible culprit to accelerated liver fibrosis; thus, leading to cirrhosis and HCC [139]. Zylberberg *et al*[140] reported significantly higher yearly progression of hepatic necroinflammation and fibrosis in HCV infected as compared to non-infected recipients. Moreover, HCV in RTRs also increases the incidence of infection, glomerulopathy, vasculitis and post-transplant diabetes mellitus[141-144].

In the past, IFN was contraindicated in RTRs due to inferior virological response, low tolerance and increased risk of graft rejection[137,145]. A meta-analysis reported 18% SVR and drop-out rate of 35% in recipients treated with IFN-based regimen[146]. Occasional studies, mostly case reports, documented beneficial response of IFN in renal transplant population[147,148]. Early in DAAs era, SOF was not recommended in patients with eGFR < 30 mL/min due to possible accumulation of SOF and its metabolites causing renal dysfunction[71]. However, various studies have reported favorable response of SOF with RBV in RTRs[149,150]. We have observed 89.2% end-of-treatment response (ETR) and 100% SVR12 in our renal transplant population treated exclusively with SOF and RBV combination. Moreover, we also reported resolution of liver-related ascites in two out of four decompensated recipients[151].

Multiple studies reported 90% to 100% SVR 12 in recipients treated with 2 different class of DAAs [152,153-155]. We also treated our 79 treatment-naive and treatment-experienced RTRs. Majority received SOF and RBV (78.5%) while remaining received SOF, DAC and RBV combination. ETR and

SVR12 were achieved in 98.7% and 96.2%, respectively[151] Coral-1 study evaluated liver and RTRs. The study population including 12 non-cirrhotic RTRs received PrOD with and without RBV in genotype 1a and genotype 1b, respectively. RTRs achieved lower SVR of 75% with premature treatment discontinuation as compared to liver transplant recipients[156]. However, Scott *et al*[157] in a multicenter randomized trial documented 98% SVR 12 in 114 RTRs with genotypes 1 and 4, treated with SOF-LDV for 12 or 24 wk.

A multicenter, prospective observational trial, HCV-TARGET, demonstrated efficacy and safety of SOF-based regimen in transplant population. The cohort included 347, 60 and 50 Liver transplant, kidney transplant and dual liver kidney transplant recipients, respectively. The regimen included SOF-LDV, SOF-DAC and PrOD with or without RBV. In RTRs, trial reported 94.5% SVR12 and acute rejection in two recipients[158]. MAGELLAN-II trial documented safety and efficacy of GLE/GDP combination in liver and kidney transplant population. The population included 20 RTRs with genotypes 1, 3 and 4, among which four were treatment-experienced with IFN-based regimen. The study documented 100% SVR12 with no virological relapse[124]. Long-term follow-up documented by Zhang *et al*[159] reported no virological relapse at 24 and 96 wk post-treatment in eight RTRs treated with SOF-based regimen.

One of the major apprehensions for use of DAAs in transplant population was the drug- drug interaction, thus leading to graft rejection. In a Spanish renal transplant registry, 55.3% of study population required immunosuppression adjustment. Although renal function remained stable during treatment, 2.9% developed acute allograft rejection[160]. Similarly, Scott *et al*[157] reported immunosuppressive dose alteration in 18% of RTRs treated with SOF-LDV combination. Özer Etik *et al*[149] reported 100% SVR in RTRs but 45% of transplant recipients required increased dose of calcineurin inhibitors. The authors attributed the increased requirement to improved liver function; thus, enhanced drug metabolism. However, various studies did not report immunosuppressive dose modification with SOF-based regimen. AASLD latest guidance recommends not to co-administer cyclosporine with EBR-GZR combination or with GLE/PIB combination. However, Tacrolimus level may need to be adjusted with GLE-PIB combination[4].

Although, multiple studies have documented effective HCV eradication, no graft rejection and stable renal function during and after DAAs therapy[161,162]. Other authors have documented worsening proteinuria in transplant recipients with higher pretreatment levels[155]. Thus, although DAAs are efficacious and safe in RTRs but caution should be practiced with monitoring of calcineurin inhibitor levels, renal functions and proteinuria.

To counteract the shortage of kidney donors, researches have focused on utilizing HCV positive kidneys in HCV negative RTRs[141]. Even with favorable results, the American Transplant Society (ATS) and KDIGO recommend that HCV infected organs can be transplanted into HCV NAT negative recipients as a research protocol only with an informed consent and approval from ethical committee [163,164]. However, KDIGO guidelines recommend HCV NAT-positive kidney to be transplanted to NAT positive recipients with the aim to decrease the organ wastage. Nevertheless, liver fibrosis stage and availability of effective DAAs prior to transplantation should be ensured[163].

Prior to 2000, various studies suggested increased incidence of hepatitis and subsequently poor graft survival in anti-HCV positive kidney recipients transplanted with anti-HCV positive organs[165,166]. Moreover, negative recipients receiving positive donors were associated with higher liver-related complications[167,168]. The effective response to DAAs in post-transplant period had led to address the issue of discarded HCV-positive organs.

To expand the donor pool, researchers have reported response of HCV viremic donors to HCV-negative recipients. The first prospective trial, THINKER-1 in 2017 followed by THINKER-2 in 2018 reported 100% SVR in aviremic RTRs who received NAT-positive organs. All the recipients received EBR/GZR combination for 12 or 16 wk depending on NS5A resistance-associated substitutions (RASs). Although among a total of 20 recipients, two developed proteinuria due to FSGS but all achieved SVR12 [169,170]. Moreover, EXPANDER trial evaluated preemptive treatment regimen in NAT-positive donors to aviremic recipients. All recipients received one dose of EBR/GZR followed by EBR/GZR × 12 wk with or without SOF depending on genotype. In total, 30% of the study population had detectable viremia post-transplant. However, all achieved SVR12[171]. La Hoz *et al*[172] documented no statistically significant difference in graft survival and acute cellular rejection (ACR) in aviremic recipients receiving HCV-positive or HCV negative kidney.

Recently, Jandovitz *et al*[133], in a single center prospective study, evaluated the response of GLE-PIB, SOF-LDV and SOF-VEL in 64 RTRs with positive donors and negative recipients. The author reported 95% detectable viremia post-transplant with SVR12 in 41/58 recipients. The result of 17 recipients was awaited at the time of publication. Moreover, two patients developed fibrosing cholestatic hepatitis (FCH), which was successfully treated with DAAs. The study documented 98% patient and graft survival.

HEART AND LUNG TRANSPLANT RECIPIENTS

In the pre-DAAs era, despite the fact that heart and lung tissue are not reservoirs for HCV, utilization of HCV-positive organs was controversial. Studies have reported lower patient survival as compared to recipients with aviremic donors[173,174]. However, the benefits of procedure outweigh the morbidities associated with no transplants in this special group[128].

Abdelbasit *et al*[175] reported first case series of five lung recipients transplanted with viremic lungs. The recipients responded to SOF-based regimen with 100% SVR 12 and 100% patient survival 12 mo after transplant. Recently, Cypel *et al*[176] compared *ex vivo* lung perfusion with or without ultraviolet C radiation (UVC) in 22 NAT-negative lung recipient transplanted with positive donors. In 20 recipients with detectable viremia, 96% achieved SVR12 with SOF/VEL combination. The relapse in two patients including one with FCH was successfully treated with SOF-VEL-VOX and RBV combination.

Similarly, various studies reported beneficial response of DAAs in heart transplant recipients with NAT-positive donors with > 90% SVR12[133,177,178]. Kilic *et al*[179] and Reyentovich *et al*[180] reported no statistically significant differences in 1-year survival in heart recipients transplanted with viremic or aviremic donors. To increase the donor pool, studies have evaluated transplantation of positive donors in NAT-negative recipients, also called HCV aviremic recipients. Bethea *et al*[132] evaluated 20 HCV non-viremic heart recipients treated with first dose of GLE-PIB combination prior to transplantation followed by 8 wk of therapy after transplantation. The author reported 100% SVR12 and 100% graft and patient survival for median of 10.7 mo.

Schlendorf *et al*[181] reported favorable response of SOF-VEL and SOF-LDV in 11 HCV aviremic heart recipients transplanted with HCV-viremic donors. Out of which, nine developed post-transplant viremia, among which eight successfully achieved SVR12. Remaining one recipient was under treatment at the time of publication. Similarly, DONATE HCV trial evaluated the response of four weeks of SOF-VEL combination, started within few hours of transplantation, with NAT-positive donors. The trial included 44 HCV aviremic recipients; 36 underwent lung transplants while eight received heart transplants. Till the time of study publication, 35 recipients achieved SVR12 and reported excellent graft survival at follow-up of six mo[182].

To decrease the risk of infection transmission, trials have been focused on preemptive and shortened DAAs course. Feld *et al*[183] suggested shortest pan-genotypic DAA course in recipients receiving viremic donors. The authors evaluated 30 HCV NAT-negative recipients who received viremic organs which included; 6 hearts, 13 lungs, 10 kidneys and one dual kidney-pancreas. All recipients received one dose of ezetimibe and GLE/PIB followed by only 7 d of treatment course. All recipients achieved SVR12 with genotypes 1-3.

Although, favorable short-term outcomes has been reported for HCV NAT positive and NAT negative donors, the long-term effects of the virus, the infected organs and drug interaction are not known. Hence, during consideration of accepting a HCV viremic donor, the risk of HCV complications including FCH and HCC, insurance policy and availability of pan-genotypic DAAs should be addressed in the informed consent.

FIBROSING CHOLESTATIC HEPATITIS (FCH)

Fibrosing Cholestatic Hepatitis (FCH), a dreaded complication of HCV recurrence, has been described in liver[184,185], renal[186] and heart[187] transplant recipients. It is seen in around 2%–15% of liver transplant recipients and causes significant morbidity and mortality[188,189]. This rapidly progressive disease is characterized by cholestatic jaundice with a high HCV viral load[190,191]. A low threshold of suspicion along with histopathological diagnosis is needed for its prompt management. In pre-DAAs era, despite contraindication in RTRs, KDIGO had recommended IFN in FCH considering the risk-to-benefit ratio. However, this treatment was associated with a low tolerance rate and a poor outcome [141]. The standard of care in transplant population is reduction or withholding immunosuppression followed by anti-viral therapy. Historically treatment with IFN was associated with lower success rate and higher side effects[191,192].

Xue *et al*[193] reported 80% SVR 12 in 10 transplant recipients treated with SOF/RBV combination with PegIFN. The SOLAR 1 and SOLAR 2 trials reported 100% SVR 12 in 6 and 5 transplant recipients, respectively with FCH treated with SOF-LDV and RBV combination[119,120]. Cypel *et al*[176] reported successful treatment of FCH with SOF-VEL-VOX and RBV combination in a lung transplant recipient. Leroy *et al*[194] documented 96% SVR12 in 23 Liver transplant recipients treated with either SOF/RBV or SOF/DAC combination. Moreover, 4 recipients in this study population had concomitant HIV infection. Shinzato *et al*[195] reported a case of post renal transplant FCH treated with GLE/PIB combination. The patient expired due to progressive hepatic failure despite decreased HCV viral load. Jandovitz *et al*[133] reported successful treatment with DAAs in 2 aviremic renal recipients transplanted with HCV-positive donors. Hence, it is proven that the use of DAAs can be beneficial in FCH in post-solid organ transplant recipients.

GLOBAL ERADICATION

From a low virological response to an almost curative treatment for all genotypes, therapy for HCV has evolved markedly in recent years. However, the greatest challenge is yet to be overcome, that is the availability of treatment for everyone. The WHO aims to eliminate viral hepatitis including HCV by 2030 since its poses a major public health threat. In order to implement this, various strategies have been devised to reduce the incidence of viral hepatitis by 90% and decrease liver-related mortality due to these viruses by 65%. To achieve this target, WHO has enlisted five core interventions that need to be focused by all countries globally. These interventions includes vaccination for HBV, prevention of HBV transmission from mother to child, use of screened blood products and safe use of injections, harm reduction in drug users, testing and treatment of HBV and HCV[196]. Despite WHO's support, only a few countries have been able to develop an effective hepatitis control program while even fewer are currently on track to achieve the elimination goal[197]. Egypt, with highest prevalence of chronic HCV infection in the world few years back, conducted a successful HCV screening program that covered more than 50 million people and treated more than 4 million. It is poised to be the first country in the world to eliminate HCV within its borders. The lessons learned from this experience can inform the elimination plans of other LMICs with high HCV burden[198].

Interestingly, DAAs with their high efficacy and short duration of therapy have provided hope on achieving this target but a higher cost of therapy, lack of insurance coverage and un-availability of therapy in many LMICs have become a major obstacle[199]. Some LMICs have heavily subsidized the DAAs for achieving the ambitious goals of HCV elimination. In addition to this, in several countries, effective diagnostic facilities are expensive. Other challenges include inadequate surveillance data, limited coverage of preventive programs and lack of focused leadership to combat HCV menace. However, the major obstacle seen globally is the lack of financial support in hepatitis programs[200,201].

Therefore, there is an urgent need to strengthen the healthcare system and develop a national plan against hepatitis in low-, middle- and even high-income countries. Moreover, support from civil societies, pharmaceutical and medical companies is also required to help the governments of various countries to combat this deadly disease.

CONCLUSION

Significant advances have been made in the fields of diagnostics and therapeutics for optimal management of chronic HCV infection. However, the disease still remains a formidable challenge for all stakeholders, particularly in developing countries. Many hurdles remain to be tackled before the disease is eliminated as envisaged by WHO's goal of eradication of hepatitis by 2030. Concerted and focused global efforts are needed to tackle and eliminate this silent killer effectively.

FOOTNOTES

Author contributions: Farina M Hanif, Zain Majid, Nasir Hassan Luck, Abbas Ali Tasneem, Syed Muddasir Laeeq, Muhammed Mubarak and Luck NL conceived the study; Tasneem AA and Laeeq SM designed the study; Hanif FM and Majid Z performed the research; all authors participated in primary and final drafting; all authors have read and approve the final manuscript; all authors significantly contributed to the study.

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EVIDENCE REVIEW

Evidence-based approach to management of hepatic encephalopathy in adults

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Abstract

Hepatic encephalopathy (HE) is a reversible syndrome of impaired brain function and represents one of the many complications of portal hypertension and decompensated liver disease. Although ammonia is clearly implicated in the pathogenesis of HE, the pathogenesis of HE is multifactorial with numerous mechanisms that results in functional impairment of neuronal cells. The initial management of HE focuses on supportive care and stabilization which includes providing appropriate nutritional support. Thereafter, focus should be on identifying and treating the precipitating factors. There are many therapeutic agents available for the management of HE, most of which are directed towards lowering the gut nitrogen load and thus the serum ammonia level. This review aims to provide an update on the conventional and emerging treatment options for HE.

Key Words: Hepatic encephalopathy; Lactulose; Rifaximin; Fecal microbiota transplant; Zinc; L-ornithine L-aspartate

Core Tip: Hepatic encephalopathy (HE) is a reversible syndrome of impaired brain function and represents one of the many complications of portal hypertension and decompensated liver disease. This review provides an update on the conventional and emerging treatment options for HE. The different conventional and emerging therapeutic options available to date are detailed in the manuscript. We have elaborated all the data available in the literature about the use of fecal microbiota transplant in the treatment of HE.

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INTRODUCTION

Hepatic encephalopathy (HE) is a reversible syndrome of impaired brain function and represents one of the many complications of portal hypertension and decompensated liver disease. It is estimated to be present in 50% to 70% of patients with liver cirrhosis[1]. It is a debilitating disease that affects the quality of life of both the patients and their caregivers and contributes to significant health care resource utilization making it an economic burden on health care facilities[2]. In 2009, 23000 patients were admitted for HE, with an average length of stay of 8.5 d exhausting $ 63108 per case[2]. The pathogenesis of HE is multifactorial with numerous mechanisms that results in functional impairment of neuronal cells, none of which are clearly understood[3].

Ammonia, which is a gut-derived nitrogenous toxin produced by the bacterial metabolism of urea from dietary proteins, has been considered the primary pathophysiologic mechanism of HE. It is normally metabolized by the liver and cleared mostly by the kidney and to a lesser extent in the muscle. In patients with cirrhosis and portal hypertension, the hepatic metabolism of ammonia is impaired and there is shunting of ammonia-rich portal blood to the systemic circulation without detoxification. In the brain, ammonia crosses the blood-brain barrier and is metabolized in the astrocytes by glutamine synthetase, which converts ammonia and glutamate to glutamine. Accumulation of glutamine in astrocytes creates an osmotic gradient, resulting in astrocyte swelling and generation of reactive oxygen species, thereby contributing to the cerebral dysfunction. Ammonia also binds to gamma-aminobutyric acid (GABA) receptors on astrocytes, leads to neurosteroids activation, which further contribute to the occurrence of HE. Moreover, the dysbiosis and increased gut permeability seen in cirrhotic patients causes an increase in the production of multiple inflammatory cytokines, which leads to increased blood-brain barrier permeability and cerebral edema[3-5].

Although ammonia is clearly implicated in the pathogenesis of HE, additional factors include inhibition of neurotransmission in the central nervous system *via* GABA receptors and alteration in other CNS neurotransmitters and circulating amino acids .The precipitating factors for HE include liver failure causing decreased metabolism of ammonia, hypoxia and increased ammonia load due to gastrointestinal bleeding, sepsis, alterations in gut flora, hepatocyte necrosis, neuroinflammation, and structural and functional changes in the brain due to other disease process, presence of spontaneous or iatrogenic portosystemic shunt, and other conditions such as hypokalemia, hyponatremia and use of sedatives.

HE has varying degrees of severity and is commonly divided according to the West Haven criteria into covert HE (CHE) and overt HE (OHE). CHE can be either minimal HE (MHE) or grade I HE, while OHE includes grade II-IV[3,6]. OHE is a spectrum of neuropsychological abnormalities that can usually be detected by bedside clinical tests in contrast to CHE, where specific psychometric tests are needed to discern them because of quasi-normal mental status of the patient at bedside. OHE is present in 30%-45% of patients, with a yearly cumulative risk of development in 20% of patients with cirrhosis. Around 60%-80% of patients diagnosed with liver cirrhosis have evidence of cognitive dysfunction or MHE[7].

The 2014 American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver clinical practice guidelines for HE management[8] recommend classifying HE based on 4 factors detailed in Figure 1: (1) Underlying disease (Type A: HE due to acute liver failure, type B: HE due to portosystemic shunts and type C: HE due to cirrhosis); (2) Severity; (3) Time course; and (4) Precipitating factors.

The initial management of HE focuses on supportive care and stabilization which includes providing appropriate nutritional support to maintain an energy intake of 35-40 kcal/kg/d, with a protein intake of 1.2-1.5 g/kg/d, correction of precipitating causes which include dehydration and electrolyte abnormalities such as hypokalemia and metabolic alkalosis[9,10]. Thereafter, focus should be on identifying and treating the precipitating factors underlined in Figure 1. There are many therapeutic agents available for the management of HE, most of them are directed towards lowering the gut nitrogen load and thus the serum ammonia levels. This review aims to provide an update on the

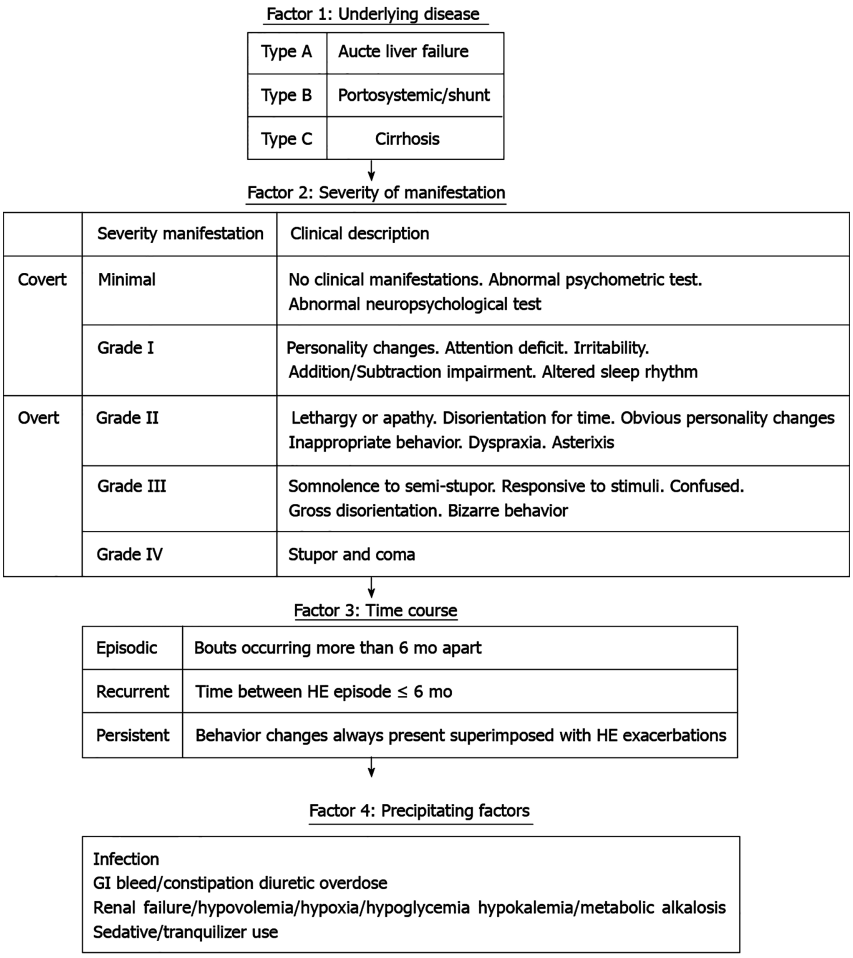

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Figure 1 Classification of hepatic encephalopathy based on 4 factors. Portions of this figure are adapted from the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver clinical practice guidelines for hepatic encephalopathy management.

conventional and emerging treatment options for HE.

GUT FLORA MODIFYING AGENTS

Nonabsorbable disaccharides

The nonabsorbable disaccharides lactulose and lactitol are considered the first-line therapeutic agents for treating HE. They are shown to significantly improve cognition and quality of life in patients with MHE, with lactitol having fewer side effects compared to lactulose[11,12].

It reduces intestinal ammonia production and absorption by four main mechanisms[13-15]: (1) The catabolism of lactulose by bacterial flora in the colon decreases the pH. The acidic pH leads to the formation of nonabsorbable NH^{4+} from NH3. The trapping of NH^{4+} (impermeable to membranes) in the colon reduces the plasma ammonia concentration; (2) It causes a laxative effect by increasing intraluminal osmolality as well as gas formation leading to a reduction in gastrointestinal transit time; thus, reducing the time for ammonia absorption and increasing the fecal nitrogen excretion; (3) It promotes an increase in ammonia uptake by colonic bacteria as a nitrogen source for protein synthesis and decrease in the formation of potentially toxic short-chain fatty acids; and (4) The acidic pH caused by lactulose modifies the colonic flora by displacing the urease- producing bacteria involved in ammonia synthesis with non-urease-producing Lactobacillus.

A systematic review published by the Cochrane collaboration in 2016 included 38 randomized controlled trials (RCTs) with a total of 1826 patients and compared lactulose *vs* placebo. Results were promising and showed a positive effect of non-absorbable disaccharides on mortality [relative risk (RR) = 0.59, 95% confidence interval (CI): 0.40-0.87] and HE (RR = 0.58, 95%CI: 0.50-0.69)[16].

Lactulose (or lactitol in some countries) is often used as the first-line treatment for OHE, at a dose of 30-45 mL (20-30 g) of lactulose syrup every 1-2 h until at least two soft bowel movements are produced. Subsequently, the dosing is of lactulose is titrated to two to four times a day to achieve and maintain

two to three bowel movements per day[8]. An approximately equivalent dose of lactitol is 67-100 g lactitol powder diluted in 100 mL of water. Lactulose and lactitol may be given as enemas, if patients are unable to take them orally, as 1-3 L of a 20 percent solution.

Antibiotics

Antibiotics with activity targeting urease producing gut bacteria have an ammonia lowering effect. These antibiotics include rifaximin, neomycin, vancomycin and metronidazole.

Rifaximin: Rifaximin has low systemic absorption, wide antimicrobial spectrum, and low occurrence of side effects[17]. The dose of rifaximin is 550 mg orally twice daily or 400 mg orally three times daily. It is typically used as a combination therapy with lactulose to treat acute encephalopathy and prevent recurrent HE when response to lactulose monotherapy is not inadequate. A randomized trial where 299 patients with cirrhosis and documented HE who were in remission at the start of the trial were administered 550 mg of rifaximin twice daily *vs* placebo for a total of 6 mo found that rifaximin was more effective than placebo in preventing recurrent episodes of HE[18]. Another randomized trial comparing the combination of rifaximin and lactulose with lactulose alone in 120 patients hospitalized with OHE showed that patients who received the combination therapy were more likely to have complete resolution of HE and lower mortality[19].

A recent systematic review of five randomized and five observational studies involving 2276 patients by Wang *et al*[20] comparing combination therapy (rifaximin + lactulose) *vs* lactulose alone showed that combination therapy significantly increased clinical efficacy compared with lactulose alone in HE patients [risk difference (RD) = 0.19, 95%CI: 0.09-0.29, P = 0.0002] with a number needed to treat (NNT) of 5 in primary analysis. Combination therapy also significantly reduced the mortality in HE patients compared with lactulose alone (RD = 0.11, 95%CI: -0.19 to -0.03, P = 0.009) with an NNT of 9 in primary analysis. Rifaximin has a place mostly in prevention of recurrence of HE when lactulose alone fails; however, recent studies showed that combination therapy with lactulose might be more beneficial[20].

Neomycin: Neomycin acts by inhibiting the activity of glutaminase, consequently decreasing ammonia production from glutamine in the intestinal mucosa. Although widely used for the treatment of HE in the past, neomycin has a significant side effect profile including ototoxicity, nephrotoxicity, and enterocolitis. The AASLD guidelines[8] recommend neomycin as an alternative for the treatment of OHE[21].

Vancomycin and metronidazole: Vancomycin and metronidazole have also been studied alone or in conjunction with lactulose. In one study[22] involving 12 patients with cirrhosis and encephalopathy who were given 2 g of vancomycin, all 12 patients showed a remarkable clinical improvement after treatment. Another study[23] showed that 19 patients with varying grades of encephalopathy that were treated with 1 wk of metronidazole had significant improvement in mental status scores and asterixis, similar to neomycin. Therefore, the authors concluded that metronidazole may be as effective as neomycin. The serious side effect of metronidazole (neurotoxicity) and vancomycin (bacterial resistance) has limited the use of these agents. Hence, long term treatment with these agents is not recommended.

Probiotics

Probiotics are formulations of microorganisms that modify gut flora to acid resistant, non-urease producing flora, resulting in diminished ammonia production and absorption. Prebiotics include compounds such as lactulose and fermentable fiber which promote the growth of beneficial gut flora. The most beneficial species of gut flora in the treatment of HE appears to be Lactobacilli and bifidobacterial[24]. Most commercial probiotic products are derived from food sources, especially cultured milk products. The Cochrane review of 21 trials with 1420 participants that compared probiotics in any dosage with placebo, or with any other treatment in people with HE, concluded that compared with placebo, probiotics probably improve recovery and may reduce recurrences of OHE, quality of life, and plasma ammonia concentrations. Probiotics, however, have no effect on mortality or significant clinical outcomes when probiotics were compared with lactulose[25]. Probiotic groups had reduced plasma ammonia concentrations compared with the placebo/no intervention groups, but not when compared with lactulose groups. Additional studies are needed before probiotics can routinely be recommended for the treatment or prevention of HE.

Fecal microbiota transplant

Cirrhosis is a leading cause of HE. Compared to healthy individual, the fecal microbiome of cirrhotic patients has prevalent pathogenic bacteria such as *Enterobacteriaceae* and *Streptococcaceae* and reduced beneficial bacteria such as *Lachnospiraceae* and *Ruminococcaceae*[26]. When the human gut microbiome was compared in patients before and after developing a HE episode, it was found that that there was a significant change in microbial abundance[27]. Subsequent studies conducted on this topic included investigating the fecal microbiome in this subgroup of patients and evaluating if fecal microbiota transplant (FMT) in patients with HE might treat or prevent further episodes of HE and improve cognitive outcomes.

A summary of the pertinent published studies and abstracts investigating the use of FMT in patients with HE till this date is shown in Tables 1 and 2. There was a total of 4 RCTs,1 case series and 1 case report included in this analysis.

In the phase I study conducted in 2017[28] and subsequently in 2019[29], the number of hospitalizations as well as HE episodes was significantly lower in the patients that underwent FMT. Another study[30] showed that 2/10 patients required hospitalization and 3/10 developed an encephalopathy episode after FMT at 20 wk. FMT also appeared to be safe and well tolerated[31].

Cognition was assessed using EncephalApp Stroop test in 4 studies[28,29,32]. Psychometric Hepatic Encephalopathy Score (PHES) was utilized in three studies[28,29]. Improvement in the time taken to complete the EncephalApp Stroop test and improvement in the PHES score was demonstrated in these studies. There was a remarkable improvement in PHES total score (P = 0.003) and EncephalApp Stroop (P = 0.01) in the FMT group compared to baseline[28], possibly indicating that FMT might also reverse cognitive impairment in patients with HE.

The PHES and EncephalApp-Stroop test are validated tests for HE-related cognitive function tests and improvement in these tests in patients who underwent FMT is promising. FMT has also been shown to influence lowering serum ammonia levels in three studies[30,32,33]. Although FMT looks promising, additional larger RCTs are needed to validate the results.

NUTRITION, DIETARY MODIFICATION AND SUPPLEMENTATION

Zinc

It has been demonstrated that zinc deficiency is common in patients with liver cirrhosis[34] and lower serum zinc level has also been a precipitating factor for HE[35]. Zinc deficiency results in decreased activity of muscle glutamine synthetase, an important enzyme in reducing serum ammonia levels making zinc an important factor in ammonia detoxification[36].

Chavez-Tapia *et al*[37] published a metanalysis that included four RCTs of 233 patients evaluating the effect of oral zinc supplementation *vs* placebo or standard therapy over HE. Three studies showed an improvement in performance on number connection test in the zinc group compared to placebo or standard therapy. This improvement suggests a beneficial effect of oral zinc in encephalopathic patients. However, there was no beneficial effect on HE recurrence. Shen *et al*[38] also published a metanalysis that included four RCTs of 247 patient's and concluded that a combination treatment of zinc and lactulose over 3-6 mo significantly improved performance in the number connection test compared to lactulose alone. The effect of short-term (10 d) oral zinc supplementation (zinc sulfate 600 mg/d) on HE, was assessed in a double-blind, crossover trial involving fifteen cirrhotic patients with stable, chronic HE. Serum zinc was significantly raised after oral zinc administration and reached the levels observed in cirrhotics without HE. Despite this, the study failed to confirm that short-term oral zinc supplementation improves chronic HE[39]. Zinc supplementation cannot be recommended for treatment of HE in the absence of larger sample size study.

Branched-chain amino acids

In cirrhotic patients, it has been clarified that there is an imbalance between aromatic amino acids (AAA) and branch-chain amino acids (BCAA) where serum concentrations of AAA are increased and BCAA are decreased. These alterations are thought to increase brain levels of aromatic amino acid precursors for monoamine neurotransmitters which contribute to altered neuronal excitability and development of HE[40].

In 2017, the Cochrane collaboration published a systemic review of 827 patients in 16 RCTs in which use of oral (eight trials) or intravenous BCAAs (seven trials) was compared with placebo, diet, lactulose, or neomycin. BCAAs were found to have a beneficial effect on manifestations of HE. More specifically, when excluding trials on lactulose or neomycin, BCAA had a beneficial effect on HE. However, when analyzing trials with a lactulose or neomycin control, it was found that there was no statistically significant benefit of BCAA over lactulose or neomycin. Gastrointestinal discomfort was the main adverse reaction observed while using BCAA with no serious adverse events reported and this intervention did not seem to influence mortality and quality of life[41]. In summary, it was found that oral, but not intravenous, BCAAs may have beneficial effects. BCAA supplementation may be considered in severely protein-intolerant patients as no benefit was observed in protein-tolerant patients.

Acetyl-L-carnitine

Carnitine is a metabolite in the degradation pathway of the essential amino acid lysine and is a substance natural to the body. Acetyl-L-carnitine (ALC) is readily formed in cells by the enzymatic addition of an acetyl group to carnitine. The major difference between ALC and carnitine is that ALC is more easily absorbed from the gut, and more readily crosses the blood-brain barrier. Carnitine is a carrier for short chain fatty acids across the mitochondrial membrane and is thought to have neuroprotective properties. A systematic review[42] concluded that ALC was effective in improving serum

Table 1 Table summarizing characteristics of all studies involving fecal microbiota transplant in cirrhotic patients

Ref.	Study		Intervention	Route of FMT administration	*n*	Mean MELD score	Follow-up
Bajaj *et al*[29], 2019	Phase I	Treatment arm	FMT + SOC	Enema	10	12.5	5 mo
		Control arm	SOC		10	12.5	5 mo
Bajaj *et al*[28], 2017	Phase I	Treatment arm	FMT	Enema	10	13.2	5 mo
		Control arm	SOC		10	12	5 mo
Woodhouse *et al*[33], 2019	Phase III	Treatment arm	FMT + Lactulose	NJ tube	13	12.88	3 mo
		Control arm	SOC		5	12.88	3 mo
Bloom *et al*[81], 2019	Phase II	Treatment arm	FMT	Oral	7	13.9	1 mo
		Control arm	SOC		3	NA	1 mo
Mehta *et al*[30], 2018	Case series		FMT	Colonoscopy	10	18	20 d
Kao *et al*[32], 2016	Case report		FMT	Colonoscopy	1	10	7 d

FMT: Fecal microbiota transplant; SOC: Standard of care; MELD: Model for end-stage liver disease.

Table 2 Table summarizing the findings of all studies involving fecal microbiota transplant in cirrhotic patients

Ref.	MELD or MELD delta (last visit-baseline)	SAE	No. of hospitalized patients	Ammonia level (mean μ/dL)	HE episodes	Cognitive assessment (s): Encephaloapp stroop test (low is good) (t = time, Δ t: Change in time)	Cognitive assessment (points): PHES score (high is good) (p = points, Δ p: Change in points)
Bajaj *et al*[29], 2019	2.8 + (-4.5)	1	0 at median 1 yr	NA	0 at median 1 yr	Day 0: 245; day 20: 200; t > 1 yr: 225	Day 0: -7; day 20: -4; *P* > 1 yr: -6
	2.78 + (-4.7)	3	10 at median 1 yr	NA	1.5 at median 1 yr	Day 0: 260; day 20: 250; t > 1 yr: 250	Day 0: -8; day 20: -8; *P* > 1 yr: -11
Bajaj *et al*[28], 2017	0.1 + (-2)	2	1	NA	0 at 0.4 yr	Day 0: 247; day 0-20: 29.1 ± 27.9	Day 0: -7.4; day 0-20: (-3.1) ± (-2.1)
	-0.2 + (-2.7)	8	9	NA	6 at 0.4 yr	Day 0: 282; day 0-20: (-43.5) ± (-95.7)	Day 0: -8.6; day 0-20: 0.0 ± 3.1
Woodhouse *et al*[33], 2019	No significant change	4	4 at day 30	Day 0: 71; day 30: 51	NA	NA	NA
	No significant change	1	1 at day 30	Day 0: 54; day 30: 73	NA	NA	NA
Bloom *et al* [81], 2019	14.3 ± 3.3	1	NA	No significant change	NA	Day 0: 56; day 30: 22	Day 7: 2.6; day 30: 3.9
	NA	NA	NA	No significant change	NA	NA	NA
Mehta *et al* [30], 2018	15	4	2 by 20 wk	Day 0: 96; day 20: 74	3 by 20 wk	NA	NA
Kao *et al*[32], 2016	NA	0	1	Day 0: 75; day 20: 45	0	Day 0: 250.9; day 7: 203.4	NA

MELD: Model for end-stage liver disease; HE: Hepatic encephalopathy; NA: Not available.

ammonia level (weighted mean difference 25.90, 95%CI: 20.89-30.91, $P < 0.05$) and number connection test completion time (weighted mean difference: 16.62, 95%CI: 9.88-23.36, $P < 0.05$), and thus a promising treatment for HE. However, in 2019, the Cochrane collaboration published a systemic review of 398 patients in 5 clinical trials that compared ALC plus standard care (*e.g.*, antibiotics, lactulose) *vs* placebo or standard care in participants with cirrhosis with covert or OHE. The review showed that ALC reduces serum ammonium levels compared with placebo however no information was found about all-cause mortality, serious adverse events, or days of hospitalization. No clear differences were found between ALC and placebo regarding quality of life, fatigue, and non-serious adverse event[43]. In

summary, further RCTs are needed to assess ALC *vs* placebo.

CENTRAL NERVOUS SYSTEM ACTING AGENTS

Flumazenil

Flumazenil is a short acting benzodiazepine receptor antagonist that was described in multiple trials to benefit patients with HE by antagonizing and eliciting a negative allosteric modulatory effect on the central benzodiazepine receptors[44-46]. The Cochrane collaboration published in 2017 a systematic review[47] of 14 RCT that included 867 patients comparing flumazenil *vs* placebo. The duration of follow-up was less than 1 d in the majority of the RCT and it was shown that flumazenil was associated with a beneficial effect on HE but with no beneficial effect on mortality, serious adverse events, or health-related quality of life. Although flumazenil yielded short term improvement, all except one of the RCT were described as having high-risk bias. Flumazenil is not recommended for routine clinical use, though it may be considered for select patients who have received benzodiazepine therapy.

Dopamine agonists

Bromocriptine, a dopamine receptor agonist, has been studied as a potential treatment for HE. In one study, 6 patients with cirrhosis and severe HE unresponsive to standard therapy were given oral bromocriptine up to 15 mg daily. All patients showed significant improvement clinically as well as improvement in cerebral blood flow and cerebral glucose consumption which led the authors to conclude that bromocriptine is a useful treatment for chronic HE when conventional therapy fails[48]. A recent metanalysis of 5 trials including levodopa and bromocriptine reported no beneficial effects on HE and mortality[49]. The available clinical data are insufficient to assess the benefit of dopamine agonists; however, they might be useful in patients not responding to first line therapies[48,50].

AMMONIA SCAVENGING AGENTS

L-ornithine L-aspartate

L-ornithine L-aspartate (LOLA) is a combination of two endogenous amino acids. In patients with cirrhosis, the activities of carbamoyl phosphate synthetase and glutamine synthetase are impaired. Ornithine activates both the enzymes, ornithine and aspartate increase ammonia removal *via* stimulation of glutamine synthesis. LOLA has thus been shown to have ammonia lowering actions *via* stimulation of urea synthesis by residual periportal hepatocytes and ammonia removal by glutamine synthesis in skeletal muscle[51].

The first published analysis considered[52] five double-blind, placebo-controlled RCTs in 246 patients with cirrhosis (Child-Pugh status A or B) and compared intravenous infusions of 20-40 mg of LOLA over 4-8 h for a 7-d period to placebo. LOLA treatment showed a 3.22-fold greater chance of resolution of OHE and significant reduction of post-prandial serum ammonia after 7 d of therapy compared to placebo. Subsequently, a high quality meta-analysis of three randomized trials that included 212 patients with cirrhosis[53], a meta-analysis of 8 RCTs with 646 patients with cirrhosis[54], and a meta-analysis of 15 RCTs with 1023 patients[55] showed that LOLA was significantly more effective than placebo in patient with OHE. In another met analysis[56], LOLA was shown to significantly reduce serum ammonia level (MD = 17.50, 95%CI: -27.73 to -7.26), regardless of its formulation, compared to placebo. When compared to other ammonia lower agents, LOLA was noted to cause decreases in serum ammonia levels compared to lactulose[57], and improvement of psychometric test scores compared to rifaximin and probiotics[58,59].

A very recent metanalysis published by the Cochrane collaboration[55] in 2018 of 36 RCTs encompassing 2377 patients showed that LOLA had beneficial effect on HE compared with placebo. In patients with MHE, LOLA was found to be comparable to lactulose and rifaximin for both reversal of deficits in psychometric test scores and for slowing of progression from MHE to OHE[51]. Treatment with LOLA is beneficial compared with placebo, but trials comparing LOLA with lactulose are needed. Overall, LOLA has been evaluated to be a safe, effective, and well-tolerated. It is routinely given to patients with HE outside of the United States.

Ornithine phenylacetate, phenylbutyrate and sodium benzoate

L-ornithine combined with phenylacetate as L-ornithine phenylacetate (OPA) lowers ammonia level by L ornithine stimulating synthesis of glutamine from ammonia in skeletal muscle and phenylacetate binding to glutamine and excreting the compound as phenylacetylglutamine through the kidneys in the urine[60]. A study performed by Stravitz *et al*[61] included 47 patients with acute liver injury/acute liver failure and ammonia level above 60 μM showed that this therapy is safe and well-tolerated in patients with acute liver failure. A metanalysis published by the Cochrane database in 2019[62] failed to show beneficial or harmful effects of OPA *vs* placebo. Up to this date, there is no clear clinical evidence linking

OPA to HE.

Phenylbutyrate (PB), a prodrug of phenylacetate is rapidly oxidized to phenylacetate and acts in the same way[63]. A phase II trial of 178 patients[64] compared glycerol PB (GPB) to placebo and concluded that GPB decreased the frequency of HE events as well as the ammonia levels in patients with cirrhosis and HE and had a comparable safety profile to placebo. However, larger RCTs are needed to confirm these results.

Sodium benzoate is generally used in food and beverage preservative. It conjugates with glycine in the liver and the kidney to form hippuric acid which carries waste nitrogen and is then excreted by the kidneys[13,65]. This low-priced adjunctive agent has shown promising results but cannot be used as first-line therapy until additional randomized trials are conducted. It can be considered as an alternative in patients with good creatinine clearance who are unable to tolerate standard of care regimen or have failed to improve despite the standard regimen[65,66]. A small RCT performed in 1992 by Sushma *et al* [67], however, compared sodium benzoate to lactulose and concluded that that sodium benzoate is as safe and effective as lactulose.

Spherical carbon microsphere adsorbent (AST-120)

This is an orally administered, engineered carbon microsphere. Compared to activated charcoal, it possesses a better adsorptive capacity for certain organic compounds. It binds to ammonia in the gastrointestinal lumen and facilitate its excretion[62,68]. In rats, it has shown to lower serum ammonia levels and normalize brain water content[68].

Polyethylene glycol 3350-electrolyte solution

Prior to the introduction of lactulose as a therapeutic option for HE, laxative agents were used, suggesting that catharsis might be an effective treatment of HE. However, since the adoption of lactulose for the treatment of HE, studies of cathartic methods have largely been abandoned until recently when a growing interest has developed over a safe, commonly used, and highly effective laxative: Polyethylene glycol 3350-electrolyte solution (PEG)[69]. Multiple RCTs[69-71] studied the effect of PEG *vs* lactulose for the treatment of HE. These studies showed that PEG led to a more rapid improvement of the HE scoring algorithm score in 24 h and shortened the hospital say. Larger trials are needed to confirm these results before recommending PEG as a routine treatment for patients with cirrhosis and encephalopathy.

Molecular absorbent recirculating system

Molecular absorbent recirculating system (MARS) is an extracorporeal hepatic support system that integrates the mechanisms of dialysis, ultrafiltration, and adsorption. It utilizes an albumin dialysate across a semi-permeable high-fluid membrane to remove protein-bound and water-soluble toxins[72]. MARS was first approved by the United States Food and Drug Administration in 2005 for use in drug overdoses and an additional approval was granted in 2012 for use in HE due to decompensated chronic liver disease[73].

The RELIEF trial that compared standard therapy *vs* MARS in 189 patients with acute on chronic liver failure showed that the patients treated with MARS had a significant improvement in symptoms of HE (38.2% *vs* 62.5%, respectively). Specifically, patients with HE treated with MARS improved from grade III-IV to grade 0-I. The study was statistically significant (P = 0.07)[74].

MARS is an expensive therapy necessitating specific skill set and expertise to operate. It is offered only in a few institutions in the United States and Europe. Survival benefit has not been demonstrated. Larger RCTs are essential to rationalize its usage at a greater scale.

VASCULAR/INTERVENTIONAL MANAGEMENT

Embolization of portosystemic shunts

Patients who fail medical management are referred to as having refractory HE. These patients may harbor large spontaneous portosystemic shunts (SPSS), mainly splenorenal shunt, leading to sustained HE episodes. A few studies have investigated embolization of these shunts in selected patients and have noticed beneficial results in the treatment of HE episodes. In a multicenter study of 37 patients with diagnosed refractory HE and SPSS, 18 patients managed to remain free of HE for about 2 years, there was also an overall improvement in autonomy and a decrease in the number of hospitalizations[75]. In another retrospective study[76] involving 20 patients who were eligible for embolization of SPSS, all the patients had immediate improvement by day 7, and 67% of the patients were free from HE related hospitalization over 1 year. Therefore, SPSS embolization may be a treatment option in a select group of patients with refractory HE.

Although HE is not an indication for liver transplant, liver transplantation remains the definitive treatment for reversal of the complications related to cirrhosis. Studies have shown that patients become free of HE following transplantation; follow up studies have also shown that HE may become

irreversible despite liver transplant[77-80].

CONCLUSION

Management of HE, since its initial description, has seen great advancement. However, there still exists a wide discrepancy in delivery of care and patient outcomes. Our understanding of the underlying pathophysiologic mechanisms is still limited. Further research into the pathogenesis of the disease may lead to development of more definitive as well as targeted treatment options.

FOOTNOTES

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MINIREVIEWS

Direct oral anticoagulant administration in cirrhotic patients with portal vein thrombosis: What is the evidence?

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Abstract

In recent years, the traditional concept that cirrhosis-related coagulopathy is an acquired bleeding disorder has evolved. Currently, it is known that in cirrhotic patients, the hemostatic system is rebalanced, which involves coagulation factors, fibrinolysis and platelets. These alterations disrupt homeostasis, skewing it toward a procoagulant state, which can lead to thromboembolic manifestations, especially when hemodynamic and endothelial factors co-occur, such as in the portal vein system in cirrhosis. Portal vein thrombosis is a common complication of advanced liver cirrhosis that negatively affects the course of liver disease, prognosis of cirrhotic patients and success of liver transplantation. It is still debated whether portal vein thrombosis is the cause or the consequence of worsening liver function. Anticoagulant therapy is the mainstay treatment for acute symptomatic portal vein thrombosis. In chronic portal vein thrombosis, the role of anticoagulant therapy is still unclear. Traditional anticoagulants, vitamin K antagonists and low-molecular-weight heparin are standard-of-care treatments for portal vein thrombosis. In the last ten years, direct oral anticoagulants have been approved for the prophylaxis and treatment of many thromboembolic-related diseases, but evidence on their use in cirrhotic patients is very limited. The aim of this review was to summarize the evidence about the safety and effectiveness of direct oral anticoagulants for treating portal vein thrombosis in cirrhotic patients.

Key Words: Dabigatran; Rivaroxaban; Apixaban; Edoxaban; Bleeding

Core Tip: The role of anticoagulant therapy in portal vein thrombosis is still unclear, especially in partial, chronic and asymptomatic thrombosis. Vitamin K antagonists and low-molecular-weight heparin were demonstrated to be safe and effective, with a positive influence on liver function, portal hypertension and mortality. Direct oral anticoagulants are a new approach to treat portal vein thrombosis in patients with cirrhosis and have many advantages compared to classic anticoagulants, although evidence is still limited. In patients awaiting liver transplantation, dabigatran may be promising for preventing thrombosis progression because of the low rate of hepatotoxicity, predominant renal metabolism and reversibility in perioperative management.

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INTRODUCTION

Portal vein thrombosis

Portal vein thrombosis (PVT) is defined as the presence of a thrombus within the portal vein, either in the main trunk or intrahepatic branches, which can extend to the splenic or superior mesenteric vein (SMV). Based on the degree of obstruction, PVT can be characterized as occlusive or nonocclusive. Based on onset, PVT can be classified as acute or chronic. Acute PVT includes a symptomatic onset and the exclusion of portoportal collaterals with imaging, while chronic PVT is defined as previously diagnosed PVT or as PVT associated with long-lasting signs of thrombosis such as cavernoma. A temporal cutoff dividing acute and chronic DVT has not been defined[1,2].

Prevalence and incidence

The heterogeneity of PVT incidence and prevalence is related to multiple factors, among the most important of which are cirrhosis severity, the clinical presentation of PVT and diagnostic techniques used to identify PVT. The analysis of a large multicenter study, which included 1243 cirrhotic patients with Child-Pugh A (863) or B (380), showed that the cumulative PVT incidence was 4.6%, 8.2% and 10.7% at the 1-, 3- and 5-year follow-up, respectively[3]. However, in advanced cirrhosis, the annual incidence was higher and ranged from 10% to 15%[1].

In the "Portal vein thrombosis Relevance On Liver cirrhosis: Italian Venous thrombotic Events Registry" (PRO-LIVER) prospective multicenter study, the PVT prevalence in 753 cirrhotic patients who underwent Doppler ultrasound was 17%, and Child-Pugh B or C, hepatocellular carcinoma (HCC), previous PVT and gastrointestinal bleeding were independently associated with PVT, suggesting that there was a correlation between the progression of disease and PVT[4]. Interestingly, in this study, 45 patients who developed PVT received anticoagulant therapy. According to Zhang *et al*[5], the prevalence was higher in advanced cirrhosis with acute decompensation than in compensated cirrhosis (9.36% *vs* 5.24%). Acute PVT seems to be more common than chronic PVT in cirrhosis[6]. This is likely related to the clinical presentation of acute PVT *vs* chronic PVT.

In the setting of liver transplantation, the prevalence of PVT ranges from 2% to 26%[7]. Francoz *et al*[8] described a prevalence of 8.4% at the time of listing for transplantation and an annual incidence of 3.2% in patients without PVT at the time of listing.

In another retrospective study on approximately 400 Liver transplant candidates, the prevalence of PVT was 10.3%, of which 25% had PVT at the time of listing for transplantation, 17.5% developed PVT while waiting for transplantation and 57.5% were diagnosed with PVT during surgery[9].

Pathophysiology and risk factors for PVT

In noncirrhotic patients, PVT is uncommon and can occur more frequently in association with inherited or acquired thrombophilia. Major risk factors for PVT in noncirrhotic patients are myeloproliferative disorders, prothrombin gene G20210A mutation and antiphospholipid syndrome[10].

In cirrhotic patients, multiple systemic and local factors contribute to an increased risk of PVT. Recent evidence changed the traditional understanding that cirrhotic patients acquired bleeding disorders due to reduced levels of procoagulant factors. In chronic liver disease, the fragile rebalance of the hemostatic system involves coagulation factors, platelets and fibrinolysis. Regarding the coagulation system, a parallel modification of both prohemostatic and antihemostatic factors takes place. Antithrombin and protein C reductions[11,12] and factor VIII increases were shown[12], and low fibrinogen levels and low factor II, V, VII, IX, X and XI levels were demonstrated[12]. Concerning platelets, thrombocytopenia due to sequestration, a shortened half-life and reduced production[13] may shift the balance toward

bleeding. Instead, high levels of von Willebrand factor (vWF) and reductions in its cleavage factor, ADAMTS 13[14], promote thrombosis. Finally, the fibrinolytic system is rebalanced, with some alterations, such as low plasmin inhibitor levels promoting fibrinolysis, and other alterations, such as low plasminogen contrast fibrinolysis[14]. In liver cirrhosis, fibrinogen production is relatively unchanged, but functional fibrinogen levels are reduced. This functional defect is called acquired dysfibrinogenemia and is caused by the inadequate removal of excess sialic acid residues from fibrinogen, resulting in fibrin polymerization impairment[15,16].

How is procoagulant imbalance in this setting possible? In 2011, Tripodi *et al*[17] demonstrated that protein C reduction (caused by reduced liver synthetic activity) and factor VIII increases (caused by vWF increases), which binds and protects factor VIII and reduces low-density lipoprotein-related protein and triggers resistance to thrombomodulin activity, which is one of the most important anticoagulant factors. Thus, it is not surprising that a decrease in protein C (PC) causes an increase in factor VIII (FVIII) levels, and the FVIII/PC ratio predicts unfavorable outcomes in cirrhotic patients[18]. However, recent developments in this field suggest that in reality, coagulopathy in cirrhotic patients is much more complicated than previously thought (as described by the classic view), and classic tests used to determine this state are inaccurate. Therefore, new tools to detect cirrhosis-related coagulopathy, which consider antithrombin, protein C and FVIII, are needed. One of the most promising tests is the thrombin generation assay[19].

Hemodynamic factors play an important role in PVT development. A decrease in portal vein blood flow velocity of less than 15 cm/second is closely related to PVT development in liver cirrhosis[20-22]. Considering this, all conditions that reduce the velocity of portal flow can promote PVT development, such as nonselective beta blockers (NSBBs) or the presence of portosystemic shunts. NSBBs reduce the portal pressure gradient by decreasing cardiac output and inducing unopposed alpha-1 adrenergic-mediated splanchnic vasoconstriction, and they are widely used for the primary and secondary prophylaxis of variceal bleeding[23,24]. A recent meta-analysis showed that NSBBs significantly increased PVT risk in cirrhosis. In this study, the authors suggest ultrasound follow-up to estimate portal vein blood flow in patients treated with long-term NSBBs[25]. Portosystemic shunts open when portal pressure increases to deviate the portal flow to the inferior vena cava through various collateral circles. The convergence of portal blood flow into these vessels, called the "steal effect", slows the portal flow velocity and is associated with a major risk of PVT, as found by Maruyama *et al*[26].

Inherited thrombophilic disorders, such as prothrombin gene G20210A polymorphisms[27], deficiencies of antithrombin, protein C and protein S, factor V Leiden[28], or lupus anticoagulant[29], increase PVT risk in patients with cirrhosis[30], but the low prevalence of these conditions does not justify screening to search for these alterations[31,32]. Other risk factors were associated with PVT in cirrhosis. Some evidence has demonstrated that the presence of endothelial damage predisposes patients to thrombosis[33]. This damage could be related to higher intestinal permeability and higher gut-derived bacterial lipopolysaccharide (LPS)[34], which also stimulate endothelial cells to produce and release factor VIII[35].

Intrabdominal surgery, especially splenectomy, significantly affects the development of PVT[5]. The etiology of liver disease may be associated with major PVT risk, such as nonalcoholic fatty liver disease (NAFLD)[21] or with a lower risk of PVT in cases of alcoholic cirrhosis, which might be correlated with the effect of alcohol on coagulant function and vitamin status[36].

Sarin *et al*[37] proposed a model to assess the pretest probability of PVT. It is based on major criteria, such as Child-Pugh B or C, PVT history and presence of prothrombotic risk mutations, and minor criteria, such as new onset or worsening of portal hypertension, reduction in portal flow velocity < 15 cm/second, evidence of portosystemic shunt, active HCC, history of VTE, recent abdominal intervention, and acute abdominal clinical manifestations. The presence of 2 major, 1 major and 2 minor, or the presence of 4 minor criteria, suggests a high risk for PVT development[37]. This score could help clinicians understand which patient could benefit from anticoagulant prophylaxis, but prospective trials are needed to establish the score's predictive role.

Clinical manifestations

The clinical presentation of PVT depends mainly on two factors: the extent of thrombotic occlusion, partial or complete, and the time of thrombus formation, acute or chronic.

Acute PVT typically presents with gastrointestinal symptoms (due to splanchnic congestion), such as abdominal pain, nausea and vomiting, up to severe gastrointestinal complications, such as bleeding, sepsis and lactic acidosis[38]. Splenomegaly is frequent, ascites is rare[39]. The symptoms can be more severe and prognosis unfavorable in cases of complete mesenteric thrombosis[6].

Chronic PVT is often asymptomatic and is usually accidentally discovered during radiological examinations performed for other reasons[40,41]. The clinical presentation of chronic PVT is related to manifestations of portal hypertension, such as ascites, hepatic encephalopathy, gastroesophageal variceal bleeding[6] and hypersplenism with pancytopenia[39]. In addition, neovessel formation and cavernomatosis can alter the anatomy of biliary ducts. The effects of these alterations can manifest with portal cholangiopathy, characterized by pruritus, obstructive jaundice and cholangitis, or "pseudocholangiocarcinoma", a tangle of neovessels mimicking cholangiocarcinoma cancer[39].

Diagnosis and staging of PVT

Doppler ultrasound is the most common diagnostic technique for PVT, with high sensitivity and specificity[40]. Generally, diagnosis with ultrasound occurs during screening for HCC in asymptomatic patients but should be performed in patients with suggestive symptoms[42] or in patients with deteriorating hepatic decompensation[1]. Normal PV flow excludes PVT, while positive results need further evaluations with second-level imaging techniques, such as CT or MRI, to confirm the presence of acute or chronic PVT[22], to exclude the presence of a neoplastic thrombus and to examine thrombus extension. Sherman *et al*[43] proposed a scoring system called A-VENA, which considers venous expansion, thrombus enhancement, neovascularity, tumors adjacent to the thrombus and alpha-fetoprotein levels to distinguish a tumor thrombus from a nonneoplastic thrombus in HCC patients being evaluated for liver transplantation.

A recent review and meta-analysis investigated the diagnostic value of contrast-enhanced ultrasound (CEUS) to differentiate PVT from neoplastic invasion in HCC. It was demonstrated that CEUS has excellent accuracy and could be considered a valid alternative to second-level imaging techniques[44]. In some cases, it is necessary to perform a histological exam of the thrombus to distinguish a nontumor thrombus from HCC vascular invasion. In this cases, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) represents a feasible and safe tool for selected patients as an alternative to classic transabdominal ultrasound-guided fine-needle aspiration[45].

Despite the use of multiple imaging techniques, PVT diagnosis can occur during surgery for liver transplantation. In the retrospective study conducted by Bert *et al*[9], incidental PVT diagnoses during surgery occurred in more than half of the PVT cases in the entire cohort.

The staging of PVT extension is very important to select treatments and to predict the potential response to treatment. However, a comprehensive classification of PVT does not exist. In the setting of liver transplantation, Yerdel's classification[46] divided PVT into four categories based on the degree of main portal vein obstruction and proximal and distal SMV extension. Each stage correlates with a different portal reconstruction approach, and for stages 2-4, with a lower graft survival. In 2016, Sarin *et al*[37] proposed a new anatomico-functional classification of PVT in cirrhosis, which considers the site and extension of the thrombus, obstruction degree, duration and presentation, and functional relevance of the thrombosis; the aim of this classification is to allow for standardization in future research in this field.

Natural history and prognosis

The evolution of untreated PVT is still unclear. Three possible scenarios exist: spontaneous resolution, stabilization, or progression of the thrombus. Data regarding the occurrence of these possibilities are highly variable[22]. Spontaneous resolution or stabilization of the thrombus is the most frequent evolution of PVT and occurs in 45% to 70% of cases[41]. Currently, data on the predictive factors for PVT progression are still lacking. Evidence suggests that the degree of occlusion and extension of the PVT do not correlate with the evolution of thrombosis[47].

Regarding prognosis, PVT seems to be related to a worse prognosis and to negatively influence the decompensation of cirrhosis and long-term survival[48]. Amitrano *et al*[27] showed that PVT is associated with increased overall mortality risk in cirrhosis. The same result was described in more recent studies, which reported that PVT is associated not only with an increased mortality risk[5,49] but also with a major incidence of ascites[49] and major variceal bleeding risks[5]. It is still unclear whether PVT is the cause or the consequence of liver deterioration, and the data are controversial because PVT is clearly associated with more severe portal hypertension and advanced cirrhosis[3].

Conversely, in compensated cirrhosis, the development of PVT is independent of liver disease progression and is not related to decompensation or lower OLT-free survival[50]. These findings might be explained by the fact that the population considered in these studies included a majority of patients with Child-Pugh A, who have fewer risk factors for PVT and a reduced mortality rate than patients with advanced cirrhosis.

Regarding patients who are candidates for orthotopic liver transplantation (OLT), PVT can be detected at the time of listing for liver transplantation or can be diagnosed while patients are on the waiting list. The presence of PVT at the time of listing is associated with worse posttransplant survival [51] and with graft failure after OLT[52].

Although PVT is not a major contraindication for liver transplantation, the presence of a thrombus can reduce surgical feasibility, which is associated with a poor prognosis when nonphysiological reconstruction is performed[53,54]. When end-to-end anastomosis is performed, the survival rate at 1 and 5 years is similar between patients with or without PVT[55]. Conversely, the risk of portal vein rethrombosis, gastrointestinal bleeding and small bowel obstruction is higher when nonphysiological anastomosis is performed[56].

PVT is also associated with a prolonged duration of transplantation surgery (especially when incidentally discovered at the time of surgery), prolonged hospitalization after surgery, and lower 1-year survival, which is independent of the time of detection[9]. The negative impact of PVT on post-OLT survival was documented by a recent meta-analysis, which reported significantly higher 30-day and 1-year mortality in patients with pre-OLT complete PVT than in those with partial PVT or without PVT

[57].

The presence of PVT before transplantation is a risk factor for PVT recurrence after liver transplantation[7]. The onset of PVT after liver transplantation is associated with reduced graft and patient survival[58].

ANTICOAGULANT THERAPY IN PVT WITH CIRRHOSIS

Anticoagulant agents are the mainstay of therapy in many cases of thromboembolism, such as for the treatment of lower limb venous thrombosis, pulmonary embolism, or stroke prevention in atrial fibrillation.

The role of anticoagulants in cirrhotic patients with portal vein thrombosis is still unclear, especially in chronic asymptomatic PVT and in nonliver transplant candidates. Current guidelines do not propose definitive evidence-based treatment strategies for cirrhotic patients affected by portal vein thrombosis. The American Association for the Study of Liver Diseases (AASLD) suggests that the indication for treatment, anticoagulant type and duration of therapy should be considered on a case-by-case basis[59]. The European Association for the Study of the Liver (EASL) recommends starting anticoagulation with low-molecular-weight heparin (LMWH) in the absence of major contraindications for anticoagulant therapy, switching to vitamin K antagonist (VKA) treatment for at least 6 mo and ensuring that there is prior adequate prophylaxis for gastrointestinal bleeding. No indications were provided for the use of direct oral anticoagulants in this setting[2,59].

The classic anticoagulants commonly used in PVT in cirrhotic patients are LMWH and VKAs, which each has advantages and limitations. LMWH does not require monitoring and has an effect for a limited time. However, subcutaneous injection may reduce compliance, and low antithrombin III levels in cirrhotic patients may compromise the LMWH mechanism of action.

VKAs are usually used for long-term anticoagulation. Their advantages are oral administration and reversibility with vitamin K supplementation. Conversely, VKAs require INR monitoring (which is altered in patients with cirrhosis and probably does not reflect the real hemostatic status) and induce a decrease in anticoagulant proteins C and S, which are already reduced in cirrhotic patients. Fondaparinux, an indirect factor X-activated inhibitor, seems to be effective and safe in advanced cirrhosis, but very little evidence is available[60].

Efficacy and safety of classic anticoagulants in PVT with cirrhosis

A body of evidence suggests that anticoagulant treatment of PVT in cirrhosis is effective and safe. In a recent meta-analysis that included 1696 cirrhotic patients with PVT, anticoagulation therapy was significantly associated with portal vein recanalization, a decrease in PVT progression, and an improvement in survival, especially when treatment was started early[61]. According to these results, other recent systematic reviews and meta-analyses reported a pooled response rate to anticoagulation therapy that was considerably higher than that of the control group (66.7% *vs* 26%)[62].

Recanalization of the portal vein in patients treated with anticoagulants is associated with decreased portal hypertension and related complications, with higher OLT-free survival[63].

Instead, the discontinuation of therapy in patients with previous PVT, which is itself considered a risk factor for recurrence[47], is associated with a high PVT recurrence risk[64] (rethrombosis rate of 46.7%) after stopping anticoagulation[61]. Therefore, the duration of anticoagulation after portal vein recanalization is controversial.

Regarding safety, anticoagulant therapy in cirrhotic PVT is not associated with a significant increase in bleeding risk compared with that in untreated cirrhotic patients[62-64].

Regarding the incidence of bleeding, Mohan *et al*[62] reported a pooled rate of bleeding that was similar in patients treated with anticoagulant and the corresponding controls (7.8% *vs* 15.4%). Upper gastrointestinal bleeding in patients with cirrhosis on anticoagulation has the same severity and mortality as in patients with cirrhosis without anticoagulation treatment[65]. In support of these findings, Wang *et al*[61] demonstrated that anticoagulation did not influence overall bleeding and is, therefore, not a predictive factor for bleeding events.

In cirrhotic patients who are candidates for liver transplantation and are affected by PVT, the goal of anticoagulant therapy is to prevent PVT progression and to promote portal and superior mesenteric vein recanalization, allowing end-to-end anastomosis, which is associated with better outcomes. Available guidelines support the use of anticoagulant treatment in cirrhotic patients with PVT who are candidates for transplantation[2,59]; a recent study demonstrated a trend toward recanalization and a beneficial trend toward 1-year survival in cirrhotic patients with PVT awaiting LT who were treated with anticoagulant therapy[9]. No consensus exists regarding anticoagulation therapy after LT. A short course of anticoagulant therapy should be administered to reduce the risk of rethrombosis, while prolonged therapy should be recommended when nonphysiological reconstruction of portal anastomosis is performed[66].

As Ponziani *et al*[39] suggested, the best recommendation for the future is to avoid PVT-related complications by identifying patients at a high risk for PVT and introducing prevention strategies and

adequate prophylaxis. In this field, only one prospective study demonstrated that prophylactic anticoagulation with LMWH in Child-Pugh B or C was associated with decreased hepatic decompensation and better survival[67]. Gaballa *et al*[68] proposed a scoring system to predict and stratify the risk of PVT in cirrhosis. This score, called the PVT risk index (PVT-RI), was developed to predict the incidence of PVT in liver transplant candidates and considers five variables associated with a higher PVT risk: Age, African American descent, the Model for End-Stage Liver Disease (MELD) score, moderate/severe ascites and nonalcoholic steatohepatitis (NASH). A PVT-RI < 2.6 has a negative predictive value of 94% and could be used to establish the time of ultrasound surveillance. A PVT-RI > 4.6, with a positive predictive value of 85%, could identify a high-risk population that would benefit from anticoagulant prophylaxis[68].

In this review, neoplastic PVT, which occurs as a complication of HCC, was not considered since anticoagulation therapy is not recommended. Instead, the treatment of choice for neoplastic PVT includes surgical resection, radiotherapy, TACE and systemic therapy[69]. Nonneoplastic PVT occurs in approximately a quarter of patients with HCC, but no evidence exists about the role of anticoagulants in this setting[47].

Direct oral anticoagulants

In the last ten years, direct oral anticoagulants (DOACs) have been increasingly prescribed to prevent stroke in nonvalvular atrial fibrillation and to treat thromboembolic disorders, such as venous thromboembolism and pulmonary embolism, after their approval[69,70].

In regard to pharmacodynamic properties, DOACs can be divided into two categories: Factor X-activated inhibitors, such as rivaroxaban, apixaban and edoxaban, and factor II-activated inhibitors, such as dabigatran. Compared with classic anticoagulant molecules (LWMHs and VKAs), among the advantages of DOACs are their oral administration in fixed doses, poor interaction with other drugs and predictable pharmacokinetic profiles and anticoagulant effects; therefore, they do not need laboratory monitoring. Rivaroxaban is metabolized by cytochrome P450 without forming active metabolites and is mostly eliminated by renal excretion. Apixaban and edoxaban are metabolized by cytochrome P3A4 without forming active metabolites. Apixaban is eliminated by renal excretion (approximately 25% of the absorbed dose) and hepatic metabolism but mainly by intestinal excretion (approximately 55%). Edoxaban is eliminated by the hepatobiliary (approximately 65%) and renal (approximately 35%) systems. Rivaroxaban, apixaban and edoxaban act independently of endogenous antithrombin. This could be useful in cirrhosis where antithrombin is reduced. Dabigatran is an oral prodrug metabolized by esterase in various organs, including the liver, but not by hepatic cytochrome, and approximately 80% of it is eliminated by renal excretion[71]. Renal impairment is the main factor that influences the pharmacokinetics of DOACs. Regarding hepatic function, clinical recommendations or contraindications are based on a small amount of evidence because cirrhotic patients have usually been excluded from trials of these drugs[47]. Experience from the long-term use of DOACs in this setting is still limited. All DOACs can be used in patients with mild hepatic dysfunction (Child-Pugh A) without a significant bleeding risk. In patients with moderate hepatic dysfunction (Child-Pugh B), dabigatran, apixaban and edoxaban can be used with caution, while rivaroxaban should not be used because of increased plasma concentrations and pharmacodynamic effects[72]. In severe hepatic dysfunction (Child-Pugh C), DOACs are not recommended[73].

Regarding hepatotoxicity, a recent systematic literature review reported two new cases of hepatocellular liver injury in patients treated with rivaroxaban[74], in addition to a case report by Liakoni *et al*[75], who described his experience with ximelagatran, which was withdrawn two years after approval because of severe hepatotoxicity[76]. However, the real hepatotoxic effect of new oral anticoagulants is still unknown. All new oral anticoagulants can lead to hepatotoxicity with an idiosyncratic mechanism, but this adverse event is very rare[77]. A recent meta-analysis considering patients treated with DOACs demonstrated that the incidence of drug-induced liver injury (DILI) was insignificant when the data of each drug were individually analysed[78]. A prospective study showed that dabigatran, rivaroxaban, apixaban and edoxaban are associated with a lower incidence of liver injury than warfarin, and among these, dabigatran seems to be the safest[79], probably due to its pharmacokinetic characteristics.

When the EASL published guidelines about PVT treatment in cirrhosis in 2016, no specific indications were described for the use of DOACs, and they emphasized the need for randomized trials to assess the efficacy and safety of DOACs in cirrhosis[2]. These recommendations have been confirmed by the most recent AASLD guidelines. The lack of evidence is the result of patients with signs of liver disease being excluded from clinical trials with DOACs[59].

Safety of DOACs in cirrhosis: current evidence

Evidence regarding the safety of DOACs in cirrhotic patients affected by atrial fibrillation or venous thromboembolism suggests that DOACs may be safe in patients with mild to moderate chronic liver disease, with rates of bleeding similar to those of traditional anticoagulants[80]. In a recent publication, Violi *et al*[47] concluded that DOACs may be considered for the treatment of deep venous thrombosis or for prophylaxis in patients with atrial fibrillation when cirrhotic patients are not eligible for VKAs.

In a more recent extended systematic review and meta-analysis, Menichelli *et al*[81] investigated the safety of DOACs compared to VKAs in patients with advanced liver disease who received anticoagulants for atrial fibrillation or deep vein thrombosis. The primary endpoints were any bleeding, major bleeding, gastrointestinal bleeding, and intracranial hemorrhage. Considering more than forty thousand patients, the authors concluded that treatment with DOACs compared to VKAs is associated with a lower risk of major bleeding, intracranial hemorrhage, and all types of bleeding (pooled hazard ratios 0.39, 0.48 and 0.73, respectively), with no difference in gastrointestinal bleeding. Subsequently, the subanalysis of only cirrhotic patients showed no difference in safety outcomes between the DOAC and VKA groups[81]. In accordance with this study, a retrospective longitudinal analysis conducted by Serper *et al*[82] also demonstrated that DOACs were associated with a significantly lower incidence of bleeding than VKAs in a cohort of cirrhotic patients with atrial fibrillation. Moreover, both anticoagulant classes have been proven to be capable of reducing all-cause mortality and the incidence rate of hepatic decompensation when compared with any anticoagulant therapy.

Regarding the safety of DOACs in cirrhotic patients with PVT, one of the first studies was conducted by De Gottardi *et al*[83], who compared the rate of bleeding in cirrhotic patients with that in noncirrhotic controls. In this study, 36 patients affected by mild to moderate liver cirrhosis treated with DOACs for a mean of 9.6 mo were included. Major or minor bleeding was reported in 5 cirrhotic patients (13.9%); however, in 58 noncirrhotic patients treated with DOACs, minor and major bleeding was reported in 9 (15.9%) patients.

Regarding the safety of DOACs compared to traditional anticoagulants, Intagliata *et al*[84] reported a comparable bleeding rate in patients affected by mild to moderate cirrhosis. In this study, the rate of bleeding was analyzed in 20 cirrhotic patients prophylactically or therapeutically treated with rivaroxaban or apixaban compared with 19 cirrhotic patients treated with traditional anticoagulants. The indications for anticoagulant therapy were atrial fibrillation or VTE, including PVT. The total bleeding and major bleeding rates were not significantly different between the two groups[84]. Similarly, Hum *et al*[85] investigated the difference in bleeding events between DOACs and traditional anticoagulants in cirrhotic patients. Twenty-seven patients treated with rivaroxaban or apixaban and 18 patients treated with warfarin or LMWH affected by atrial fibrillation and venous thromboembolism, including PVT, were included. Total bleeding was similar in the two groups: 10 events in the traditional group and 8 in the DOAC group (P = 0.12). Major bleeding was significantly higher in the traditional group than in the DOAC group (5 *vs* 1, P = 0.03).

Table 1 summarizes the evidence about DOAC safety in cirrhosis. The main limitation in assessing DOAC safety in patients with cirrhosis is the lack of uniformity in outcome definitions. In the studies examined, different bleeding definitions were used. To address this lack of uniformity, Nisly *et al*[86] conducted a systematic review and meta-analysis considering only studies in which the primary safety outcome was major bleeding according to the definition of the International Society on Thrombosis and Haemostasis (ISTH). In these studies, pooled analysis demonstrated the absence of a statistically significant difference between DOACs and traditional anticoagulants for ISTH major bleeding in cirrhotic patients treated for stroke prevention or venous thromboembolism[86].

Efficacy of DOACs in PVT: Current evidence

Studies regarding the efficacy of DOACs to treat PVT in cirrhosis are very limited (Table 2). Ai *et al*[87] studied the efficacy of rivaroxaban and dabigatran[87]. In this prospective study, 80 patients with chronic PVT were enrolled and divided into two groups: 40 patients were treated for 6 mo with DOACs, 26 patients with rivaroxaban 20 mg once daily, 14 patients with dabigatran 150 mg twice daily, and 40 control patients were not treated with anticoagulant therapy. At 0, 3 and 6 mo, patients were tested with ultrasound and pulsed Doppler to establish the portal blood flow rate and CT portal angiography to examine thrombus extension. Regarding efficacy, in treated patients, a significant response in terms of complete/partial recanalization and improved portal blood flow velocity compared with the control group was demonstrated, which was superior at 6 mo than at 3 mo. The majority of recanalized patients were Child-Pugh A, and none of them were Child-Pugh C. Regarding safety, no significantly different bleeding rates in the treated *vs* the control group were shown. In this study, patients with moderate to severe esophageal varices and platelet counts below 50×10^9/L were excluded.

Comparing DOACs with classic anticoagulants, Hanafy *et al*[88] designed a randomized, controlled, interventional study in which they compared the efficacy and safety of rivaroxaban with warfarin to treat acute portal thrombosis in HCV-related cirrhosis. Eighty patients were enrolled. After 3 days of enoxaparin 1 mg/kg every 12 h, forty patients continued therapy with rivaroxaban 10 mg twice daily; instead, controls were treated with warfarin at variable dosages to maintain the international normalized ratio (INR) between 2 and 2.5. Regarding efficacy, the primary outcome was partial or complete PVT recanalization; the secondary outcome was the absence of recurrence after the end of therapy. Regarding safety, the main outcome was major bleeding. The results showed that rivaroxaban was more effective than warfarin in terms of complete or partial recanalization, time to recanalization, recurrence of PVT and safety, with a significantly lower risk of major bleeding[88].

In a retrospective analysis, Nagaoki *et al*[89] evaluated the efficacy and safety of edoxaban compared with warfarin to treat PVT in cirrhotic patients after 2 wk of danaparoid sodium. Twenty patients were enrolled in the edoxaban group and received 60 mg or 30 mg once daily depending on renal function,

Table 1 Safety of direct oral anticoagulants in cirrhosis

Ref.	Drug administrated (*n* of patients)	Child-Pugh score at baseline	Indication for anticoagulant therapy	Definition of events	Events in cases *vs* control
Ai *et al*[87]	Rivaroxaban 20 mg once daily (26 pts)	Mean 7.2	PVT	No definition of events	3 *vs* 1 (P = 0.616); 1 hematuria in dabigatran; 1 hemoptysis in rivaroxaban; 1 melena in rivaroxaban
	Dabigatran 150 mg twice daily (14 pts)				
	No anticoagulant (40 pts)	Mean 7.4			
Hanafy *et al*[88]	Rivaroxaban 10 mg twice daily (40 pts)	Mean 6.4	PVT	Major bleeding	0 *vs* 17 (P = 0.001)
	Warfarin (40 pts)	Mean 6.2		Death bleeding related	0 *vs* 8 (P = 0.001)
Nagaoki *et al*[89]	Edoxaban 60 mg or 30 mg once daily (20 pts)	Child-Pugh A; (15 pts); Child-Pugh B; (5 pts)	PVT	Adverse events of grades 3/4 according to Common Terminology Criteria for Adverse Events version 4.0	3 *vs* 2 (P = 0.335)
	Warfarin (30 pts)	Child-Pugh A; (15 pts); Child-Pugh B; (10 pts); Child-Pugh C; (5 pts)			
De Gottardi *et al*[83]	Rivaroxaban (30 pts); Dabigatran (4 pts); Apixaban (2 pts)	Mean 6	PVT (22 pts); Budd Chiari syndrome (5 pts); Cardiac Arrhythmia (5 pts); DVT (2 pts); Other (2 pts)	Major bleeding	1
				Minor bleeding	4
Intagliata *et al*[84]	Apixaban 5 mg or 2.5 mg twice daily or; Rivaroxaban 20 mg or 10 mg daily (20 pts)	Child A; (9 pts); Child B; (11 pts)	PVT (12 pts); Non-splanchnic VTE (4 pts); Atrial fibrillation (4 pts)	Major bleeding	1 *vs* 2 (P = 0.6)
	LMWH or VKA (19 pts)	Child A (9 pts); Child B (10 pts)		Moderate bleeding	1 *vs* 1
				Mild event	2 *vs* 1
Hum *et al* [85]	Rivaroxaban 15 mg daily (17 pts); Apixaban 5 mg twice daily (10 pts)	Child A; (11 pts); Child B; (12 pts); Child C; (4 pts)	PVT (4 pts); DVT (12 pts); Atrial fibrillation (15 pts)	Major bleeding	1 *vs* 5 (P = 0.03)
	LMWH or WKA (18 pts)	Child A; (7 pts); Child B; (9 pts); Child C; (2 pts)		Moderate bleeding	4 *vs* 5 (P = 0.45)
				Mild bleeding	3 *vs* 0 (P = 0.26)
Goriacko *et al*[91]	Dabigatran (35 pts); Rivaroxaban (29 pts); Apixaban (11 pts)	Child A; (48 pts); Child B; (26 pts); Child C; (1 pts)	Atrial fibrillation	Major bleeding	3.3% *vs* 3.9% (P = No significance)
	Warfarin (158)	Child A; (56 pts); Child B; (93 pts); Child C; (9 pts)			

PVT: Portal vein thrombosis; DVT: Deep vein thrombosis; VTE: Venous thromboembolism; LMWH: Low molecular weight heparin; VKA: Vitamin K antagonist.

body weight and concomitant drug administration. Thirty patients were enrolled in the control group treated with warfarin, and the INR target was 1.5-2. The duration of the study was 6 mo. Efficacy was evaluated in terms of PVT volume and PVT reduction rate at 2 wk and 1, 3 and 6 mo, as assessed with dynamic CT. Safety was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Additionally, in this study, the findings demonstrated the effectiveness of DOACs compared to warfarin, showing a significant reduction in thrombus volume after 6 mo of treatment and a higher prevalence of complete response. Regarding safety, there were no significant dissimilarities between the two groups[89].

AREAS OF UNCERTAINTY

Although evidence has shown the noninferiority of DOACs compared with traditional anticoagulant therapy, the studies examined varied in design, and no universal outcome definition was used.

Table 2 Efficacy of direct oral anticoagulants in portal vein thrombosis treatment in cirrhosis

Ref.	Study design	Drug administrated (*n* of patients)	Duration of treatment	PVT type	Results (cases *vs* controls)
Ai *et al* [87]	Prospective cohort study	Rivaroxaban 20 mg once daily (26 pts) Dabigatran 150 mg twice daily (14 pts) No treatment (40 pts)	6 mo 6 mo	Chronic PVT;	At 3 mo:; Complete/partial recanalization: 5 *vs* 0 (P = 0.026); At 6 mo:; Complete/partial recanalization: 11 *vs* 1 (P = 0.003)
Hanafy *et al*[88]	Randomized, controlled, interventional, open-label study	Rivaroxaban 10 mg twice daily (40 pts) Warfarin (40 pts)	Until recanalization and prolonged for 1 or 2 mo in complete recanalization of PVT which non-involvement/ involvement of SMV and for 6 mo in case of positive thrombogenic assay or partial recanalization;	Acute PVT	Complete recanalization: 34 *vs* 18 (P = 0.001); Partial recanalization: 6 *vs* 0 (P = 0.001)
Nagaoki *et al*[86]	Retrospective cohort study	Edoxaban 60 mg or 30 mg once daily (20 pts) Warfarin (30 pts)	6 mo	PVT	Complete recanalization: 14 *vs* 6 (P < 0.001); Partial recanalization: 4 *vs* 3 (P = 0.312)

PVT: Portal vein thrombosis.

Furthermore, in these studies, no uniformity in dosage strategy, treatment duration, clear predictor efficacy or evidence on the ideal time of initiation and duration of anticoagulant therapy were described. This poses a challenge for establishing the real effect and benefit of anticoagulant therapy with DOACs in terms of portal recanalization.

Regarding safety, the definition of bleeding events varied between studies. However, the safety of DOACs appears comparable or superior to that of classic anticoagulants. In addition, a major limitation, which is shared in these studies, regards the characteristics of the patients included. Most of the patients considered were affected by compensated cirrhosis. Insufficient data are reported about the safety and efficacy of DOACs in patients affected by advanced liver cirrhosis.

No evidence exists about the role of prophylactic anticoagulant therapy. Villa *et al*[67] demonstrated that prophylactic anticoagulant therapy with LMWH has some beneficial effects on the deterioration of liver function and survival. Most likely, DOACs may contribute to reducing liver damage, especially in early cirrhosis stages, and superior drug tolerance makes them suitable for wider use. The pharmacodynamic and pharmacokinetic characteristics of DOACs could be an important tool for portal vein thrombosis prophylaxis, but patients who would benefit most from this therapy have not yet been identified. A defined stratification of the portal vein thrombosis risk is still lacking. There is a need to validate scores to establish PVT risk and subsequent prophylactic anticoagulant therapy.

In the setting of liver transplantation, anticoagulant therapy with DOACs in patients with PVT on a waiting list is a potential option to allow recanalization of the portal vein and to allow physiological reconstruction of vessels. The major advantage for patients who are waiting for liver transplantation is the possibility of counteracting the anticoagulant effect with reversal agents at any time, such as idarucizumab for dabigatran or andexanet alfa for rivaroxaban. The main limitations are the high cost, availability, and lack of evidence about their use in cirrhotic patients, especially with decompensated disease.

CONCLUSION

This review emphasizes that DOACs could represent a valid alternative to the currently poorly defined standard of care for portal vein thrombosis. However, we show that the lack of evidence and inhomogeneity of studies regarding outcome definitions to evaluate efficacy and safety poses challenges to clinical trial design to evaluate DOACs and, as consequence, its use in clinical practice.

As shown here, in cirrhotic patients with mild hepatic function impairment, the safety and efficacy of new oral anticoagulants seems to be noninferior compared with classic anticoagulants, especially in patients with a low bleeding risk (platelet count >100,000/mm^3 and no high-risk esophageal varices). No significant differences between dabigatran, rivaroxaban, and edoxaban have been observed, while data on apixaban for treating portal vein thrombosis in cirrhotic patients are limited[90]. In patients with moderate liver dysfunction, anticoagulant drugs need to be selected with caution, especially those metabolized by liver cytochromes. Considering this, molecules with a predominantly renal metabolism

might be preferred in more advanced liver disease. In patients awaiting liver transplantation, dabigatran may be promising in preventing thrombosis progression because of the low rate of hepatotoxicity, predominant renal metabolism and reversibility by idarucizumab in perioperative management. Well-designed randomized controlled trials are needed to further evaluate the safety and efficacy of DOACs to treat PVT in cirrhotic patients, especially in patients listed in the OLT setting.

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FOOTNOTES

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MINIREVIEWS

Noninvasive diagnosis of periportal fibrosis in schistosomiasis mansoni: A comprehensive review

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Abstract

Schistosomiasis mansoni is a neglected disease and key public health problem, mainly due to its high prevalence, the scarcity of public policies, and the severity of some clinical forms. Periportal fibrosis (PPF) is the commonest complication of chronic schistosomiasis mansoni and its diagnosis requires different techniques. Even though wedge biopsy of the liver is considered the gold standard, it is not justified in non-surgical patients, and percutaneous liver biopsy may be informative but does not have sufficient sensitivity. Noninvasive PPF tests mostly include biological (serum biomarkers or combined scores) or physical assessments (imaging assessment of fibrosis pattern or tissue stiffness). Moreover, imaging techniques, such as ultrasound, computed tomography, magnetic resonance imaging, and elastography are applied not only to support the diagnosis of schistosomiasis, but also to assess and detect signs of portal hypertension and organ damage due to chronic schistosomiasis. A combination between a comprehensive history and physical examination with biomarkers for liver fibrosis and imaging methods seems to offer the best approach for evaluating these patients. In addition, understanding their strengths and limitations will allow a more accurate interpretation in the clinical context and can lead to greater accuracy in estimating the degree of fibrosis in patients with *Schistosomiasis mansoni (S. mansoni)* infection. This review will discuss the different noninvasive methods that are currently available for the evaluation of PPF in *S. mansoni* infection, and their application, advantages, and limitations in clinical practice.

Key Words: Schistosoma mansoni; Parasitic liver diseases; Liver fibrosis; Biomarkers; Elasticity imaging techniques; Ultrasonography

Core Tip: Schistosomiasis mansoni is a neglected and key public health problem and periportal fibrosis (PPF) is its commonest complication. Noninvasive PPF tests mostly include biological or physical assessments. Imaging techniques have been currently applied to assess and detect liver damage due to chronic schistosomiasis. A combination between these biomarkers, a comprehensive history and physical examination, laboratory tests, and imaging methods seems to offer the best approach for evaluating these patients. We herein discuss the different noninvasive methods that are currently available for evaluating PPF in *Schistosomiasis mansoni* infection, and their application, advantages, and limitations in clinical practice.

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INTRODUCTION

Schistosomiasis mansoni is a neglected parasitic disease of chronic evolution and a key public health problem, mainly due to its high prevalence, the scarcity of public policies, and the severity of some clinical forms. As it is endemic in over 78 resource-constrained countries, schistosomiasis is considered one of the indicators of poverty[1,2].

The World Health Organization (WHO) estimates that at least 241 million people required preventive treatment[3]. In Brazil, the economic burden of schistosomiasis mansoni is high and results in loss of productivity, and it is a big challenge to public health[4]. Approximately 200 thousand deaths are attributed to this disease annually and they occur in up to 29% of those who present bleeding varices even with full hospital care. Community-based studies suggest that periportal fibrosis (PPF), which is the term more frequently used after the advent of ultrasonography in schistosomiasis, is the commonest complication of *Schistosomiasis mansoni (S. mansoni)* chronic infection[5].

Additionally, Symmers fibrosis or "pipestem fibrosis" is the advanced PPF form that occurs in patients with hepatosplenic schistosomiasis (HSS) and it is defined as a moderate to advanced PPF, with or without hepatomegaly, but always with splenomegaly, on ultrasound (US). In endemic areas, it is possible to find patients with advanced PPF (Symmers fibrosis) without splenomegaly, also known as the hepatic form of the disease. On the other hand, a few individuals with splenomegaly without PPF can also be found, which is due to other causes of splenomegaly, and if there were no US, these patients would be misdiagnosed, based on the clinical examination, as HSS.

Hence, Symmers fibrosis is the most prominent feature of liver pathology in schistosomiasis mansoni and it is represented by a process of portal fibrosis that extends from the smallest to the largest portal spaces[6]. Granulomas around the parasite eggs can be seen in abundance in the portal spaces, at the beginning of the disease, which disappear when it becomes chronic and the fibrosis remains. There are also obstructive vascular lesions secondary to granulomas, thrombosis, phleboscleorosis, and fibrous intimal thickening. The major liver pathology of egg granulomas results from physical obstruction and tissue compression, while splenomegaly results from both chronic passive congestion and reactive hyperplasia of the reticuloendothelial system due to immune dysregulation[7]. The main clinical manifestation of HSS is portal hypertension (non-cirrhotic) and porto-systemic collateral circulation, notably esophageal varices[8,9].

The hepatic parenchyma maintains its usual acinar structure, and this is reflected in patients exhibiting a normal hepatocyte function despite the signs of portal hypertension, without stigmata of chronic liver disease, unlike cirrhosis. However, in some cases, compensated HSS may turn into decompensated HSS with the presence of stigmatas, ascites, muscular loss, and hepatic failure[8]. These cases of progression from schistosomiasis to liver cirrhosis, with the capacity of the synthesis of hepatocytes being impaired, are sometimes observed in clinical practice in patients who have recurrent episodes of digestive bleeding and repeated necrosis of hepatocytes.

In these decompensated cases, it should also be remembered that there is the possibility of co-infection of schistosomiasis with hepatitis B, C, and E viruses, with the non-alcoholic steatohepatitis or chronic alcohol abuse[10-12].

Table 1 Major advantages and limitations of noninvasive periportal fibrosis markers in *Schistosomiasis mansoni* infected patients

Feature	Liver biopsy		Serum markers	Imaging techniques		
	Wedged	Percutaneous		US	pSWE	TE
Invasiveness	High	High	Minimal	None	None	None
Post-procedural risk	Possible	Possible	Minimal	None	None	None
Accuracy for PPF prediction	High	Low	Medium to high	High	Good	Good
Sensitivity	High	Low	Medium	High	Medium	Medium
Interpretation	Subjective	Subjective	Objective	Subjective	Objective	Objective
Observer variability	High	High	Low	High	Not yet evaluated	Not yet evaluated
Costs	High	Medium	Depends	Medium	Medium	Medium
Limitations by anthropometric features	High	High	None	Medium	Medium	Medium
Suitability for monitoring PPF	Low	Low	High	High	High	High

PPF: Periportal fibrosis; pSWE: Point shear wave elastography; TE: Transient elastography; US: Ultrasound.

Schistosomiasis mansoni is commonly diagnosed due to detecting parasite eggs in stool using the Kato-Katz technique. Immunological methods are favored for monitoring communities in areas with low infection rates, and for patients with mild and chronic infections where parasitological tests are negative. Polymerase chain reaction-based diagnostic techniques are more sensitive, but expensive. The Point-of-care Circulating Cathodic Antigen test method appeared to be a promising feature in the diagnosis, but many false positive cases were reported in low endemic areas[2,3,13].

Recently, studies in low prevalence areas for schistosomiasis in Brazil have shown that the primary diagnostic approach performed by the Schistosomiasis Control Country Program may underestimate the real prevalence of this infection. In addition, there have been reports of a high proportion of infected individuals in the urban area, the presence of infected snails, a random distribution of vectors, and the absence of an association between classical risk factors and the human infection[14]. In low endemic countries, the determination of infected patients should combine different methods and algorithms for accurately estimating the prevalence and the indicators that could be used in the control of schistosomiasis[2].

Moreover, diagnosing PPF requires different techniques. Wedge liver biopsy is considered the gold standard, but it is not justified in non-surgical patients. Alternatively, percutaneous liver biopsy may be informative but does not have sufficient sensitivity[15,16]. Imaging techniques like US, computed tomography (CT), magnetic resonance imaging (MRI), and elastography, on the other hand, are used not only to support the diagnosis of schistosomiasis but also to assess and detect signs of portal hypertension and organ damage due to chronic schistosomiasis disease[17-20]. A combination between a comprehensive history and physical examination with biomarkers for liver fibrosis, and imaging methods seems to offer the best approach for evaluating these patients[7,17].

In this review, we will discuss the different noninvasive methods that are currently available for evaluating PPF in chronic schistosomiasis mansoni, and their application, advantages, and limitations in clinical practice.

NONINVASIVE MARKERS OF PPF

Noninvasive markers of liver fibrosis can be divided into two groups: Serum biomarkers and imaging techniques. Table 1 summarizes the main features of these methods. Besides the clear advantage of being noninvasive, some of them can offer a more objective interpretation of numerical test results and may overcome the intra- and inter-observer variability of US techniques.

Serum biomarkers

Serological biomarkers of liver fibrosis are frequently classified as direct or indirect biomarkers. Whereas direct biomarkers reflect extracellular matrix (ECM) turnover and the changes in the fibrogenic cell type, indirect biomarkers mostly estimate the degree of fibrosis[21].

In advanced stages of fibrosis, the liver contains approximately six times more ECM components than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronic acid (HA), and proteoglycans[22]. Therefore, qualitative and quantitative ECM changes in liver fibrosis can be measured in the blood or urine using these serological biomarkers[23].

Table 2 Major advantages and limitations of noninvasive periportal fibrosis markers in *Schistosomiasis mansoni* infected patients

PFF markers	Advantages	Limitations
Direct markers		
PICP	Elevated levels in patients not treated yet with praziquantel and related to the stage of fibrosis and necroinflammation	Not reliable for establishing fibrosis grade
P3NP	Use for complicated patients who developed hypertension and with more severe liver diseases	Low sensitivity in mild cases
Serum type VI collagen	Correlated with liver fibrosis, splenomegaly, portal vein dilatation and the presence of portosystemic collaterals	Low sensitivity
Hyaluronic acid	Marker for the initial phase of liver fibrosis and it is able to assess the severity of liver disease	High levels in different etiologies of liver disease, barely accessible
Indirect markers		
APRI	Low cost, good sensitivity, high diagnostic accuracy for cirrhosis	Interference of hepatic comorbidities
Blood platelet count	Low cost and sensitive marker. It is a marker of portal hypertension and inversely correlated with advanced PPF and the diameter of the spleen	Interference of coagulopathies, some drugs and other live disorders
GGT	Low cost. Correlated with more advanced PPF, faster fibrosis progression rate and indicates intrahepatic alterations	Interference of hepatobiliary alterations
Coutinho Index	Simplicity of calculation and low cost	Requires more tests for use in mild and moderate fibrosis

HA: Hyaluronic acid; PICP: Procollagen type I carboxy-terminal peptide; P3NP: Procollagen type III amino-terminal peptide; PPF: Periportal fibrosis; APRI: Aspartate aminotransferase to platelet ratio index; GGT: Gamma glutamyl transferase.

Direct and indirect markers may be used alone or in combination to produce composite scores, which can be relatively simple or can be based on complicated formulas[21]. These markers should be reliable and sensitive so as to indicate advanced lesions and also have prognostic value for identifying early disease[17]. Table 2 summarizes the major advantages and limitations of these markers.

Direct markers

Direct markers are usually fragments of the components of the ECM of the liver produced by hepatic stellate cells during the ECM remodeling process and they usually reflect its deposition or removal[24]. However, they do not indicate the extent of ECM protein distribution[21] and since they are not liver specific, they have a tendency to be more elevated when associated with high inflammatory activity and tend not to be detected in the presence of minimal inflammation[24].

These biomarkers are classified into three groups: Those that measure matrix deposition [procollagen I carboxy terminal peptide, procollagen III amino terminal peptide, tissue inhibitors of metalloproteinase (MMP), transforming growth factor beta and tenascin], those that reflect matrix removal or degradation (procollagen IV C peptide, procollagen IV N peptide, collagen IV, MMP, undulin, urinary desmosine, and hydroxylysylpyridinoline), and those that cannot clearly determine the relationship to the matrix deposition or removal (HA, chitinase-3-like protein 1, and laminin)[23].

Even though direct markers are less useful in clinical practice due to their high cost and difficult procedures, their potential role in the diagnosis and assessment of PPF in schistosomiasis warrants further investigation. The most promising and studied serum markers for the evaluation of PPF are HA, collagen type III, chitinase-3-like protein 1, and laminin[17,23,25].

Since the latest studies referring to these markers have been previously described in complete review articles[17,23,24], the present paper will not be well on them but rather will concentrate on the most recent markers.

Indirect markers

Indirect markers include molecules synthesized, regulated, excreted by the liver or released into the blood in response to liver inflammation, or impairment of the liver function[24].

These markers include common clinical biochemistry tests, such as enzymes, proteins, platelets, and coagulation factors, which do not necessarily reflect ECM turnover or fibrogenic cell changes[24]. Moreover, most indirect biomarkers of fibrosis are integrated with one or more biomarker panels that predict fibrosis.

The most prevalent markers to assess PPF are the platelet count, aspartate aminotransferase to platelet ratio index (APRI)[26,27], Fibrosis-4 (FIB-4)[4], gamma glutamyl transferase (GGT)[17,28], and the Coutinho Index[29,30]. Table 3 summarizes the performance of these markers that has been reported in the literature.

Table 3 Performance of indirect periportal fibrosis markers in *Schistosomiasis mansoni* infected patients

Marker	Parameters	Performance in *S. mansoni* infected patients					
		Severe PPF			Mild/significant PPF		
		Cut-off	Sn (%)	Sp (%)	Cut-off	Sn (%)	Sp (%)
Platelet count[17,26]	Platelet count/mm^3	141000[17]	78.5	60	171000[17], 108500 [26]	80, 91	91.7, 85
APRI[17,26]	(AST/ULN)/platelet count	1.066	58.5	71.1	0.349[17], 0.440[26]	90, 96	83.3, 85
GGT[17]	GGT/ULN	> 1.55	60.0	75.6	> 0.84	74.6	83.3
Coutinho index[29, 30]	(ALP/ULN)/platelet count	≥ 0.330[29], ≥ 0.316 [30]	98, 67.4	94.7, 68.3	≥ 0.300, ≥ 0.228	70.8, 68.6	89.5, 46.3

APRI: Aspartate aminotransferase to platelet ratio index; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; PPF: Periportal fibrosis; Sn: Sensitivity; Sp: Specificity; ULN: Upper limit of normality; *S. mansoni*: *Schistosomiasis mansoni*.

Table 4 Image pattern classification of periportal fibrosis according to the World Health Organization[37]

IP	Description
A	Normal
B	Diffuse echogenic foci ("starry sky"), minimal wall thickening of portal and segmental branches
C	Ring echoes around vessels in cross-section; pipe-stems parallel with portal vessels
D	Echogenic ruff around portal bifurcation and main stem; main portal vessel wall thickening
E	Hyper-echogenic patches expanding into parenchyma
F	Echogenic bands and streaks extending from main portal vein and its bifurcatin to liver surface; may retract the surface of the organ
X	Cirrhosis
Y	Fatty liver
Z	Other hepato-biliary diseases

IP: Image pattern.

In addition, Nascimento *et al*[4] (2018) noted a correlation tendency between the scores for APRI and FIB-4 (Spearman $r = 0.87$, $P < 0.0001$), transient elastography (TE) and FIB-4 ($r = 0.70$, $P = 0.01$), and TE and GGT ($r = 0.68$, $P < 0.01$) in a small sample of 17 patients with schistosomiasis. Therefore, further studies with a larger sample are required to confirm these results and to determine if this correlation can be used clinically.

The Coutinho index is the alkaline phosphatase (ALP) to platelet ratio {[ALP/upper limit of normality (ULN)]/platelet count (10^6/L) × 100}. This index was developed to identify patients with more advanced fibrosis (Niamey's US classification D, E, and F patterns) in endemic areas, without the need for US. These patients, once recruited, were evaluated and, if necessary, sent to a referral hospital. Barreto *et al*[29] initially studied 120 patients at the referral hospital in Pernambuco, Brazil. After analyzing different enzymes that change during the course of this disease, they developed this index. Subsequently, in another study, this index was validated in an endemic area of the same state, with 378 patients who took a parasitological test that was positive for *S. mansoni*[30].

Moreover, Gunda *et al*[31] have also indicated a positive correlation between the presence of esophageal varices and higher APRI levels (> 1.5 m/s, $P < 0.001$) with a higher sensitivity (82.5%) and specificity (80.1%) in discriminating varices among patients with PPF due to schistosomiasis mansoni. The most common diagnostic approaches to stratify patients based on the risk of variceal bleeding include the use of US and platelet count[32].

In this context, Agha *et al*[33] in a case-control study in Saudi Arabia, and Xu *et al*[34] in a cohort in China, investigated, independently, the platelet count/spleen diameter ratio and reported high specificity (83% to 92%) and sensitivity (85% to 100%) for the presence of esophageal varices in HSS patients.

Although the results of some of these studies are promising with regard to evaluating PPF with these indices, more studies with a larger number of patients will be necessary in order to demonstrate the validity of applying these indices in clinical practice.

Imaging techniques

The early detection of serious complications is vital in the management of chronic schistosomiasis. Thus, making use of diagnostic imaging modalities, such as US, CT, and MRI, is very important as each of them has a vital role in diagnosing and assessing the severity of target organ involvement[35,36].

Since US is a convenient and reliable method, it is routinely used in the diagnosis and evaluation of patients with schistosomiasis and it has become the most well-established tool for evaluating PPF. It can be applied to demonstrate classical features of schistosomal hepatic damage and to grade schistosomiasis disease patterns and status, based on criteria published by the WHO[37].

US is a simple, inexpensive, and safe tool and the technique is more sensitive with respect to diagnosing advanced PPF (Symmers fibrosis) than its milder stages. Since the advent of the WHO protocol[37] and later the Niamey-Belo-Horizonte Protocol (2001), US has become the gold standard in the diagnosis of PPF and in the classification of its intensity, which is mainly based on the use of pictorial image patterns (Table 4 and Figure 1). Other previously used protocols, such as Cairo and Managil, were abandoned due to flaws in classifying mild forms of the disease[38,39].

Santos *et al*[14] demonstrated moderate to substantial reproducibility in the PPF classification according to the WHO protocol, mainly using image patterns. In field studies, using these patterns is reported to be simpler and more reproducible than applying other measurements, such as the thickness of the wall of the portal branch and the diameter of the portal, which are also recommended in the WHO protocol[40,41].

Furthermore, the imaging patterns B, C, and D can also be found in other diseases, such as congenital liver fibrosis, viral and autoimmune hepatitis, primary sclerosing cholangitis, and liver cirrhosis. Consequently, a more rigorous analysis by the observer is required to find other signs that will lead to a differential diagnosis of schistosomiasis liver fibrosis[42].

More advanced portable US equipment, like color Doppler US, can characterize portal vein perfusion to predict disease prognosis and with this, decisions can be made on the best treatment options for portal hypertension complications, such as portal vein thrombosis[18].

In our opinion, as US is an operator-dependent technique, the previous experience of the ultrasonographist is very important in evaluation of the image patterns and therefore, to obtain more consistent results. That is the reason why it is unsuitable for the PPF evaluation by US scan to be performed by non-physicians in rural areas, even if they are well trained. Well-trained physicians who know about other diseases that may affect the liver would apply the Niamey protocol in the evaluation of PPF in patients with *S mansoni*. However, in endemic areas, this protocol is not widely applied by every physician that usually performs US, but only by the few who are trained and interested in research projects.

CT and MRI can also be applied to diagnose PPF. The main features of CT in the fibrotic liver with HSS are round, low-density periportal zones enhanced after contrast administration, and linear bands in longitudinal sections of portal veins[43]. On the other hand, MRI is described as a more sensitive imaging technique than US in the diagnosis of Symmers fibrosis, because it can differentiate fibrosis from fatty liver disease and inflammatory infiltrate.

However, these techniques are not routinely used for schistosomiasis diagnosis in resource-poor settings, due to the use of contrast and the associated risks and the costs[44-46]. In addition, some places do not have an adequate structure for installing these sophisticated devices.

Nevertheless, Voieta *et al*[47] aimed to classify schistosomal PPF intensity and observed a moderate agreement between imaging techniques (US and MRI) (κ = 0.41). However, it was only after re-grouping the grades (absent and slight *vs* moderate and intense) that it was observed that the concordance was substantial (κ = 0.63). On the other hand, the agreement between US and MRI and histology was poor.

Moreover, Silva *et al*[15] described a poor correlation between US and MRI in PPF assessment using WHO patterns (κ = 0.14), and even after grouping image patterns such as "A-D", "Dc-E" and "Ec-F", the agreement between US and MRI remained weak (κ = 0.39). The authors recommended that new patterns should be constructed to better reflect MRI findings. Accordingly, Scortegagna *et al*[48] reported that MRI presented a good reproducibility in the evaluation of PPF in later stages of schistosomiasis, with a global interobserver agreement of 70%. However, the correlation between MRI and US was poor, showing only a 30% agreement.

Recently, elastography-based imaging techniques, such as TE and point shear wave elastography (pSWE) using acoustic radiation force imaging (ARFI), have received substantial attention with respect to the non-invasive assessment of the mechanical properties of tissue. These techniques have emerged as complementary to US images in the study of liver fibrosis in many hepatic diseases[49,50] including schistosomiasis mansoni[19,20,51-54].

These techniques take advantage of changes in the elasticity of soft tissue according to the involvement of certain organs, including the liver and spleen, as this yields qualitative and quantitative information that could be used for diagnostic and prognostic purposes[55].

Recent studies have suggested an association between the degree of liver fibrosis in chronic hepatitis C virus (HCV) infection, reflected by the shear wave speed in the liver, and the severity of liver disease (advanced, compensated, decompensated, or hepatocellular carcinoma). Additionally, in 358 patients with schistosomiais mansoni, Carvalho Santos *et al*[19] evaluated the PPF by hepatic pSWE (ARFI), thus revealing that this technique could be useful in the diagnosis of advanced forms of schistosomiasis

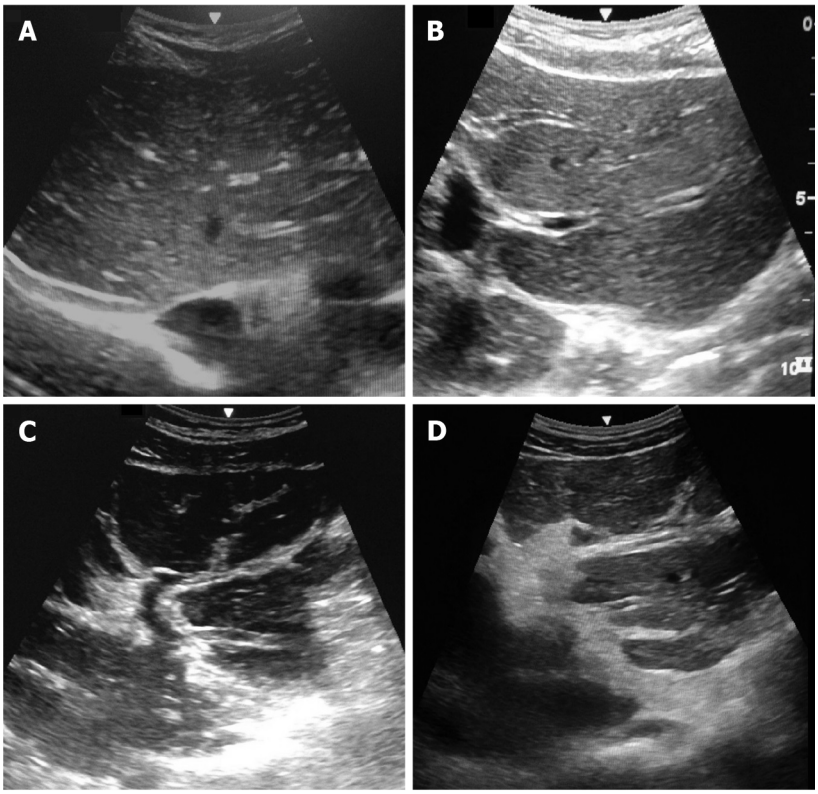

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Figure 1 Ultrasound-based liver images obtained from patients with periportal fibrosis due to *Schistosomiasis mansoni* infection, by a Siemens scanner based on Niamey's pattern, Pernambuco, Brazil, 2020. A: Pattern C; B: Pattern D; C: Pattern E; D: Pattern F.

mansoni. The ARFI was able to accurately differentiate mild PPF from significant PPF. Table 5 summarizes the performance reported in the literature of noninvasive PPF imaging techniques in *S. mansoni* infected patients.

Similarly, an association has also been reported between splenic elastography and signs of portal hypertension, including the presence of esophageal varices, in some chronic liver diseases[56]. However, these are still initial studies in assessing schistosomiasis portal hypertension[52].

In fact, recent studies have shown that the measurement of spleen stiffness by TE and pSWE correlates with that of liver stiffness and of portal hypertension in patients with HSS[20,52]. Accordingly, spleen stiffness may also be a predictor of variceal bleeding in HSS and may be further investigated for predicting HSS complications[57].

Furthermore, it is relevant to highlight that, in schistosomiasis, massive splenomegaly occurs due to the hyperplasia of the reticuloendothelial system. Hence, there is an increase in blood flow in the splenic vein and also obstruction for the venous flow by intra-hepatic granulomas resulting in portal hypertension[9,58]. The pathophysiology of portal hypertension in (non-cirrhotic) schistosomiasis should contribute to more changes in splenic parenchyma stiffness compared to cirrhosis, since the spleen undergoes passive congestion (without hyperplasia). As a result, splenic elastography should be more accurate than liver elastography for evaluating disease morbidity and portal hypertension signs in patients with schistosomiasis mansoni[59,60]. Table 6 summarizes the advantages and limitations of noninvasive PPF techniques that are frequently used in clinical practice with regard to *S. mansoni* infected patients.

COST-EFFECTIVENESS OF NONINVASIVE MARKERS OF FIBROSIS

Despite their increased use in clinical practice, Non-invasive tests were not designed to follow longitudinal changes in fibrosis or disease activity nor to reflect the dynamic process of fibrogenesis and differentiate between adjacent disease stages regardless of their increased application in clinical practice. Comprehending their strengths and limitations will result in a more accurate interpretation in the clinical context[61].

Therefore, liver fibrosis biological markers allow an objective interpretation of results and significantly reduce the risk of bias due to variability that occurs in liver biopsy, in addition to which these markers have the advantage of not being invasive. Associating the various non-invasive methods

Table 5 Performance reported in the literature of noninvasive periportal fibrosis imaging techniques in *Schistosomiasis mansoni* infected patients

Marker	Parameters	Performance in schistosoma infected patients		
		Severe PPF		Mild/significant PPF
		Cut-off		Cut-off
		HS	HSS	HIS
US[37]	Image interpretation (Niamey sonographic protocol)	D	E/F	-
TE[20,51,53,57]	Wave propagation speed (kPa)	-	9.6 kPa[57], 8.9 kPa[51], 9.7 kPa[20], 9.5 kPa[53]	-
Pswe[19,51,42]	Wave propagation speed (m/s; kPa)	1.33 m/s[51]	1.39 m/s[19], 1.53 m/s[52]	1.11 m/s[19], 1.29 m/s[52]
2D-SWE[54]	Wave propagation speed (m/s; kPa)	-	14.9 kPa[54]	-

HIS: Hepatointestinal schistosomiasis; HS: Hepatic schistosomiasis; HSS: Hepatosplenic schistosomiasis; PPF: Periportal fibrosis; pSWE: Point shear wave elastography; TE: Transient elastography; US: Ultrasound.

Table 6 Advantages and limitations of noninvasive periportal fibrosis techniques frequently used in clinical practice with regard to *Schistosomiasis mansoni* infected patients

Techniques	Advantages	Limitations
Blood Platelet Count	Low cost, routine laboratory test, easy access	Difficult to diagnose patients with initial PPF
APRI	Low cost, based on routine laboratory tests, easy access	More frequently used to diagnose patients with portal hypertension and esophageal varices, less sensitive for PPF
Coutinho index	Low cost, based on routine laboratory tests (alkaline phosphatase and platelet count), easy access, lets advanced PPF be identified	These tests need to be validated in other centers
Ultrasound	Low cost, safe and based on the Niamey-WHO protocol	Operator dependent
MRI/CT	MRI is more sensitive than ultrasound at diagnosing PPF	Expensive, use of radiation, not available in endemic areas, no relation with the Niamey-WHO protocol
Liver elastography	Good accuracy, distinguishes mild from significant PPF	Expensive, not available in endemic areas
Spleen elastography	Related to portal hypertension	Expensive, not available in endemic areas, needs further studies
Wedge liver biopsy	Gold standard used to diagnose Symmers fibrosis	Only for surgical patients
Percutaneous liver biopsy	Can be used in differential diagnosis between schistosomiasis and other liver diseases	Insufficiently sensitive and so may fail to diagnose PPF

CT: Computer tomography; PPF: Periportal fibrosis; MRI: Magnetic resonance imaging; WHO: World Health Organization.

can lead to greater accuracy in estimating the degree of fibrosis in patients with schistosomiasis mansoni disease[4].

Crossan *et al*[62] in a cost-effectiveness study of non-invasive tests for assessment and monitoring of liver fibrosis and cirrhosis in patients with chronic liver disease in the United Kingdom, reported that high-quality studies with a low risk of bias are required to allow sufficient validation of specific cut-offs to stage fibrosis in different disease etiologies.

Moreover, Crossan *et al*[62] suggested that, for HCV and hepatitis B virus (HBeAg-negative) infected patients, treating all patients without prior diagnostic testing and regardless of fibrosis level was the most cost-effective option. Nevertheless, the findings from these models may not be transferable to a resource setting where funds are limited and the ability to treat all patients is not a realistic option[63].

In rural areas with few resources, it becomes very difficult to implement imaging methods, such as elastography, for example, or even for there to be a trained physician available to perform US and define the fibrosis pattern using the Niamey-Belo Horizonte protocol. Certainly, the use of serum markers, especially the platelet count and the Coutinho index, should be the most cost-effective, straightforward, and objective noninvasive way to identify and select patients who may have the most advanced forms of PPF.

CONCLUSION

By undertaking this literature review, we have observed that noninvasive PPF tests mostly include biological (serum biomarkers, indices, or combined algorithms) or physical assessments (imaging assessment of the fibrosis pattern or tissue stiffness). Even though currently available approaches have shown some advantages with respect to overcoming the limitations set out in the previous section, the reason for requesting a test is what will determine the best one for each case.

To date, the platelet count and the Coutinho index appear to be the most applicable noninvasive tests for evaluating PPF in endemic zones where schistosomiasis mansoni occurs. These tests are simple and inexpensive and thus are used to select the cases that will need more accurate evaluation (US assessment of the fibrosis pattern or tissue stiffness). However, they are not always available in endemic areas. Therefore, in such cases, hepatosplenic patients should be referred, if necessary, to hospitals where more specialized imaging tests, such as MRI and CT, can be conducted.

FOOTNOTES

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Review on hepatitis B virus precore/core promoter mutations and their correlation with genotypes and liver disease severity

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Abstract

Of 350 million people worldwide are chronically infected with hepatitis B virus (HBV) and are at risk of developing cirrhosis and hepatocellular carcinoma (HCC) later in life. HBV is the most diverse DNA virus, and its genome is composed of four open reading frames: Presurface antigen/surface antigen gene (preS/S), precore/core gene (preC/C), polymerase gene (P), and the X gene (X). HBV produces quasispecies naturally or in response to antiviral agents because of the absence of proofreading activity amid reverse transcription and a high replication rate. The virus has 10 genotypes (A to J) with different geographical distributions. There are various HBV mutations in the HBV genome, including preC/C mutations, preS/S mutations, P gene mutations, and X gene mutations. The core promoter region plays a vital part in the replication, morphogenesis and pathogenesis of the virus. The precore region also plays a crucial role in viral replication. Both core promoter and precore mutations rescue the virus from host immune surveillance and result in the formation of mutated strains that may have altered pathogenicity. preC/C mutations are associated with liver disease progression. Precore mutations stop hepatitis B e antigen (HBeAg) production and basal core promoter mutations downregulate HBeAg production. Mutations in the basal core promoter are also associated with increased HBV replication and an increased incidence of advanced liver diseases such as cirrhosis and HCC. The emergence of antiviral-resistant mutations is the main reason for treatment failure. This review focuses mainly on preC/C promoter mutations and their correlation with genotypes and liver disease severity. Thorough perception and knowledge of HBV genetic variety and mutants could be vital to discover techniques for the prognosis and control of HBV infection.

Key Words: Hepatitis B virus; Hepatitis B virus e antigen; Hepatocellular carcinoma; Basal core promoter; Core promoter region; Precore region; Fulminant hepatitis; Acute hepatitis

Core Tip: Worldwide, 350 million people are chronically infected with hepatitis B virus and are at risk for cirrhosis and hepatocellular carcinoma. Both core promoter and precore mutations help rescue the virus from host immune surveillance and result in the formation of mutated strains that may have altered pathogenicity. Precore/core promoter (preC/C) mutations are associated with advanced liver disease. We discuss mainly preC/C mutations and their correlation with genotypes and liver disease severity.

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INTRODUCTION

The hepatitis B virus (HBV) epidemic exists as a leading public health issue worldwide even with the accessibility of effective vaccines[1,2]. Worldwide, almost 350 million people are chronically infected with HBV and it accounts for more than 1 million deaths as a result of progressive liver diseases like cirrhosis and hepatocellular carcinoma (HCC)[3]. Persistent HBV infection leads to various clinical manifestations like inactive carrier state, chronic hepatitis, cirrhosis, and HCC[3].

HBV is an enveloped virus of the *Hepadnaviridae* family. The genome of this virus has partially double-stranded circular DNA of around 3200 nucleotides. There are four overlapping open reading frames (ORFs) that make up the polymerase (P), core (C), surface antigen (S), and X protein[4-7].

Based on an intergroup divergence of more than 7.5% in its complete genome, this virus can be divided into nine genotypes (A to I)[8,9], and based on nucleotide divergence of more than 4%, these genotypes are further subdivided into subgenotypes[8,9]. A to H have long been established as individual genotypes. Two new genotypes (I and J) were suggested more recently[10,11]. Evidence supports that HBV genotypes play a significant role in deciding HBV disease severity. Several studies have revealed that genotype A evolves more rapidly than genotype D, which causes challenges for treatment[12]. In addition, genotype-C-infected patients have more advanced liver disease than patients infected with genotype B[13]. Genotype-F-infected patients have higher mortality than patients infected with genotype A or D[14]. In an Indian study, genotype D was related to more advanced liver disease than other genotypes were[15]. In a study from the United States, genotype D acted as an independent risk factor for fulminant hepatitis (FH)[16]. In another study, genotype-F-infected patients had a higher rate of liver-related death than genotype-D-infected patients had[17]. Generally, patients infected with genotype C and D are more associated with liver disease severity (HCC) than patients infected with genotype A and B[18].

Replication in HBV occurs by reverse transcription of RNA intermediates. This virus has a high error rate during reverse transcription, leading to various mutations in the HBV genome[19]. These mutations have clinical and epidemiological significance. Various mutations (preC/C and preS/S deletion) are related to advanced liver disease and the possibility of HCC[19]. The basal core promoter (BCP) and precore mutations are frequently appearing natural variants of HBV. In this review, we discuss preC/C mutations and their correlation with genotypes and severity of liver disease.

HBV CORE PROMOTER MUTATIONS AND RELATEDNESS WITH GENOTYPE AND ADVAN-CEMENT OF LIVER DISEASE

Core promoter region

The CP region (nt 1575-1849) of the virus genome plays a vital part in the replication and morphogenesis of the virus[20]. CP region controls transcriptional initiation for the synthesis of the precore mRNA and pregenomic RNA (pgRNA). The CP region includes BCP (nt 1743-1849) and upper regulatory region (URR, nt 1613-1742). BCP has a crucial role in the replication of virus by promoting the formation of precore and pgRNAs[20]. The URR contains positive and negative regulatory elements that regulate activity of the promoter[20]. Hence, core promoter mutations may have an impact on virus gene expression and/or replication, and contribute to viral pathogenesis. Now, we discuss various core promoter mutations and their correlation with genotypes and the advancement of liver disease.

A1762T/G1764A double mutation

A1762T/G1764A double mutation is the most commonly observed core promoter mutation and it is associated with hepatitis B e antigen (HBeAg) negativity and one of the causes of advanced liver disease. There are various observations associated with this double mutation described below.

Table 1 Qualitative and quantitative factors and the level of each factor associated with hepatitis B virus-related hepatocellular carcinoma

Patient group	Qualitative factors	Quantitative factors
Low risk	Genotype A/B	Low level of HBV-DNA in serum; low level of HBsAg in serum
High risk	Genotype C/D; BCP mutation (A1762T/G1764A); pre-S deletion	High level of HBV-DNA in serum; high level of HBsAg in serum

HBV: Hepatitis B virus; BCP: Basal core promoter; HBsAg: Hepatitis B surface antigen.

Kim *et al*[21] observed the role played by the precore mutation G1896A and the BCP double mutation A1762T/G1764A in the progression of HBV-related liver diseases. BCP double mutation A1762T/G1764A may prevent the formation of HBeAg by inserting a premature stop codon into the concerned ORF or may enhance the pgRNA transcription by evacuating of the nuclear-receptor-binding motif and led to the feeble immune response that causes HCC[22,23]. From transfection studies, it was observed that HBeAg negativity caused by BCP double mutation A1762T/G1764A was linked with serious liver disease[24].

BCP mutant may increase the host immune response towards HBV-infected hepatocytes by reducing circulating HBeAg, leading to apoptosis and regeneration of hepatocytes, which contribute to liver injury[22,25]. In an *in vivo* study, A1762T/G1764A mutant was related to cytoplasmic positioning of intracellular HB core antigen, which were strongly linked with active necroinflammation of hepatocytes [26]. The A1762T/G1764A double mutation was found to influence the amino acid sequence of X gene of HBV, upregulating S-phase kinase-associated protein 2 and downregulating its target, cyclin kinase inhibitor p21. The combined action of the above-mentioned events may lead to inhibition of precore mRNA and an increase in pgRNA transcription, followed by increased viral replication, which may finally lead to HCC[27].

It was found that A1762T/G1764A mutation usually appeared almost 10 years before the identification of HCC and could be an early episode in hepatocarcinogenesis[28]. The BCP double mutation A1762T/G1764A was found to be related to more progression of HCC[29] independently of HBV genotype[24,30] and viral load[30,31]. The mutations A1762T, G1764A, and A1762T/G1764A played a vital part in the progression of chronic HBV infection to HCC[25,32,33]. In a study from East Kalimantan, Indonesia, BCP mutations, mainly the double mutation A1762T/G1764A and T1753V, were linked with the risk of advanced liver disease (ALD)[34].

HCC patients with genotype C had more chance of carrying the A1762T/G1764A double mutation than those with genotype B[35]. In China, A1762T/G1764A mutation is a familiar mutation and found generally in HBeAg-negative chronic hepatitis B patients with genotype C and may be related to the progression of chronic HBV infection[36]. In a study on HBV-infected children in China, the frequency of BCP mutations in genotype C samples was significantly higher than that of genotype B samples[37]. From a Taiwanese study, genotype C and A1762T/G1764A mutations may be the cause of liver cirrhosis and could act as molecular markers for the diagnosis of the clinical symptoms of chronic HBV disease [38]. From a North Indian study, genotype-D-infected patients had more advanced liver disease and higher frequency of BCP mutations A1762T/G1764A than patients infected with genotype A had[39].

A1762T/G1764A mutation also acted as a significant viral marker for cirrhosis development[40]. From a study of chronic HBV infection, it was observed that A1762T/G1764A mutation was an independent factor for advancement to cirrhosis instead of HCC[41]. Table 1 showed various qualitative and quantitative factors associated with HBV-related HCC.

1753 mutation

1753V (V represents C/A/G) is one of core promoter mutations that is associated with advanced liver diseases like cirrhosis and HCC. In a study from East Kalimantan, Indonesia, BCP mutations, mainly the double mutation A1762T/G1764A and T1753V, were linked with the risk of ALD[34]. BCP T1753V mutation was associated with an increased risk of HCC[28]. The T1753C mutation was treated as one of the hot spot mutations of the protein-X encoding gene[28] and was shown to increase the transactivation and antiproliferation activity of protein X in HBV genotype D and led to carcinogenesis[42]. In a study on HBeAg-positive genotype C1 carriers, 1753 mutation was detected as a prognostic factor for HCC [28].

In a study from China, T1753V mutation in combination with BCP A1762T/G1764A double mutation was assumed to be related to HCC progression, notably in HBV/C1-infected patients[43]. In a Mongolian study, patients infected with HBV/D, C1752, and/or T1753V mutation were found to be related to HCC[42]. In a study from Southern Guangxi, China, BCP mutations were associated with HCC risk and the add-on impacts of A1762T/G1764A double mutations and T1753V mutations along with dietary aflatoxin B1 exposure for HCC risk[44]. In a study from Chiba, Japan, preC mutation (A1896) and CP mutations (T1753V and 1754) were observed more often in FH patients than in acute self-limited hepatitis patients[45]. In a study from Taiwan, the frequency of genotype C and frequency

Table 2 Prevalent basal core promoter mutations and their clinical importance

Nucleotide position	Clinical applicability
A1762T + G1764A (BCP double mutant)	Chronic hepatitis; FH; decline in HBeAg formation and enhanced virus replication; high ALT; found in patients with HBV genotypes who have 1858C (*i.e.*, genotype C)
A1762T	HBeAg seroconversion; histological inflammation
A1764A	Marginally decline in replication ability of the virus
1653T	Jointly with 1762T + 1764A in FH and HCC patients
1753-1757	Jointly with the 1762T + 1764A mutation in FH and HCC patients; ALT level changes and histological changes
1764A/T + 1766A/G	Jointly with 1810T + 1811T double mutation in active and inactive disease; 1762A1766A mutation, jointly with 1762T mutation, was observed in FH and HCC patients; 1764T + 1766G mutation was observed in a patient with recurrent FH after liver transplantation, although was not observed in FH patients
1766T + 1768A	FH; along with A1762T + G1764A, in recurrent hepatitis patients following liver transplantation; jointly with G1764A in HBeAg-negative asymptomatic carrier; exacerbation of HBV infection

ALT: Alanine aminotransferase; BCP: Basal core promoter; FH: Fulminant hepatitis; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma.

of various mutations (A1762T/ G1764A, T1753C, T1766/A1768, and A1896) increased and the frequency of various mutations (T/G1752, T1773, G1799, and C1858) declined with advancing liver diseases[38].

T to C/A mutation at nt 1753 in BCP and G to A mutation at nt 1899 in the precore region were more frequently found in liver cirrhosis patients than in the other clinical states[46]. 1653T/1753C mutation may be observed as a marker for hepatic failure[47].

1757 mutation

1757 mutation is one of the core promoter mutations. It is associated with the severity of liver disease. G1757 mutation and BCP A1762T/G1764A double mutation are directly linked with the high level of serum HBV DNA[48]. Various CP mutations (M1386, T1485, B1499, A1613, T1653, G1727, A1757/T1764/G1766, G or C1753, T1773, and T1766/A1768) are related to progression to HCC. These mutations individually and/or in combination are prognostic markers of HCC[49-51].

1764/1766 mutation

1764/1766 mutation is one of the core promoter mutations. It is associated with patients with severe liver disease. 1762/1764/1766 and 1753/1762/1764/1766 mutations showed increased viral replication and decreased HBeAg expression compared with 1762/1764 mutations alone; however, 1753/1762/1764 mutant showed only a small decrease in HBeAg expression like that of 1762/1764 mutant. So, core promoter mutations other than mutation (1762 and 1764) can have an influence on viral DNA replication and HBeAg expression[52].

1809-1812 mutation

1809-1812 mutations (Kozak region mutations) are responsible for HBeAg negativity and are found mainly in genotype A; subgenotype A1. Several mutations can reduce HBeAg expression: 1762T1764A mutation at the transcriptional level; 1809-1812 mutations at the translational level and 1862T mutation at the post-translational level. A1762T/G1764A mutation and 1809-1812 mutations cumulatively reduce HBeAg expression[53]. In a study from Kenya, mutations in the Kozak region (1809-1812) or precore start codon (1814-1816) were responsible for HBeAg-negativity in patients with subgenotype A1[54]. Table 2 shows the clinical potency of frequently found BCP mutations.

HBV PRECORE MUTATIONS AND RELATEDNESS WITH GENOTYPE AND ADVANCEMENT OF LIVER DISEASE

Precore region

The precore gene spans the length from nt 1814 to 1901. This region has the start site for synthesis of pgRNA, direct repeat 1, and RNA encapsidation signal. The precore region plays a crucial role in viral replication. The presence of mutations in this region disrupts viral replication and results in the formation of defective replicative intermediates. These replicative intermediates (which are not encapsidated) in hepatocytes, increase the chances for integration of HBV DNA into chromosomal DNA and lead to advanced liver disease like HCC[55].

Table 3 Various precore/core promoter mutations and their clinical outcomes

Mutation type	Clinical symptoms	Particular mutation
PC/CP mutations	HBeAg-negative. Hepatitis	T1753C[52], A1762T[82], G1764A[82], C1766T[52], T1768A[52], G1896A[59,83], G1899A[59,83]
	HCC	C1653T[63], T1753C[63], A1762T[63,65,84], G1764A[63,65,84], G1896A[63], G1899A[63]
	Fulminant hepatitis	A1762T[85], G1764A[85], G1862T[71,85], G1896A[85]

PC: Precore; CP: Core promoter; HBeAg: Hepatitis B e antigen; HCC: Hepatocellular carcinoma.

1896 mutation

1896 mutation is the one of most important precore mutations. It causes HBeAg negativity and advanced liver diseases like HCC. Moreover, the precore mutation G1896A and BCP double mutation A1762T/G1764A play a significant part in the progression of HBV-related liver diseases[21]. The precore region mutation G1896A is one of the most frequently observed mutations in ALD and chronic HBV patients[56].

The G1896A mutation gives rise to a premature stop codon and reduces HBeAg synthesis. This mutation stops HBeAg formation by checking the translation of HBeAg, and the existence of a stop codon mutation may be a cause of immune evasion[56,57]. The most commonly observed precore mutation, G1896A, stops HBeAg formation by substitution of a tryptophan residue at amino acid position 28 with a premature stop codon[58]. This mutation was observed in more than 50% of individuals with chronic hepatitis B in Asia and the Mediterranean area[58]. This mutation suppresses HBeAg production, thus causing increased HBV replication and liver disease progression[21,59,60]. Precore 1858-1896 mutations are related to high serum HBV DNA levels[61].

In a meta-analysis among Asians, G1896A mutation was found to be related to a greater chance of HCC and liver disease severity[62]. Various mutations and combinations, notably at nt 1762/1764 and nt 1896/1899 in the BCP-preC region were observed to induce HBeAg negativity, liver function impairment, and HCC progression[63]. In some of the studies listed below, G1896A mutation was associated with less severity of liver disease. In a study from Taiwan, the G1896A mutation was linked with a low HCC risk[64].

1858 mutation

1858 mutation is one of the precore mutations and is related to greater liver disease severity. In a study from Vietnam, genotype-C-infected patients contained a high rate of C-1858 mutation (70%) and it could play a decisive role in generating severe chronic liver disease[65].

1858C mutation was found to be related to A, F and H genotypes, and 1858T mutation with B, D and E genotypes[66]. C-1858 strains may be more infectious, but more investigations are required to determine the pathogenesis of this strain[67]. High serum HBV DNA level was related to precore 1858-1896 mutations. These BCP/precore mutations may act as a prognostic marker for HBV DNA increase [61]. TCC at positions 1856 to 1858 of the precore region of HBV might cause more severe liver disease than other HBV strains with genotype C[68].

The presence of C rather than T at nt 1858 prevents G1896A mutation because the above-mentioned mutations diminish the stem-loop structure of the RNA encapsidation signal. So, CCC polymorphism and 1896 mutation are mutually exclusive[69]. HBV-infected patients with C at nt 1858 have a greater prevalence of core promoter mutations, while patients infected with T at position 1858 have only precore stop codon mutations[70].

1862 mutation

1862 mutation is one of the precore mutations and is one of the leading causes of HBeAg negativity and advanced liver diseases. In a study on Chinese patients, G1862T mutation caused an amino acid change in codon 17 of the precore protein; a part of the signal peptidase recognition motif. G1862T mutations were found only in genotype-B-infected patients. These variants have reduced capacity to form HBeAg and the absence of or reduced HBeAg may be a causative factor in fulminant disease. G1862 mutation is observed more in FH patients than in chronic carriers[71].

G1862 mutation is mainly found in HBV genotype A (sub-genotype A1). G1862T mutation is associated with HBeAg negativity and low viremia level in genotype-A-infected patients[21]. Saha *et al* [72] reported in an East India study that all HBV/A1 isolates had G1862T mutation regardless of HBeAg status. So, the G1862T variant might present a natural mutation in HBV/A1 isolates.

1888 mutation

1888 mutation is one of the precore mutations and is found only in subgenotype A1 and causes HBeAg negative serostatus. The linked loci 1809-1812, 1862 and 1888 are observed regularly in subgenotype A1 and they cause inhibition of HBeAg expression[53]. The 1888A mutation is related to HBV subgenotype

A1 and it expresses a start codon upstream from the core ORF and can influence the core protein expression, resulting in lowering of viral level[73].

1915 mutation

1915 mutation is one of the precore mutations and is reported as one of the HCC survival mutations. Xie *et al*[74] observed five different mutations in the preC/C region (1915, 2134, 2221, 2245 and 2288) and these mutations act as independent predictors of HCC survival. Various newly reported preC/C promoter mutations (1690A, 1695A/T/G, 1700A/C, 1703C, 1850A and 1915A/G) were observed in HBV-infected isolates of Punjab state (North India)[75].

Scanty/rare mutations

Some rare mutations in preC/C promoter region are described below, which contribute to the severity of liver disease. Mutations in the enhancer II/BCP regions (C1653T, T1674C/G, T1753V, A1762T/G1764A and C1766T/T1768A) and mutations in the precore region (G1899A, C2002T, A2159G, A2189C and G2203A/T) are significantly associated with an increased risk of HCC[76]. Various newly reported individual/combination mutations of the X/precore region in HBV genotype D1 act as markers of HCC[50]. These mutations are T1673/G1679, G1727, C1741, C1761, A1757/T1764/G1766, T1773, T1773/G1775 and C1909[50]. Numerous core promoter mutations, 1653T, 1753V, A1762T, G1764A, 1766T and 1768A, have been observed regularly in HCC patients[77].

In an *in vitro* study, individual mutations in the BCP/precore region, T1753V (C/A/G), C1766T, T1768A, G1862T and G1899A, were linked with enhanced viral replication and/or reduced HBeAg expression and related with acute liver failure in some instances[16,52,71,78]. Ren *et al*[79] observed that individual mutations, T1753V (C/A/G), A1762T, G1764A, G1896A and G1899A, were more regularly detected in hepatitis-B-related acute-on-chronic liver failure (ACLF) than chronic hepatitis B (CHB) patients. Patients with precore mutations have a greater risk of a fatal outcome.

Tsai *et al*[80] reported that triple BCP mutation 1762/1764/1766 was related to the maximum increase in pgRNA transcription, while 1762/1764 double mutation was not so effective. T1846 and A/G1913 mutations are linked with ACLF in patients infected with genotypes B and C[81]. In Table 3 various precore/CP mutations and their correlation with clinical outcomes are shown.

DISCUSSION AND FUTURE PERSPECTIVES

HBV genotypes and viral mutations have a fair connection for various clinical conditions of CHB and this information should be utilized to predict likely clinical course, *e.g.*, lengthy duration of HBeAg phase and more risk of progression to cirrhosis in genotype C and increased risk of HCC in genotypes C and F. There is evidence to confirm the role of genotypes and subgenotypes in pathogenesis and clinical symptoms of HBV infection. HBeAg seroconversion is delayed in patients infected with genotype C compared to other genotypes[86]. So, more research is needed about the pathogenicity of genotypes E, F and H.

In addition, preS/S region mutations are related to vaccine failure, immune escape, occult HBV infection, and HCC occurrence. P region mutations may create drug resistance to nucleoside analog antivirals. Mutations in the preC/C region are associated with HBeAg negativity, immune escape, and persistent hepatitis. Mutations in the X region play a vital part in developing HCC[86]. Mutations in various parts of the HBV genome could be the reason for unwanted clinical outcomes or evasion of detection by various diagnostic procedures, thus it becomes necessary to detect these mutations for proper evaluation of patients.

Revill and Locarnini[87] observed and recommended that in patients infected with genotype B or C, BCP mutation is a significant biomarker of the risk of cirrhosis. So, identification and quantification of BCP mutants should be used for proper treatment in Asian CHB patients. More work on the importance of these mutations in other genotypes is suggested.

HBV genotypes and mutations act as useful viral biomarkers for the prognosis of disease progression and also assist clinicians to diagnose patients who can be treated mostly from interferon therapy. In the future, clinical trials classified by different genotypes/mutants should be adopted and it is essential to apply individualized treatment regimens for CHB patients.

For population health prospects, proper observation of the pervasiveness of vaccine escape and S escape mutations is needed and more research will be required into vaccines that remain effective against mutant strains of HBV. Identification of resistance profile is important in deciding the exact antiviral agent to start therapy.

CONCLUSION

HBV has more genomic diversity and various HBV mutants are significantly related with response to

antiviral therapy, vaccine escape, diagnostic failure, liver fibrosis, liver cirrhosis, and HCC development. Understanding the correlation between various mutations and the clinical manifestations of HBV infection should lead to advances in diagnostic strategies and therapeutic directions.

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ORIGINAL ARTICLE

Basic Study

Assessment of periportal fibrosis in *Schistosomiasis mansoni* patients by proton nuclear magnetic resonance-based metabonomics models

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Abstract

BACKGROUND

The evaluation of periportal fibrosis (PPF) is essential for a prognostic assessment of patients with *Schistosomiasis mansoni*. The WHO Niamey Protocol defines patterns of fibrosis from abdominal ultrasonography, ^{1}H-nuclear magnetic resonance (NMR)-based metabonomics has been employed to assess liver fibrosis in some diseases.

AIM

To build ^{1}H-NMR-based metabonomics models (MM) to discriminate mild from significant periportal PPF and identify differences in the metabolite profiles.

METHODS

A prospective cross-sectional study was performed on schistosomiasis patients at a University Hospital in Northeastern Brazil. We evaluated 41 serum samples from 10 patients with mild PPF (C Niamey pattern) and 31 patients with significant PPF (D/E/F Niamey patterns). MM were built using partial least

squares-discriminant analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) formalisms.

RESULTS

PLS-DA and OPLS-DA resulted in discrimination between mild and significant PPF groups with R2 and Q2 values of 0.80 and 0.38 and 0.72 and 0.42 for each model, respectively. The OPLS-DA model presented accuracy, sensitivity, and specificity values of 92.7%, 90.3%, and 100% to discriminate significant PPF. The metabolites identified as responsible by discrimination were: N-acetylglucosamines, alanine, glycolaldehyde, carbohydrates, and valine.

CONCLUSION

MMs discriminated mild from significant PPF patterns in patients with *Schistosomiasis mansoni* through identification of differences in serum metabolites profiles.

Key Words: Metabolomics; Portal hypertension; *Schistosoma mansoni*; Biomarkers; Neglected disease; Nuclear magnetic resonance

Core Tip: In this study, we demonstrated a metabolic signatures and metabolic pathway disturbances that allowed to discriminate mild from significant periportal fibrosis in 41 patients with *Schistosomiasis mansoni*. Partial least squares-discriminant analysis (PLS-DA) and OPLS metabonomics models provided a clear separation between the groups. PLS-DA model presented accuracy, R2 and Q2 values equal to 0.85, 0.80 and 0.38, respectively, while OPLS model had R2 and Q2 values equal to 0.717 and 0.417, respectively. We also identified some metabolites responsible by discrimination which are associated with changes related to liver function and amino acids metabolism.

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INTRODUCTION

Schistosomiasis is a neglected disease that still occurs around the world and affects about 240 million people in 78 countries[1]. In Brazil, it is caused by *Schistosoma mansoni*. It has been considered an endemic disease in the state of Pernambuco, with cases reported in 102 of 185 cities[2,3].

Periportal fibrosis (PPF), known as Symmers' fibrosis, is induced by helminth eggs deposition in the portal vein and its branches. This fibrosis can extend to the peripheral intrahepatic branches without promoting hepatocyte necrosis, making it one of the causes of non-cirrhotic portal hypertension[4].

Ultrasonography (US) scan is used for diagnosis and assessment of PPF by the Niamey-Belo Horizonte Protocol, the WHO Standard Protocol. This protocol classifies 6 PPF patterns from A (no-fibrosis) up F (very advanced fibrosis), plus mixed patterns, such as the D/C or E/C patterns[4-6].

Although the US exam enables PPF diagnosis and measurement, there are limitations for its use, including the inter-observer variation and the low sensitivity to the diagnosis of initial forms of the disease, especially if the examiner has no experience in applying the Niamey-Belo Horizonte protocol. Additionally, the device can be difficult to access in some poor regions. Due to these difficulties, alternative strategies are being studied. Some serum biomarkers, alone or in association (indexes), have been used for this purpose. Some authors reported an inversely proportional relationship between platelet count and PPF pattern, as well as a directly proportional relationship between liver enzymes serum levels and PPF patterns[5,6].

Metabonomics is an area of knowledge that uses multivariate statistical formalisms applied to spectra data of biofluids to obtain a multiparametric response to external stimuli, such as pathogens[7,8]. The biofluid, properly stored, can be analyzed at a center distant from the collection site. Batista *et al*[9] used ^{1}H-nuclear magnetic resonance (NMR)-based metabonomics for liver fibrosis assessment in patients with chronic hepatitis C. The method proved to be useful in the diagnosis of significant and advanced fibrosis in these patients. Gardini *et al*[10] developed the profile of the serum metabolome of patients with hepatocellular carcinoma in early and advanced stages. They found that ^{1}H-NMR metabolomics profiling could discriminate early from advanced hepatocellular carcinoma. The multivariate statistical

formalisms most commonly used in metabonomics assays are: principal components analysis (PCA) for exploratory analyses, since it does not depend on class information and investigates if there are outlier samples; and partial least square-discriminant analyses (PLS-DA) or orthogonal PLS-DA (OPLS-DA). which use the class information to build metabonomics models (MM) that discriminate among samples from different groups[11]. In the present study, we aimed to build ^{1}H-NMR-based MM to discriminate mild from significant PPF in patients with *Schistosomiasis mansoni* and identify differences in the profiles of the endogenous metabolites.

MATERIALS AND METHODS

Study design

This is a phase II diagnostic validation test, a cross-sectional study performed with adult patients who were diagnosed with *Schistosomiasis mansoni*, aiming to assess PPF patterns by ^{1}H-NMR-based metabonomics.

Patients

Patients aged 18 years or over diagnosed with *Schistosomiasis mansoni* were included from the Schistosomiasis Clinic of the Gastroenterology Service of the Hospital das Clínicas, Universidade Federal de Pernambuco (Recife, Pernambuco, Brazil), between March and December 2019. Schistosomiasis diagnosis was based on the clinical history of contact with water sources in endemic areas, report of previous treatment with praziquantel, and associated with finding of PPF by US scan. Exclusion clinical criteria were: presence of fatty liver disease, cirrhosis or hepatocellular carcinoma, portal vein thrombosis, HIV, hepatitis B or C virus coinfection, or history of drug-induced liver injury or alcohol abuse.

Abdominal ultrasonography scan

All patients were submitted to US scan after overnight fasting of about 8 h, by the same examiner. According to the Niamey-Belo Horizonte Protocol, PPF pattern was defined as follows: C (peripherical fibrosis), D (central fibrosis), E (advanced fibrosis) and F (very advanced fibrosis) patterns. Patients without or with a doubtful PPF (A and B pattern) were excluded of the study. All US exams were performed using a US Siemens Acuson S2000 instrument equipped with a 6C1 Ultrasound probe (Siemens Medical Solutions, Mountain View, CA, United States).

Liver function tests

Blood samples were collected from a peripherical vein after US scan. Serum was obtained after centrifugation (3500 *rpm*) using a Centurion-Laborline equipment. Liver function tests, including alanine aminotransferase and aspartate aminotransferase, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), and glycemia, were carried out using Wiener Lab® (Wiener Lab Group, Santa Fé, Argentina) kits in a Wiener Lab® autoanalyzer (Wiener Lab Group, Santa Fé, Argentina). Part of the samples were stored at minus 40 °C until the NMR analysis.

Statistical analyses

To investigate the distribution of demographic and clinical or laboratory data between groups, univariate tests were performed using GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, United States) with unpaired Student's *t*-test, Mann-Whitney, and Fisher's exact as appropriate. A P value < 0.05 was set as the level of statistical significance.

^{1}H-NMR spectra and Metabonomics modelling

All ^{1}H-NMR spectra were recorded using a VNMRSYS400 spectrometer operating at 400 MHz. After thawing, serum samples were prepared by mixing 400 µL of serum and 200 µL of D_2O and placing in NMR tubes of 5 mm id. ^{1}H-NMR spectra were performed using a sequence of radiofrequency pulses with presaturation of the water signal hyphenated to the Carr-Purcell-Meiboom-Gill pulse sequence, which was employed as a T2 filter. The following parameters were used: spectral window of 6.4 kHz, saturation delay of 2.0 s, acquisition time of 1.704 s, 90° RF pulse, temperature of 27 °C, 88 cycles, tau equal to 0.0004 s, bigtau equal to 0.07 s, and 128 scans. The line broadening used was 0.3 Hz. Baseline and phase distortions were corrected manually. The signal attributed to the methyl group of lactate (δ 1.33 ppm) was used as a chemical shift reference. Using MestreNova 9.0 software, the region between δ 4.004 and 0.772 ppm was binned into 808 bins (each 0.004 ppm-wide). The matrix was built with 41 rows (cases) and 809 variables (bins of ^{1}H-NMR spectra plus class variable), and then was submitted to multivariate analysis. The models based on PCA, PLS-DA, and OPLS-DA were constructed using MetaboAnalyst online platform 4.0[12,13]. In the preprocessing step, each sample was normalized by sum (cumulative intensity of the spectrum). This was performed to compare the spectral data, avoiding

problems with sample dilutions, for example[14]. In addition, data were pre-processed using autoscaling. The validation of the PLS-DA and OPLS-DA models was based on two methods: (1) the leave-one-out cross validation method (LOOCV), where the optimal number of latent variables for the PLS-DA model was determined, thus providing the basis for the computation of the predictive ability (Q2), determination coefficient (R2), and the classification accuracy of the model; and (2) the permutation test, which made 2000 permutations of the class label to verify the accuracy of metabonomics models. PLS-DA and OPLS-DA models provided a quantitative measure of the discriminating power of each spectral bin. Variable importance in the projection (VIP) score was used. VIP is a weighted sum of squares of the PLS loadings. These weights are based on the amount of explained variance of the dependent variable in each PLS dimension. A VIP score cut-off equal to 1 was used. Discriminatory signals were attributed to metabolites using Human Metabolome Database platform and also based on the literature[15-18]. Accuracy, sensitivity, and specificity values were obtained from a confusion matrix that was constructed considering classification of OPLS-DA model.

RESULTS

Forty-four patients were selected, but three were excluded because their samples proved to be outliers. Thus, 41 patients with PPF were included in the study: 10 patients with C, 12 patients with D, 17 patients with E, and 2 patients with F patterns, according to the Niamey-Belo Horizonte Protocol. These patients were divided into two group: mild PPF (C pattern) and significant PPF (D/E/F patterns)[19]. Table 1 shows clinical and demographic data of the patients.

Figure 1 shows a typical ^{1}H-NMR spectrum of serum obtained in the study with assigned peaks. Exploratory analyses by PCA failed to indicate separation between the groups (data not shown). Thus, MM were developed using supervised methods: PLS-DA and OPLS-DA formalisms. Figure 2 shows a score plot (A) and the performance of MM constructed using PLS-DA formalism (B). Regarding accuracy, the best performance was achieved when four latent variables were used, resulting in accuracy, R2 and Q2 values equal to 0.85, 0.80 and 0.38, respectively.

Figure 3 shows variables importance for projection (VIP) score plot (A) and the permutation test from PLS-DA model which presented *P* value equal to 0.0245 after 2000 classes permutations (B). The spectral region responsible for discrimination was between δ 1.975 and δ 2.011 ppm, which is attributed to the methyl group of N-acetylglucosamines. The serum level of N-acetylglucosamines is higher in the group with significant PPF (Significant PPF) than in the group with mild PPF (Mild PPF). In addition to this region, three more discriminatory bins can be observed: δ = 3.544, 3.692, and 3.808 ppm, which were assigned to carbohydrates. According to the VIP score plot, the serum level of these carbohydrates is higher in the Mild PPF group. The VIP score full table presents other discriminatory bins, such as δ 1.502 ppm (VIP score = 1.98) and δ 3.492 ppm (VIP score = 1.83), which were assigned to alanine and glycolaldehyde, both higher in the Significant PPF group.

Figure 4 shows a score plot and the results of permutation test of metabonomics models using OPLS-DA formalism (B). The OPLS-DA MM presented R2Y and Q2 values equal to 0.717 and 0.417, respectively, with *P* values from permutation test less than 0.01. We identified four discriminatory bins, as follows: δ 1.030 ppm, assigned to valine; δ 1.046 ppm (not assigned); δ 1.446 ppm attributed to alanine; and δ 3.692 ppm assigned to carbohydrates. The serum levels observed for valine, carbohydrates, and the unidentified metabolite (signal at δ 1.046 ppm) were higher in the Mild PPF group, while the alanine serum level was higher in the Significant PPF group.

Table 2 presents a summary of metabolites that differentiated the Mild PPF samples from the Significant PPF samples, as well as the chemometric formalism employed, the chemical shift (δ) of each metabolite, and the group in which these metabolites had higher serum level.

Table 3 shows the confusion matrix obtained from the OPLS-DA MM. Accuracy, sensitivity, specificity, positive predictive and negative predictive values are equal to 92.7%, 90.3%, 100%, 100% and 76.9%, respectively.

DISCUSSION

PPF is a mark of *Schistosomiasis mansoni* disease. Assessment of PPF intensity is crucial to determine disease morbidity and prognosis. In addition, significant PPF is associated with non-cirrhotic portal hypertension and its consequences. Generally, US scan and serum biomarkers have been used for PPF assessment in schistosomiasis patients[2,20]. Among serum biomarkers for PPF assessment, liver enzymes and platelet count alone or combined with ALP, as in the Coutinho-index, have been given importance in the literature[2,6,7]. In agreement with these authors, laboratory data found in this study indicate a higher serum level of GGT in particular, and lower platelet count in patients with significant PPF. Köpke-Aguiar *et al*[21] also reported higher serum levels of GGT in the more severe cases. Pereira *et al*[22] and Lambertucci[23] also reported lower platelets count, as well as increased spleen size in more severe *Schistosomiasis mansoni* disease.

Table 1 Demographic and laboratorial characteristic of 41 patients with *Schistosomiasis mansoni*, Pernambuco, Brazil, 2020

Characteristic	Total	Mild PPF (C pattern)	Significant PPF (D/E/F pattern)	*P* value
n	41	10	31	-
Age (yr)	57 (18-80)	48.1 (18-75)	57.2 (25-80)	0.0865[a]
Sex				0.4820[b]
Male	17 (41%)	5 (40%)	12 (39%)	
Female	24 (59%)	5 (50%)	19 (61%)	
AST (U/L)	29.0 ± 2.6	24.3 ± 1.9	30.0 ± 2.9	0.3328[c]
ALT (U/L)	29.0 ± 2.2	26.2 ± 4.5	30.0 ± 3.2	0.4584[c]
ALP (U/L)	262 ± 33	329 ± 113	238 ± 22	0.6379[c]
GGT (/LSN)	62 ± 12	35 ± 16	71.2 ± 14.0	0.0013[c]
Platelets count (/mm^3)	131 ± 12	218 ± 15	102 ± 11	0.0001[c]
Total Cholesterol (mg/dL)	169.0 ± 4.6	174.0 ± 7.2	167.8 ± 5.6	0.4626[c]
HDL (mg/dL)	45.7 ± 2.0	49 ± 5.7	44.6 ± 2.0	0.4863[c]
LDL (mg/dL)	105.0 ± 3.8	108 ± 4.7	104 ± 4.8	0.3760[c]
Glucose (mg/dL)	93.6 ± 5.2	97 ± 14	92 ± 5.1	0.9451[c]

[a]Unpaired *t* test.
[b]Fisher's exact test.
[c]Mann-Whitney test.
Data presented as Mean values ± standard deviation. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; HDL: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein cholesterol; PPF: Periportal fibrosis.

Table 2 Metabolites responsible for discrimination between mild from significant periportal fibrosis of 41 patients with *Schistosomiasis mansoni*, Pernambuco, Brazil, 2020

Metabolite	Chemical shift (δ/ppm)	Metabonomics formalism	Higher serum level
Valine	1.030	OPLS-DA	Mild PPF
No identified	1.046	OPLS-DA	Mild PPF
Alanine	1.446 and 1.502	PLS-DA and OPLS-DA	Significant PPF
N-acetylglucosamine	1.975 up to 2.011	PLS-DA	Significant PPF
Glycolaldehyde	3.492	PLS-DA	Significant PPF
Carbohydrates	3.544; 3.692; and 3.808	PLS-DA and OPLS-DA	Mild PPF

OPLS-DA: Orthogonal projections to latent structures discriminant analysis; PLS-DA: Partial least squares-discriminant analysis; PPF: Periportal fibrosis.

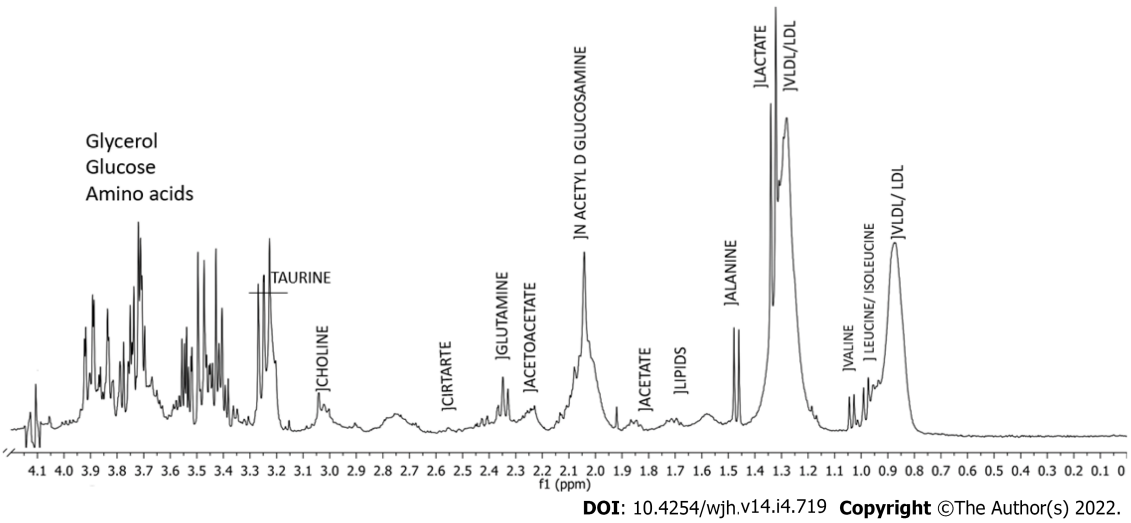
Significant PPF in the schistosomiasis patients is induced by increase in number of eggs in the intrahepatic portal veins due repeated infections and by the host's exacerbated immune response[24]. The presence of adult worms in mesenteric and portal vessels, as well as the presence of their eggs, promotes immunological stimulation and induces primary splenomegaly by reticuloendothelial system hyperplasia, which leads to pancytopenia by hypersplenism[19,22]. Splenomegaly also causes blood hyperflow in the splenic vein, which contributes to presinusoidal portal hypertension[25]. In addition, the changes in liver hemodynamics, triggered by portal hyperflow, could promote increase of GGT serum level, as observed in our study[26].

Usually, metabonomics studies begin with exploratory analyses by PCA. However, this initial analysis failed to discriminate between the groups. Therefore, discriminant analyses formalisms were employed, resulting in efficient separation between mild PPF and significant PPF groups. Serum levels of valine (δ 1.030 ppm) and alanine (δ 1.446 and 1.502 ppm) observed in the spectral data suggest that the changes in portal vein flow could trigger disorders of amino acids metabolism in hepatocytes, as reported by Li *et al*[27]. Changes in amino acid serum levels were important for discrimination in both the PLS-DA and OPLS-DA MM. These findings are in agreement with studies that correlate changes in serum levels of these amino acids in response to liver fibrogenesis caused by schistosomiasis in mice[15,

Table 3 Confusion matrix-orthogonal projections to latent structures discriminant analysis metabonomics model (mild *vs* significant periportal fibrosis) of 41 patients with *Schistosomiasis mansoni*, Pernambuco, Brazil, 2020

		Classification from WHO Niamey Protocol		
		Significant PPF	Mild PPF	*P* value[a]
Metabonomics Model	Significant PPF	28	0	< 0.0001
	Mild PPF	3	10	

[a]Fisher's exact test. 92.7% accuracy, 90.3% sensitivity, 100% specificity. PPV and NPV value equal to 100% and 76.9%, respectively.
PPF: Periportal fibrosis; WHO: World Health Organization.



DOI: 10.4254/wjh.v14.i4.719

Figure 1 Typical ^{1}H-nuclear magnetic resonance spectrum (400 MHz, D_2O, presaturation-Carr-Purcell-Meiboom-Gill) of serum from a patient with *Schistosomiasis mansoni*, Pernambuco, Brazil, 2020. Integration areas under the signal are associated with the concentration of metabolites weighted by the number of hydrogen nuclei in each chemical environment. Some assignments are presented in the spectrum.

28]. Balog *et al*[29] also reported an association between valine and alanine serum levels with the disease progression.

An increase of N-acetylglucosamines serum levels was observed in the significant PPF group, while the carbohydrate serum level was higher in the mild PPF group. Glucosamines are products of glucose metabolism, which are capable of suppressing the production of metalloproteinases. Therefore, the N-acetylglucosamines and glucose serum levels observed could be associated with liver damage, which requires glucose consumption and production of N-acetylglucosamines[30].

In clinical practice, the monitoring of patients with *Schistosomiasis mansoni* is done by US scan. Hence, it is necessary to transfer patients from rural zone to hospital unit or to bring the device to the field. In addition, an experienced examiner is necessary, since the US scan is operator-dependent.

The metabolic profile presented in this study can be strategic for monitoring the patients in endemic regions through blood samples collected and transported to a reference laboratory. ^{1}H-NMR-based metabonomics produce a "metabolic fingerprint", providing systemic metabolic information about patients. It can help to identify those with more severe forms of schistosomiasis. The main limitation of the study was the sample size and the disproportionate PPF pattern groups.

In the present study, we used ^{1}H-NMR-based metabonomics from the serum of patients with *Schistosomiasis mansoni* to discriminate those with highest intensity of PPF. Moreover, the chemometric formalisms used enabled the identification of some metabolites associated with the discrimination, such as alanine, glycolaldehyde, and N-acetylglucosamines, which presented higher serum levels in the significant PPF group, while valine and carbohydrates presented lower serum levels in the most severe cases.

CONCLUSION

The ^{1}H-NMR-based metabonomics models were able to discriminate mild from significant PPF patterns in patients with *Schistosomiasis mansoni* through identification of differences in serum metabolites

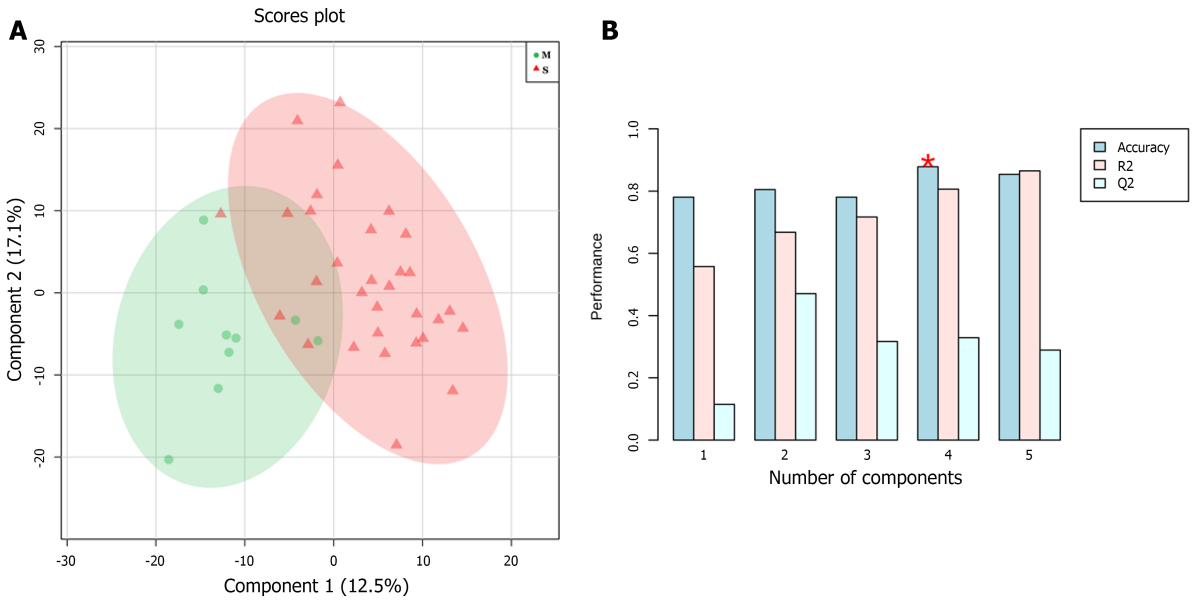


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Figure 2 Results of partial least squares-discriminant analysis modelling using 41 samples of patients with *Schistosomiasis mansoni*, Pernambuco, Brazil, 2020. A: Score plot–significant (red) and mild (green) PPF patterns; B: Performance of metabonomics models (Red star: Best number of components for modelling).

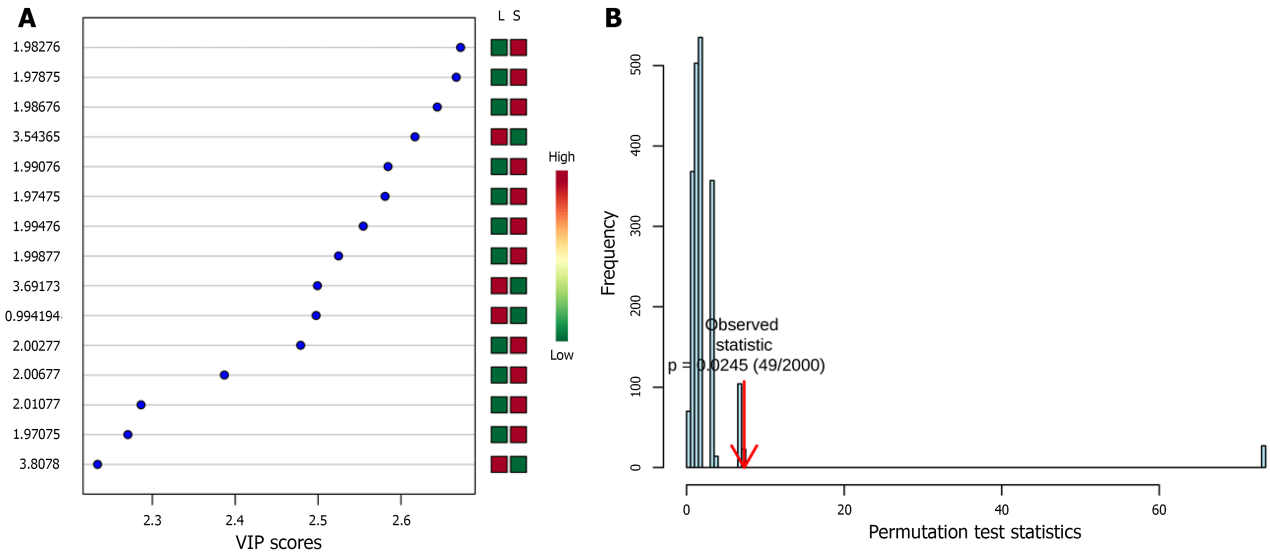

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Figure 3 Results of partial least squares-discriminant analysis modelling using 41 samples of patients with *Schistosomiasis mansoni*, Pernambuco, Brazil, 2020. A: Variable importance in the projection score plot; B: Permutation test statistic at 2000 permutations with observed statistic of the model prediction accuracy with *P* value = 0.0245.

profile. We intend to expand the study in the coming years in order to confirm the results and best understand the metabolic pathways associated to observed discrimination.

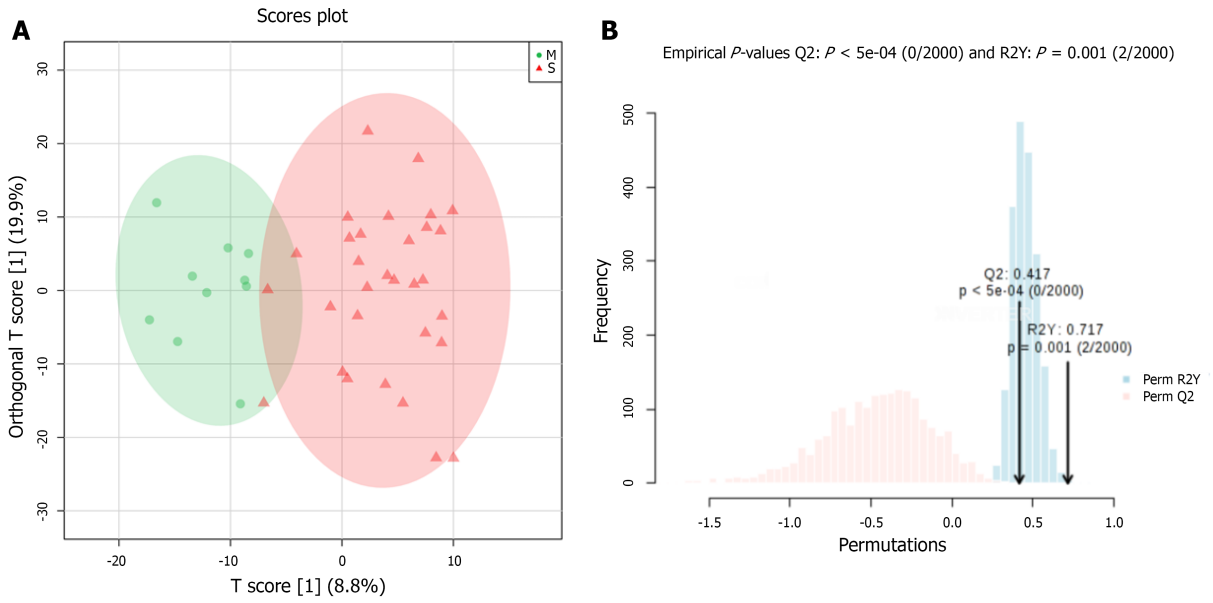

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Figure 4 Results of orthogonal projections to latent structures discriminant analysis modelling using 41 samples of patients with *Schistosomiasis mansoni*, Pernambuco, Brazil, 2020. A: Score plot–Significant (red) and Mild (green) PPF patterns; B: Permutation test statistic at 2000 permutations with observed statistic of the model prediction accuracy with *P* value = 0.001.

ARTICLE HIGHLIGHTS

Research background

Classification of the pattern of periportal fibrosis (PPF) is essential in the prognostic evaluation of patients with *Schistosomiasis mansoni*.

Research motivation

There is a need for novel minimally invasive methods and new biomarkers for the diagnosis *Schistosomiasis mansoni*.

Research objectives

To develop metabolic models, based on ^{1}H-nuclear magnetic resonance spectra, that allow the classification of the pattern of PPF and its associated metabolites in patients with *Schistosomiasis mansoni*.

Research methods

Metabonomics models (MMs) were built to differentiate requirements with mild PPF and significant PPF. An analysis of the performance of MMs was performed for the prediction of PPF, using ultrasonography as a reference standard and the description of the main metabolites present in each PPF group and their relationship with serum markers.

Research results

The partial least squares-discriminant analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) formalisms discriminated spectral regions between the groups as follows: carbohydrates and valine, more concentrated in those of the group with mild FPP; N-Acetylglycosamines, Alanine, Glycolaldehyde more concentrated in the samples of the group with significant PPF. OPLS-DA showed accuracy, sensitivity, and specificity, were equal to 92.7%, 90.3%, and 100% for the diagnosis of significant PPF.

Research conclusions

The constructed MMs were able to discriminate between mild and significant FPP in patients with schistosomiasis with good accuracy.

Research perspectives

This technique will be able to detect even low-intensity infections, overcoming the limitations of current diagnostic techniques, with the use of a single serum sample. These models can be inserted in the propaedeutic arsenal in clinical practice for the measurement of PPF in remote areas.

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FOOTNOTES

Author contributions: Rodrigues ML, Domingues ALC, Lopes EP, and Silva RO conceived and implemented the study; Rodrigues ML, Pereira CLD, and da Luz TPSR collected and performed analysis; Rodrigues ML, da Luz TPSR, Lopes EP, and Silva RO interpreted the data and drafted the manuscript; Domingues ALC, Batista AD, Lopes EP, and Silva RO critically revised the manuscript; All authors read and approved the final manuscript.

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Baicalin provides protection against fluoxetine-induced hepatotoxicity by modulation of oxidative stress and inflammation

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Abstract

BACKGROUND

Fluoxetine is one of the most widely prescribed anti-depressant drugs belonging to the category of selective serotonin reuptake inhibitors. Long-term fluoxetine treatment results in hepatotoxicity. Baicalin, a natural compound obtained from the Chinese herb *Scutellaria baicalensis* is known to have antioxidant, hepatoprotective and anti-inflammatory effects. However, the beneficial effects of baicalin against fluoxetine-induced hepatic damage have not previously been reported.

AIM

To evaluate the protective action of baicalin in fluoxetine-induced liver toxicity and inflammation.

METHODS

Male albino Wistar rats were divided into seven groups. Group 1 was the normal control. Oral fluoxetine was administered at 10 mg/kg body weight to groups 2, 3, 4 and 5. In addition, groups 3 and 4 were also co-administered oral baicalin (50 mg/kg and 100 mg/kg, respectively) while group 5 received silymarin (100 mg/kg), a standard hepatoprotective compound for comparison. Groups 6 and 7 were used as a positive control for baicalin (100 mg/kg) and silymarin (100 mg/kg), respectively. All treatments were carried out for 28 d. After sacrifice of the rats, biomarkers of oxidative stress [superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-S-transferase (GST), advanced oxidation protein products (AOPP), malondialdehyde (MDA)], and liver injury [alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, bilirubin] were studied in serum and tissue using standard protocols and diagnostic kits. Inflammatory markers [tumor necrosis factor (TNF-α), interleukin (IL)-6, IL-10 and interferon (IFN)-γ] in serum were evaluated using ELISA-based kits. The effect of baicalin on liver was also analyzed by histopathological examination of tissue sections.

RESULTS

Fluoxetine-treated rats showed elevated levels of the serum liver function markers (total bilirubin, ALT, AST, and ALP) and inflammatory markers (TNF-α, IL-6, IL-10 and IFN-γ), with a decline in total protein and albumin levels. Biochemical markers of oxidative stress such as SOD, CAT, GST, GSH, MDA and AOPP in the liver tissue homogenate were also altered indicating a surge in reactive oxygen species leading to oxidative damage. Histological examination of liver tissue also showed degeneration of hepatocytes. Concurrent administration of baicalin (50 and 100 mg/kg) restored the biomarkers of oxidative stress, inflammation and hepatic damage in serum as well as in liver tissues to near normal levels.

CONCLUSION

These findings suggested that long-term treatment with fluoxetine leads to oxidative stress *via* the formation of free radicals that consequently cause inflammation and liver damage. Concurrent treatment with baicalin alleviated fluoxetine-induced hepatotoxicity and liver injury by regulating oxidative stress and inflammation.

Key Words: Fluoxetine; Hepatotoxicity; Oxidative stress; Baicalin; Anti-inflammatory; Hepatoprotective and antioxidant

Core Tip: Prolonged treatment with the antidepressant drug fluoxetine causes severe hepatic damage. This study evaluated fluoxetine-induced liver damage in male albino Wistar rats. Oral fluoxetine was administered (10 mg/kg) for 28 d and caused significant alterations in serum and tissue biomarkers. Baicalin and silymarin were co-administered to facilitate the amelioration of oxidative stress-mediated hepatic damage and inflammation. The biochemical markers (total protein, albumin, total bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, superoxide dismutase, catalase, glutathione, glutathione-S-transferase, malondialdehyde and advanced oxidation protein products) and inflammatory markers [tumor necrosis factor-α, interleukin (IL)-6, IL-10 and interferon-γ] were markedly restored to near normal levels after treatment with the natural flavonoid compound baicalin. Histopathological examination of liver slices showing cellular degeneration and increased vacuolation in the fluoxetine-treated rats also corroborated the results obtained for biomarkers of liver function, oxidative stress and inflammation. The baicalin-treated rats demonstrated normal vacuolation and cellular pattern. Thus, baicalin acts as an antioxidant, anti-inflammatory and hepatoprotective agent in mitigating fluoxetine-induced toxicity. To the best of our knowledge, this is the first study to report the hepatoprotective efficacy of baicalin in fluoxetine-induced liver damage.

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INTRODUCTION

Fluoxetine {N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine} is the most commonly prescribed drug for depression and other neuro-psychotic disorders. It belongs to the category of selective serotonin reuptake inhibitors, and is widely used due to its higher tolerability and fewer side effects[1,2]. Fluoxetine has a long half-life, is metabolized in the liver and excreted *via* urine. The active metabolite of fluoxetine is norfluoxetine. Fluoxetine acts by inhibiting cytochrome P450 (2D6) and its other isozymes, leading to potential drug interactions[3]. Although clinically approved, prolonged use of fluoxetine may cause various adverse effects such as anxiety, sleeplessness, nausea, diarrhea, metabolic disorders and sexual dysfunction[4,5]. The metabolism of fluoxetine results in excess production of free radicals that consequently causes liver damage. In addition, inflammation in the hepatic tissue is also due to over production of superoxide, hydroxyl, and some non-radical species like hydrogen peroxide (H_2O_2) along with surplus phagocyte formation which in turn can cause further tissue damage[6]. Liver diseases resulting from the excessive consumption of drugs are a leading cause of mortality worldwide. The mammalian system has evolved several enzymatic and non-enzymatic pathways that can counter the drug induced adverse effects arising from the action of free radicals[7-9]. In the past decade, several studies have reported the adverse effects caused by fluoxetine such as

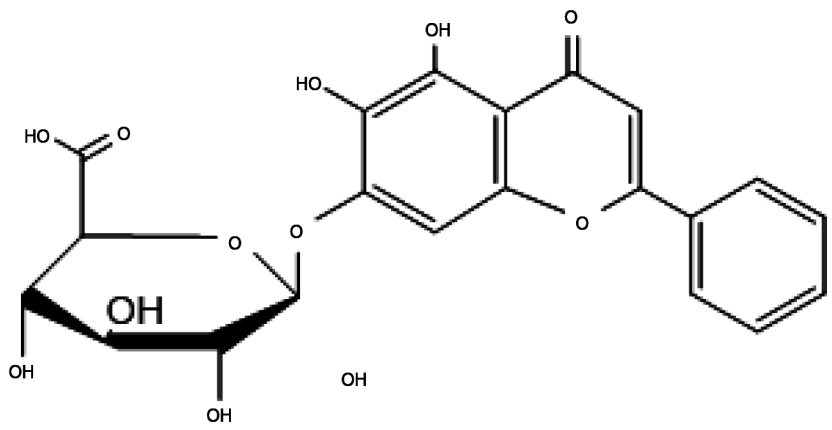

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Figure 1 Structure of baicalin.

hyperglycemic effect, hepatic damage, bipolar disorders and even organ failure in extreme cases[10-13]. Further studies also showed a similar toxicity profile of fluoxetine mediated by oxidative stress and inflammation[14].

Baicalin (5,6,7-trihydroxyflavone 7-O-β-D-glucuronide) is a flavonoid primarily obtained from the Chinese herb *Scutellaria baicalensis*. It is known to possess several pharmacological activities including anti-diabetic, anti-inflammatory, hepatoprotective, neuroprotective, antioxidant and anticancer properties[15]. Structurally, baicalin possesses a di-ortho hydroxyl functional group on its aromatic rings (Figure 1). The divalent metal ion chelating and free radical scavenging actions of baicalin can be attributed to this structural feature[16]. Baicalin extracted from *Scutellaria* roots has exhibited antidepressant properties in mice and rats[17]. Several researchers have reported the neuroprotective effects of baicalin in rats with cerebral ischemia[18,19]. The antioxidant and hepatoprotective activities of baicalin in mice have also been reported[20]. Baicalin is also capable of inducing colon cancer cell apoptosis by inhibiting onco-microRNAs[21]. To date, no studies have assessed the hepatoprotective activity of baicalin against fluoxetine-induced toxicity. Moreover, prolonged treatment with fluoxetine in depression cases may result in liver dysfunction. Thus, the present study was undertaken to demonstrate the protective ability of baicalin against fluoxetine-induced hepatotoxicity, inflammation and oxidative stress in male albino Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals

Fluoxetine hydrochloride and silymarin were procured from Sigma-Aldrich. Baicalin, thiobarbituric acid (TBA), trichloroacetic acid (TCA), chloramines-T, 5-5′-Dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), pyrogallol, bovine serum albumin (BSA), hematoxylin, and eosin were purchased from TCI Chemicals, India. Diagnostics kits were obtained from Erba Diagnostics Mannheim, Germany. Inflammatory marker kits were supplied by Krishgen BioSystems, India.

Animals

Healthy male albino Wistar rats of similar age (weight: 200-250 g) were acclimatized at 23 ± 2 °C for one week prior to the experiment. The animals were given a standard pellet diet and water *ad libitum*. The *in vivo* experiments were carried out as per the norms of the Institutional Animal Ethics Committee, University of Allahabad, Allahabad [IAEC/AU/2017(1)/003].

Animal treatment

The rats were divided into seven groups (n = 6). Oral fluoxetine was administered for 28 d to induce toxicity. Group 1 rats, treated as the normal control, were fed with feed and water. Group 2 rats were administered fluoxetine (10 mg/kg) only. Group 3 (fluoxetine 10 mg/kg + baicalin 50 mg/kg), Group 4 (fluoxetine 10 mg/kg + baicalin 100 mg/kg) and group 5 (fluoxetine 10 mg/kg + silymarin 100 mg/kg) were the drug combination groups. Group 6 and group 7 were treated as positive controls and were only given baicalin (100 mg/kg) and silymarin (100 mg/kg), respectively. Silymarin was used as a standard hepato-protectant for comparison. After completion of 28 d treatment, the rats were sacrificed by cervical dislocation. Blood and liver tissue were collected for the evaluation of enzymatic and non-

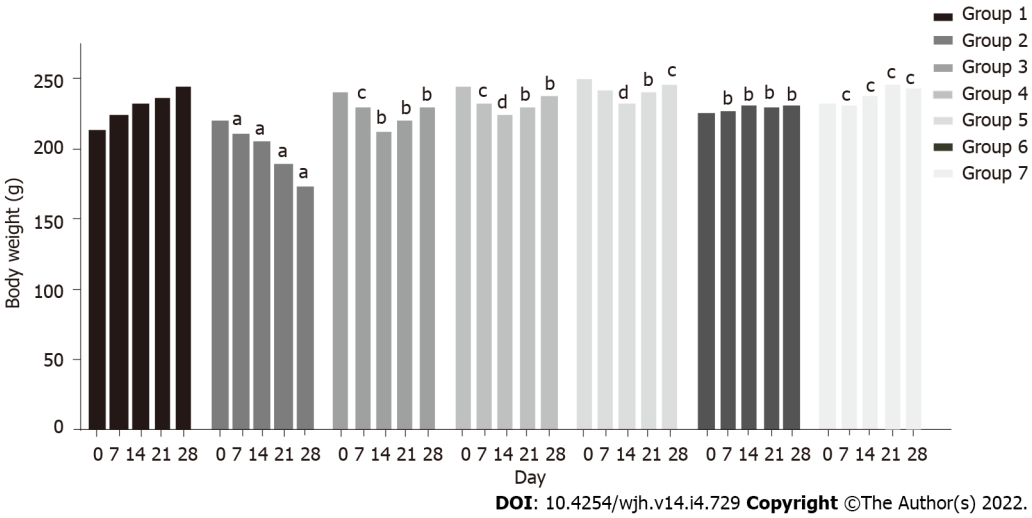

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Figure 2 Effect of baicalin on body weight of rats with fluoxetine-induced toxicity. Group 1: Control rats; group 2: Fluoxetine treated rats (10 mg/kg); group 3: Fluoxetine (10 mg/kg) + baicalin (50 mg/kg); group 4: Fluoxetine (10 mg/kg) + baicalin (100 mg/kg); group 5: Fluoxetine (10 mg/kg) + silymarin (100 mg/kg); group 6: Baicalin (100 mg/kg); group 7: Silymarin (100 mg/kg). Weight of rats is shown in grams. Data represent mean ± SD, n = 6. [a]Represents a significant difference compared with group 1, $P < 0.05$; [b]represents a significant difference compared with group 2, $P < 0.05$; [c]represents a significant difference compared with group 2, $P < 0.05$; [d]represents a significant difference compared with group 2, $P < 0.0005$.

enzymatic biochemical markers.

Measurement of body weight

The body weight of rats was measured every day for 28 d until sacrifice.

Serum and plasma collection from rat blood

At the end of experiment, approximately 5 mL blood was drawn by heart puncture. About half the blood was left to clot for 20 min at approximately 25 °C and serum was obtained at 5000 rpm for 10 min. The rest of the blood was collected in heparin containing vials. This was centrifuged in a cooling centrifuge at 4000 rpm for 20 min, and clear non-hemolyzed plasma was obtained. The plasma and serum samples thus separated were transferred to fresh microfuge tubes and preserved at -80 °C, until further assessment.

Evaluation of serum markers

The serum was used for assessment of the following enzymes: Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and non-enzymatic parameters such as total bilirubin, albumin and total protein using Erba Diagnostics kits. The levels of serum inflammatory markers tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10 and interferon (IFN)-γ were evaluated using ELISA kits (Krishgen BioSystems).

Evaluation of hepatic and oxidative stress markers in liver homogenate

Tissue homogenization: 10% (w/v) liver tissue homogenate was prepared in 0.1 M phosphate buffer with 0.15 M KCl, at pH 7.4. The clear supernatant was separated after centrifugation at 4000 × g for 15 min at 4 °C and was used for further analysis.

Evaluation of malondialdehyde in liver homogenate: The amount of lipid peroxidation in the liver tissues was determined by the method of Niehaus and Samuelsson[22]. 100 mL tissue homogenate was mixed with 2 mL TBA reagent comprising TBA 0.37%, 15% TCA and 0.25 N HCl, and the tubes were placed in a hot water bath for 10 min and cooled at room temperature followed by centrifugation. The supernatant was used for spectrophotometric assessment at 532 nm against a reference blank. An extinction coefficient of 1.56 × 105 $M^{-1}cm^{-1}$ was used and the results were denoted as nmol malondialdehyde (MDA)/mg.

Evaluation of advanced oxidation protein products in liver homogenate: The levels of advanced oxidation protein products (AOPP) were determined by the method of Witko-Sarsat[23]. To 2 mL of liver homogenate [1:5 diluted in phosphate buffer solution (PBS)], 100 mL KI (1.16 M) was added followed by 200 mL glacial acetic acid after 2 min. The absorbance of the reaction mixture was read spectrophotometrically at 340 nm against a reference blank. The blank contained the same reaction mixture, except that the homogenate sample was replaced by 2 mL of PBS. The concentrations of AOPP were denoted as μmol/L of chloramine-T equivalents.

Evaluation of reduced GSH in liver homogenate: GSH content in liver homogenates was determined by the method of Ellman *et al*[24]. 250 mL of liver homogenate was added to the reaction mixture comprising 100 mL of 6 mmol/L DTNB, 300 mL of 0.2 M phosphate buffer (pH 8.0) and 50 mL 0.3M NaOH. Absorbance of the reaction mixture was determined at 412 nm. GSH was used as a standard and the results were represented as μg mg^{-1} protein.

Determination of the activity of antioxidant enzymes in liver homogenate: The activity of glutathione-S-transferase (GST) was measured by the method of Habig *et al*[25]. The activity assay was performed in a reaction mixture of 1 mL comprising 0.1 M phosphate buffer (pH 6.5), 1 mmol/L CDNB, 1 mmol/L GSH and 100 mL supernatant of liver homogenate. The change in absorbance on account of conjugate formation of GSH and CDNB was measured at 340 nm. GST activity was represented as μmol $min^{-1}mg^{-1}$ protein.

Superoxide dismutase (SOD) activity was evaluated by the method of Marklund and Marklund[26]. Formation of the colored complex takes place due to auto-oxidation of pyrogallol. This was measured for 3 min at an interval of 60 s at 412 nm in the presence or absence of the enzyme. One unit of enzyme activity was represented as 50% inhibition of auto-oxidation of pyrogallol per minute.

Catalase (CAT) activity was assessed by the method of Beers and Sizer[27]. The decreasing absorbance of H_2O_2 consumption was measured at 240 nm at an interval of 60 s for 3 min. One unit of CAT activity was expressed in μmol of H_2O_2 decomposed per minute with an extinction coefficient of H_2O_2 of 43.6 $M^{-1}cm^{-1}$.

Total protein assay

The total protein content in serum and tissue samples was estimated by the method of Lowry *et al*[28] with BSA as standard.

Histopathological study of liver tissue

Following sacrifice of the rats, the tissue samples were washed with cold saline and fixed in 10% formalin. The samples were then processed further for the preparation of paraffin wax blocks. Sectioning was done with a rotatory microtome and stained with hematoxylin and eosin[29]. The slides were then examined under the light microscope at 40 × magnification to observe the protective effect of baicalin against fluoxetine-mediated oxidative damage in liver tissue.

Statistical analysis

The statistical analysis was performed using GraphPad Prism 5 software. An unpaired *t* test was used for statistical comparisons and the results were expressed as mean ± SD. A P value < 0.05 was considered significant.

RESULTS

Change in body weight

A marked decline in body weight was observed in group 2 rats (220 g to 173 g) within 28 d, while a constant increase was observed in group 1 rats (213 g to 245 g) ($P < 0.05$). In group 3 rats, body weight declined (240 g to 213 g) up to the second week, and increased thereafter (213 g to 229 g) ($P < 0.05$). A similar pattern was observed in group 4 (245 g to 224 g up to the second week and 224 g to 238 g thereafter) ($P < 0.005$) and group 5 (250 g to 231 g up to the second week and 231 g to 245 g thereafter) ($P < 0.0005$). Co-administration of baicalin and silymarin in groups 3, 4 and 5 helped body weight gain from day 14 onwards, after initial weight loss due to fluoxetine treatment. Groups 6 and 7 exhibited a normal pattern of body weight similar to the control group (Figure 2).

Effect of baicalin on serum liver function enzymes

To evaluate the protective effect of baicalin against fluoxetine-induced hepatic injury, liver function marker enzymes were assessed in serum. Fluoxetine treatment for 28 d caused a significant increase in serum AST levels in group 2 rats (129.29 IU/L) in comparison to the control group (64.28 IU/L). Concurrent administration of baicalin significantly alleviated serum AST in group 3 (113.49 IU/L) and group 4 (94.51 IU/L) rats (Figure 3A). Group 5 rats treated with silymarin, a standard hepato-protectant, also showed a decline in AST levels to near normal (88.82 IU/L). ALT showed a similar pattern with a significant rise in group 2 (70.40 IU/L) as compared to the control group 1 (25.63 IU/L). Oral administration of baicalin decreased ALT levels in group 3 (55.84 IU/L) and group 4 (42.67 IU/L) rats (Figure 3B). Silymarin-treated group 5 rats also showed a reduction in ALT (38.22 IU/L). Similar to ALT and AST, group 2 rats also exhibited a marked increase (218.95 IU/L) in the level of serum ALP in comparison to group 1 (102.66 IU/L). Concurrent administration of baicalin produced a little decline in serum ALP levels in group 3 (191.26 IU/L), while a significant decline in group 4 (147.33 IU/L) and silymarin-treated group 5 (134.46 IU/L) was observed (Figure 3C). The greatest ameliorative potential

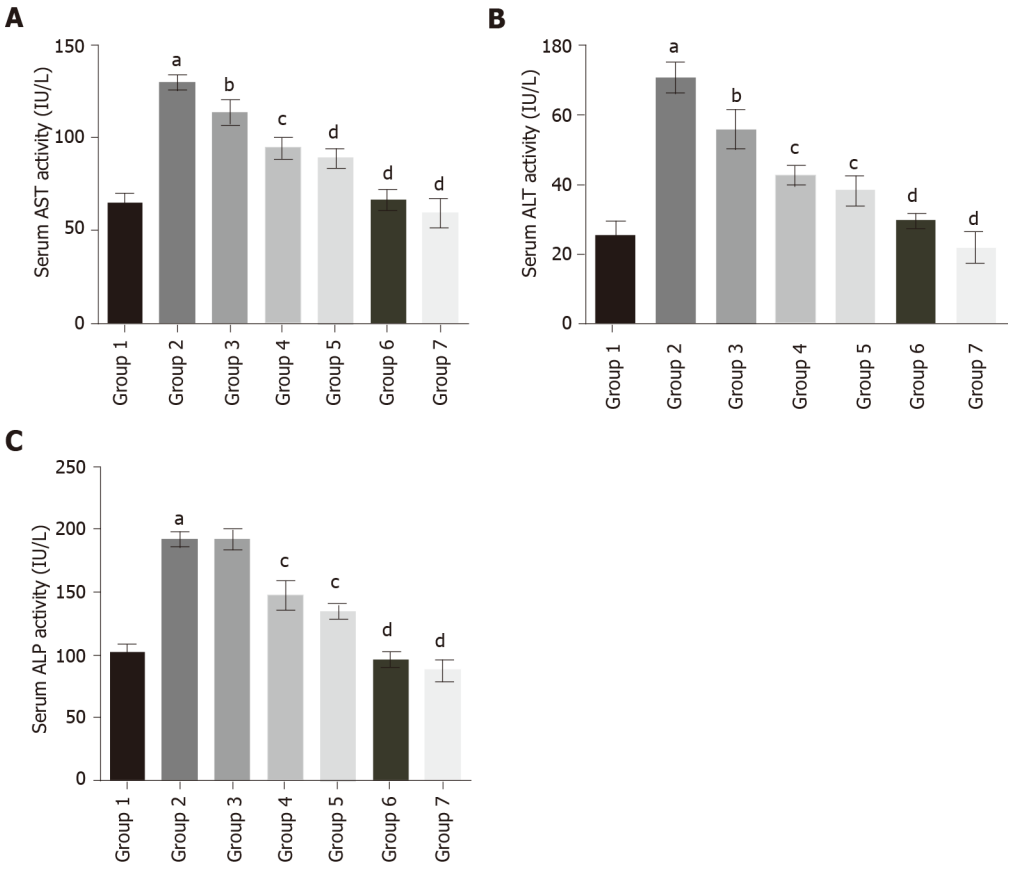

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Figure 3 Effect of baicalin on serum enzymatic markers of liver function in fluoxetine-induced hepatotoxicity. A: Aspartate transaminase; B: Alanine transaminase; C: Alkaline phosphatase. Group 1: Control rats; group 2: Fluoxetine treated rats (10 mg/kg); group 3: Fluoxetine (10 mg/kg) + baicalin (50 mg/kg); group 4: Fluoxetine (10 mg/kg) + baicalin (100 mg/kg); group 5: Fluoxetine (10 mg/kg) + silymarin (100 mg/kg); group 6: Baicalin (100 mg/kg); group 7: Silymarin (100 mg/kg). Data represent mean ± SD, $n = 6$. [a]Represents a significant difference compared with group 1, $P < 0.05$; [b]represents a significant difference compared with group 2, $P < 0.05$; [c]represents a significant difference compared with group 2, $P < 0.005$; [d]represents a significant difference compared with group 2, $P < 0.0001$. AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase.

in relation to serum enzymes was observed in group 5 rats treated with the standard hepato-protectant silymarin, while group 4 rats treated with baicalin (100 mg/kg) clearly exhibited greater restoration ability compared to group 3 rats treated with baicalin (50 mg/kg). Groups 6 and 7 served as positive controls and showed no significant changes compared to the control (group 1) for all the biomarkers.

Effect of baicalin on serum bilirubin, total protein and albumin

The serum total bilirubin levels in group 2 rats (1.44 mg/DL) were significantly increased compared to group 1 (0.35 mg/DL). Baicalin treatment along with fluoxetine led to a decline in bilirubin level in group 3 (0.81 mg/DL), and group 4 (0.64 mg/DL) (Figure 4A). Similarly, co-administration of silymarin with fluoxetine in group 5 caused a reduction in bilirubin (0.57 mg/DL). However, the level of total protein in group 2 (3.53 g/DL) was significantly decreased compared to group 1 rats (7.52 g/DL). Concurrent administration of baicalin resulted in a significant improvement in the total protein levels of group 3 (4.55 g/DL), group 4 (4.89 g/DL), and group 5 (5.52 g/DL) rats (Figure 4B). Similarly, the level of albumin was also decreased in group 2 (2.39 g/DL) compared to group 1 (3.97 g/DL), which increased with baicalin administration in group 3 (3.02 g/DL), group 4 (3.61 g/DL), and group 5 (3.75 g/DL) (Figure 4C).

Effect of baicalin on hepatic antioxidant enzymes in liver homogenate

The enzymatic activities of SOD, CAT and GST were evaluated in liver tissue homogenates of rats treated with fluoxetine, baicalin and silymarin. The changes in activities in the different groups are shown in Table 1. A 55% reduction in the activity of SOD was observed in group 2 (6.357 U/mg) compared to group 1 (14.04 U/mg). Groups 3, 4, and 5 (7.73 U/mg, 11.11 U/mg and 10.37 U/mg, respectively) showed significant restoration in the presence of baicalin and silymarin. A similar pattern was observed in CAT activity with group 2 showing a decline (3.17 U/mg) as compared to control group 1 (7.26 U/mg). Co-administration of baicalin (50 and 100 mg/kg) and silymarin resulted in improved CAT activity in groups 3, 4 and 5 (3.74 U/mg, 4.94 U/mg and 5.37 U/mg, respectively). GST

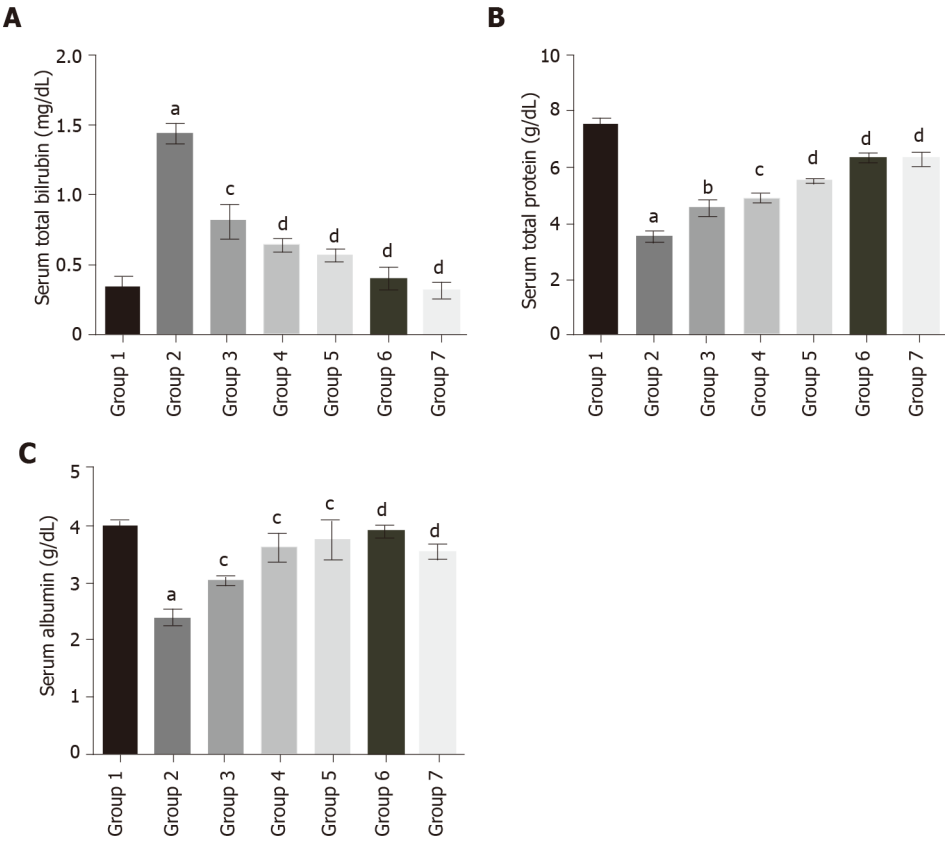

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Figure 4 Effect of baicalin on serum bilirubin, total protein and albumin in fluoxetine-induced hepatotoxic rats. A: Serum bilirubin; B: Serum total protein; C: Serum albumin. Group 1: Control rats; group 2: Fluoxetine treated rats (10 mg/kg); group 3: Fluoxetine (10 mg/kg) + baicalin (50 mg/kg); group 4: Fluoxetine (10 mg/kg) + baicalin (100 mg/kg); group 5: Fluoxetine (10 mg/kg) + silymarin (100 mg/kg); group 6: Baicalin (100 mg/kg); group 7: Silymarin (100 mg/kg). Data represent mean ± SD, $n = 6$. [a]Represents a significant difference from group 1, $P < 0.05$; [b]represents a significant difference compared with group 2, $P < 0.05$; [c] represents a significant difference compared with group 2, $P < 0.005$; [d]represents a significant difference compared with group 2, $P < 0.0001$.

activity in group 2 rats (1.29 U/mg) showed decreased activity compared to group 1 (2.14 U/mg). In groups 3, 4 and 5, the activity of GST was increased towards normal values (1.32 U/mg, 1.41 U/mg, 1.54 U/mg, respectively).

Assessment of the non-enzymatic antioxidant markers in liver tissue

Treatment with fluoxetine caused increased levels of MDA and AOPP, along with a decline in GSH as compared with the control group (Table 1). Fluoxetine administration led to a decline in GSH in group 2 animals (52.12 Nm/mg) by more than 50% compared to group 1 (115.76 Nm/mg). The levels of GSH in group 3 (78.87 U/mg), group 4 (97.02 U/mg) and group 5 (104.91 U/mg) animals were subsequently elevated to near normal levels following administration of baicalin and silymarin. The level of MDA in group 2 exhibited an almost three-fold increase (2.07 Nm/mg) compared with control group 1 (0.68 Nm/mg). This was again restored partially in groups 3, 4 and 5 (1.73 Nm/mg, 1.32 Nm/mg and 0.96 Nm/mg, respectively). The AOPP levels also exhibited an identical pattern of an almost three-fold increase in group 2 animals (1.12 Nm/mg) compared with control group 1 (0.48 Nm/mg). Groups 3, 4 and 5 treated with baicalin and silymarin showed restoration of AOPP levels to near normal (0.83 Nm/mg, 0.64 Nm/mg and 0.56 Nm/mg, respectively) suggesting the appreciable antioxidant potential of baicalin. These results were found to be comparable to silymarin.

Assessment of inflammatory markers in serum

The serum TNF-α level in fluoxetine-treated group 2 (163.75 pg/mL) significantly increased as compared to group 1 (49.9 pg/mL). Baicalin and silymarin treatments showed improvement in groups 3 (120.36 pg/mL), 4 (98.67 pg/mL) and 5 (88.8 pg/mL) (Figure 5A). Similarly, serum IL-6 was elevated in group 2 (331.81 pg/mL) relative to group 1 (200.07 pg/mL), but there was a subsequent decline in groups 3 (298.18 pg/mL), 4 (258.24 pg/mL) and 5 (260.39 pg/mL) (Figure 5B). IL-10 levels slightly increased in group 2 (84.06 pg/mL) compared to group 1 (55.24 pg/mL), while the baicalin and silymarin treated groups 3 (93.7 pg/mL), 4 (85.36 pg/mL) and 5 (77.47 pg/mL) showed comparable results to group 2 (Figure 5C). The serum IFN-γ levels followed a similar pattern. There was a rise in fluoxetine-treated group 2 (365.14 pg/mL) compared to group 1 (256.73 pg/mL), whereas groups 3

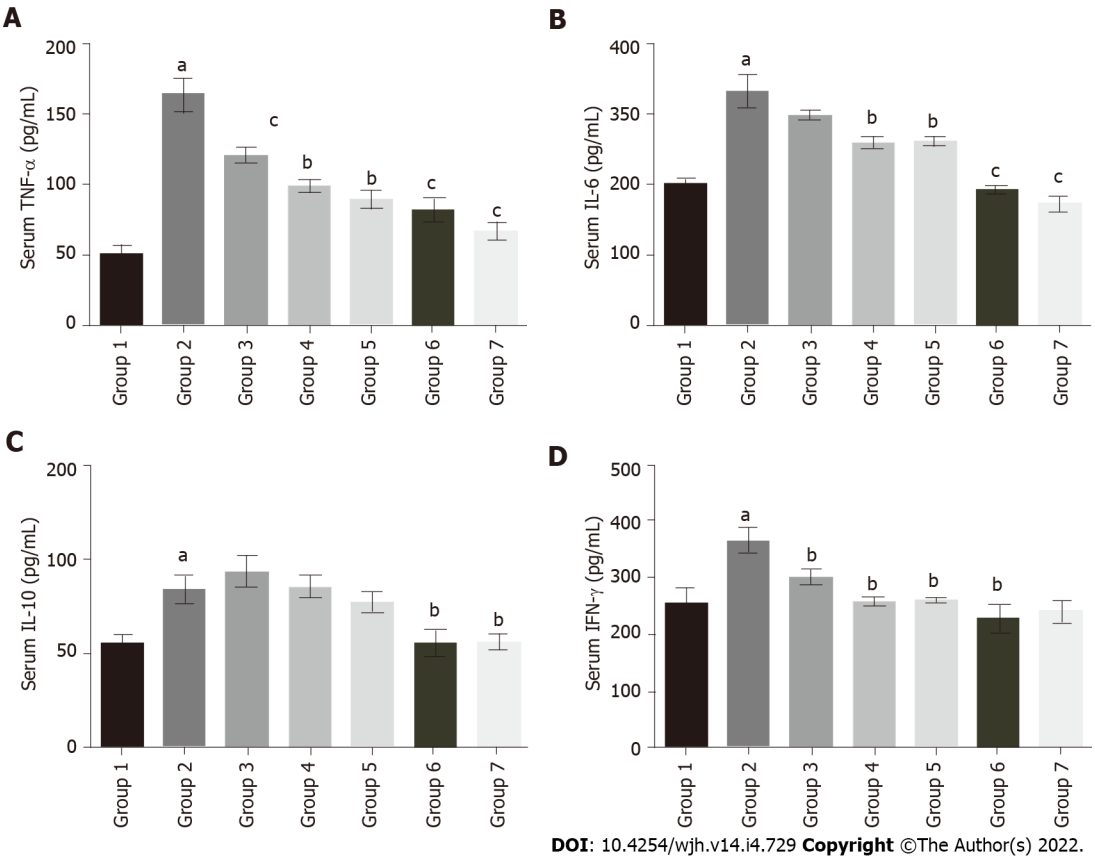

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Figure 5 Effect of baicalin on serum inflammatory markers in fluoxetine treated rats. A: Tumor necrosis factor-α; B: Interleukin (IL)-6; C: IL-10; D: Interferon-γ. The values are expressed in pg/mL. Group 1: Control rats; group 2: Fluoxetine treated rats (10 mg/kg); group 3: Fluoxetine (10 mg/kg) + baicalin (50 mg/kg); group 4: Fluoxetine (10 mg/kg) + baicalin (100 mg/kg); group 5: Fluoxetine (10 mg/kg) + silymarin (100 mg/kg); group 6: Baicalin (100 mg/kg); group 7: Silymarin (100 mg/kg). Data represent mean ± SD, $n = 6$. [a]Represents a significant difference compared with group 1, $P < 0.05$; [b]represents a significant difference compared with group 2, $P < 0.05$; [c]represents a significant difference compared with group 2, $P < 0.005$; [d]represents a significant difference compared with group 2, $P < 0.0001$. TNF: Tumor necrosis factor; IL: Interleukin; IFN: Interferon.

(298.17 pg/mL), 4 (258.24 pg/mL) and 5 (260.39 pg/mL) showed considerable restoration to near normal levels (Figure 5D). In all treatment groups, baicalin at a dose of 100 mg/kg showed better efficacy as compared to baicalin at 50 mg/kg.

Histopathological studies

Histopathological examination of the control liver section revealed normal cellular architecture with mild vacuolation, presence of the central vein and sinusoidal spaces with intact hepatic cells (Figure 6A). The fluoxetine-treated liver slices showed an irregular pattern of hepatic cells, increased vacuolation, dilation of hepatic sinusoids, inflammatory cell infiltration, cellular disintegration and the initial stage of bridging necrosis that links the terminal veins to the portal tracts (Figure 6B). Co-administration of baicalin and silymarin prevented the disarrangement of hepatic cells, and normal sinusoidal spaces were observed without abnormal alterations (Figures 6C, 6D and 6E). The positive control groups 6 and 7 did not exhibit any alterations and showed a normal cellular pattern (Figures 6F and 6G).

DISCUSSION

Liver is associated with the biotransformation of the entire xenobiotic load in the body. Drugs and various chemicals ingested are transformed with the help of cytochrome P450 in the liver. The active metabolites produced during biotransformation elevate the levels of free radicals and reactive oxygen species (ROS) thus disrupting the redox homeostasis[30,31]. Hepatic injury and toxicity can be produced by the use of numerous chemical agents or drugs, heavy metals and pesticides[32]. In the present study, the dose-dependent protective efficacy of baicalin was evaluated in fluoxetine-induced hepatotoxicity in male Wistar rats. Baicalin is well known in the treatment of several liver-related anomalies. It ameliorates the effects of estrogen-induced liver injury by up-regulating the expression of hepatic efflux transporters and down-regulating hepatic uptake transporters[33]. The neuroprotective and antide-

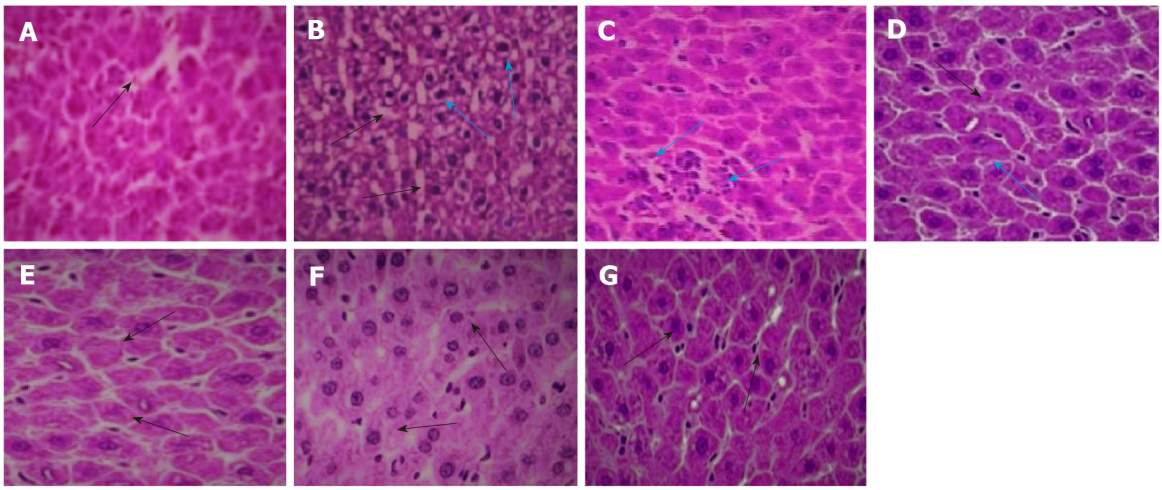

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Figure 6 Histological sections of rat liver. A: Normal control (the black arrow shows normal intercellular spaces and negligible vacuolation); B: Fluoxetine (10 mg/kg) treated (the black arrows show increased vacuolation and cellular degeneration, blue arrows indicate presence of inflammatory cells); C: Fluoxetine + baicalin (50 mg/kg) treated (blue arrows indicate a patch of inflammatory cell infiltration); D: Fluoxetine + baicalin (100 mg/kg) treated (the black arrow depicts regular cellular pattern and normal sinusoidal space, blue arrow indicates fewer inflammatory cells); E: Fluoxetine + silymarin (100 mg/kg) treated (the black arrows show normal cells and intercellular spaces similar to normal control); F: Baicalin (100 mg/kg) treated (the black arrows show normal cellular structure); G: Silymarin (100 mg/kg) treated (the black arrows depict normal cells and intercellular spaces).

pressant properties of baicalin and its derivatives have also been widely studied. Baicalin facilitates stimulation of neurogenesis, the production of neurotrophic factors and modulation of the hypothalamic-pituitary-adrenal axis, which further counter oxidative stress, and inflammation. Similar to fluoxetine and its metabolite norfluoxetine, baicalin also elicits an anti-depressant effect by regulation of the gamma-aminobutyric acid (GABA) neurotransmitter system, and upregulating GABA receptors [17,34,35].

Long-term fluoxetine treatment in male albino Wistar rats resulted in reduced food intake over 28 d thereby resulting in weight loss (50 g in 28 d). Fluoxetine increases serotonin signaling in the brain, and higher serotonin levels help in activating the satiety neurons thus decreasing appetite. In addition, the active metabolite of fluoxetine, norfluoxetine is slow to metabolize and causes anorexia[36]. However, co-administration of baicalin prevented excess weight loss from the second week onwards. Baicalin has been reported to alleviate anorexia by inhibiting the over-expression of pro-inflammatory cytokines TNF-α and IL-6. The hypothalamic region in the brain that regulates food intake is stimulated by cytokine levels thus helping to increase appetite and body weight[37].

Fluoxetine caused a significant increase in the serum levels of ALT, AST and ALP in rats. This could be attributed to the fluoxetine-induced membrane alterations resulting from ROS action leading to cellular disintegration and necrosis of hepatic cells. As these enzymes reside in the cytoplasm, their elevated levels in the serum indicate breakdown of the hepatocellular membrane[31,38]. ALT is a liver injury biomarker while ALP is a marker of hepatic biliary injury and cholestasis. These results are further corroborated by earlier reports that showed enhanced levels of ALT, AST and ALP upon fluoxetine treatment[9,11]. Oral administration of baicalin led to a substantial decrease in serum ALT, AST and ALP levels in fluoxetine-treated rats, indicating its hepatoprotective action. Baicalin at 100 mg/kg was more effective in restoring enzyme levels to near normal than at 50 mg/kg. The efficacy of higher dose baicalin treatment was comparable to the effect of silymarin (100 mg/kg), a standard hepato-protectant used in experimental studies. Silymarin can bind to the receptors on the hepatocyte membrane to prevent the entry of toxic substances in the liver. The antioxidant properties of silymarin can reduce ROS, thus inhibiting cellular damage[39]. Fluoxetine treatment led to an elevation in serum bilirubin along with a reduction in total protein and albumin. Higher serum bilirubin is an indicator of lower hepatic clearance suggesting liver function abnormality that may cause jaundice and other hepatic symptoms[40]. Following treatment with baicalin, the serum bilirubin levels were considerably restored to near normal.

The decline in total protein and albumin levels also point to an anomaly in the protein synthesis machinery leading to alterations in cellular physiology and hepatocellular function in fluoxetine-treated rats. This subsequently causes a decline in cytochrome P450 activity. Baicalin treatment increased the total protein and albumin levels, and thereby improved cellular functions. Previous studies also demonstrated the potential of baicalin as a hepato-protectant that helps in restoring the total protein and albumin levels in mice[17]. Fluoxetine treatment caused elevated AOPP levels along with increased lipid peroxidation as indicated by higher MDA content in liver tissue suggesting the overproduction of ROS and free radicals. Moreover, decreased levels/activity of GSH, SOD, CAT and GST in liver tissue

further supports enhanced oxidative stress in fluoxetine-treated rats.

GSH plays a key role in intracellular defense against free radical-induced oxidative stress. It is an intermediate in the pathways involving antioxidant enzymes such as GSH peroxidase and GSH reductase. The other important biological roles of GSH include regulation of signal transduction, transport of sulfate, modulation of cell growth and division, metabolite conjugation, protein and nucleic acid synthesis, xenobiotic detoxification, promoting metal ion chelation and enzymatic reactions[41]. Low GSH acts as an indicator of oxidative stress and tissue damage and adversely affects redox equilibrium with an increased oxidized state of the system[42,43]. GSH acts as a free radical scavenging and membrane stabilizing agent. It is capable of minimizing radical-linked membrane damage and prevents lipid peroxidation. Thus, reduced GSH content in fluoxetine-treated rats could be responsible for an increase in lipid peroxidation. Elevated levels of MDA, a product of lipid peroxidation, in the tissue also signify the oxidized state of the system that is beyond the control of the antioxidant defense system[44-48]. The higher levels of AOPP also point towards oxidative stress causing protein damage following prolonged fluoxetine treatment in rats. Proteins are often targeted by ROS that cause modification of amino acids which are measured quantitatively to determine the extent of oxidative damage. As fluoxetine is prescribed as an antidepressant in psychotic disorders, it is possible that its long-term intake causes oxidative damage to proteins in hepatocytes. Chloramines are oxidants that are produced in neutrophils by the enzyme myeloperoxidase. These oxidants result in the formation of advanced oxidation di-tyrosine cross-linked protein products. They are estimated quantitatively and act as biomarkers of protein oxidation[49]. The excessive production of AOPP is also suggestive of the onset of numerous diseases such as Alzheimer's disease, rheumatoid arthritis, muscular dystrophy and respiratory diseases[50]. Hence, the decline in MDA and AOPP in rats treated with baicalin could be attributed to its antioxidant and oxidative stress lowering potential. SOD causes dismutation of the superoxide radicals generated in tissue into H_2O_2 and O_2. CAT, in the peroxisomes further converts excess H_2O_2 produced by SOD action into water and O_2[51,52]. During the present study, rats treated with baicalin and silymarin exhibited restoration of GSH, along with enhancement of SOD, CAT and GST activities. Nuclear respiratory factor 2 (Nrf2) is a major factor involved in maintaining cellular redox homeostasis. Activated Nrf2 helps to maintain the mitochondrial redox balance, increases the expression of antioxidant enzymes, and promotes mitochondrial biogenesis by increased transcription of Nrf1[53,54]. The rise in SOD and CAT activities could be due to increased activation of Nrf2 by baicalin and silymarin[55,56]. This further supports the role of baicalin treatment in improving and maintaining the redox balance in fluoxetine-treated rats.

GST is a cytosolic enzyme that helps to detoxify the toxic metabolites generated from cellular processes. It acts as a defense mechanism against oxidative stress, and regulates GSH homeostasis *via* mitogen activated protein kinase (MAPK) pathways that is involved in cellular response to stress[57]. The decline in GST activity is an indicator of oxidative stress. In the current study, fluoxetine treatment in rats led to a reduction in GST activity suggesting increased ROS production in the system. Thus, decreased GST activity further hinders the detoxification process[58]. The co-administration of baicalin (50 and 100 mg/kg) resulted in increased GST activity signifying a reduction in oxidative stress.

Baicalin also showed considerable efficacy against the fluoxetine-induced inflammatory response. The levels of serum inflammatory markers TNF-α, IL-6, IL-10 and IFN-γ were considerably elevated following fluoxetine treatment. Baicalin administration significantly restored the levels of TNF-α, IL-6, IL-10 and IFN-γ in fluoxetine-treated rats. The anti-inflammatory effects of baicalin and its derivative baicalein have been reported in several studies[59]. Baicalin helps to reduce the elevated levels of cytokines TNF-α and IL-6 by regulating the p38 MAPK signaling cascade[60]. Notably, IL-10 is an anti-inflammatory cytokine that binds to the IL receptor proteins and induces the STAT3 signaling cascade. In previous reports, fluoxetine administration elevated the level of IL-10 in depressive patients as it inhibits the synthesis of other pro-inflammatory cytokines such as IL-6 and IFN-γ[61]. However, in this study, the IL-10 level in fluoxetine-treated rats was slightly increased compared to the control group. In liver cells, IFN-γ is produced by natural killer cells and T lymphocytes. During hepatic injury and inflammation, the IFN-γ receptor expression is up-regulated which stimulates the secretion of IFN-γ. This in turn activates the macrophages, producing other cytokines such as TNF-α in abundance[62]. Thus, the elevated levels of IFN-γ in fluoxetine-treated rats indicate hepatic injury and oxidative stress. Baicalin treatment led to restoration of the IFN-γ levels to near normal. The efficacy of baicalin was better at the dose of 100 mg/kg than at 50 mg/kg. Histopathological examination of liver sections of rats treated with fluoxetine and baicalin showed signs of improvement as indicated by reduced vacuolation in cells, decreased cellular degeneration, less inflammatory cell infiltration and regular cellular architecture.

Thus, long-term intake of fluoxetine caused hepatotoxicity due to increased production of ROS during its biotransformation in liver. Baicalin, acting as an oxidative stress mitigator, led to improved structural and functional aspects of the liver as shown by biochemical and histopathological indices. In general, baicalin administration at 100 mg resulted in an appreciable reduction in fluoxetine-induced hepatic damage and inflammation in rats by restoring liver function markers and inflammatory cytokines to near normal levels. This was comparable to the effect of silymarin, a standard hepato-protectant at the same dose. In addition, it has been reported that baicalin possesses higher oral bioavailability than silymarin. Baicalin inhibits efflux transporters to increase the bioavailability of

Table 1 The effect of baicalin and silymarin on the antioxidant status of liver tissue in fluoxetine-treated rats

Groups	SOD (U/mg)	CAT (U/mg)	GST (U/mg)	GSH (nM/mg)	MDA (nM/mg)	AOPP (nM/mg)
1	14.04 ± 0.58	7.26 ± 0.78	2.14 ± 0.22	115.76 ± 1.74	0.68 ± 0.04	0.48 ± 0.06
2	6.357 ± 0.50[a]	3.17 ± 0.33[a]	1.29 ± 0.15[a]	52.12 ± 1.90[a]	2.07 ± 0.18[a]	1.12 ± 0.17[a]
3	7.73 ± 0.65[b]	3.74 ± 0.45	1.32 ± 0.17[c]	78.87 ± 1.52[c]	1.73 ± 0.16[c]	0.83 ± 0.12[c]
4	11.11 ± 0.4[d]	4.94 ± 0.66[c]	1.41 ± 0.23[c]	97.02 ± 1.43[c]	1.32 ± 0.11[c]	0.64 ± 0.08[c]
5	10.37 ± 0.46[d]	5.37 ± 0.47[c]	1.54 ± 0.11[d]	104.91 ± 1.88[c]	0.96 ± 0.07[c]	0.56 ± 0.06[d]
6	14.73 ± 0.31[d]	7.27 ± 0.42[d]	1.86 ± 0.24[c]	110.24 ± 1.12[c]	0.56 ± 0.13[c]	0.49 ± 0.06[c]
7	13.98 ± 0.3[d]	6.66 ± 0.63[d]	1.68 ± 0.10[c]	106.58 ± 1.98[c]	0.69 ± 0.08[c]	0.50 ± 0.09[c]

[a]Represents a significant difference compared with group 1, $P < 0.05$.
[b]Represents a significant difference compared with group 2, $P < 0.05$.
[c]Represents a significant difference compared with group 2, $P < 0.005$.
[d]Represents a significant difference compared with group 2, $P < 0.0005$.
The activities of biomarkers were assessed in tissue homogenate. Superoxide dismutase, catalase and glutathione-S-transferase are expressed as U/mg. Reduced glutathione, malondialdehyde and advanced oxidation protein products are expressed as nM/mg. Group 1: Control rats; group 2: Fluoxetine treated rats (10 mg/kg); group 3: Fluoxetine (10 mg/kg) + baicalin (50 mg/kg); group 4: Fluoxetine (10 mg/kg) + baicalin (100 mg/kg); group 5: Fluoxetine (10 mg/kg) + silymarin (100 mg/kg); group 6: Baicalin (100 mg/kg); group 7: Silymarin (100 mg/kg). Data represent mean ± SD, $n = 6$. SOD: Superoxide dismutase; CAT: Catalase; GST: Glutathione-S-transferase; GSH: Reduced glutathione; MDA: Malondialdehyde; AOPP: Advanced oxidation protein products.

silymarin[63]. Therefore, baicalin can be used over silymarin as an alternative hepatoprotective compound to prevent fluoxetine-induced liver toxicity. Furthermore, it also improved the antioxidant status of the liver which consequently diminished ROS production and associated injury. Thus, co-treatment of baicalin with prolonged fluoxetine treatment proved beneficial for the liver and overall health status of the rats.

CONCLUSION

Fluoxetine is a commonly prescribed antidepressant drug used for long-term treatment. This study revealed that long-term fluoxetine treatment induced oxidative stress, hepatotoxicity and inflammation in rats. Baicalin administration prevented fluoxetine-induced liver damage and inflammation in rats by alleviating liver function biomarkers and inflammatory cytokines. Furthermore, it also prevented ROS-mediated damage by strengthening the antioxidant defense system at the enzymatic and non-enzymatic levels. Baicalin exhibited considerable hepatoprotective activity at a dose of 100 mg/kg and was found to be comparable to the standard compound silymarin at the same dose. Hence, to defend against oxidative stress and hepatotoxicity due to prolonged fluoxetine treatment, baicalin administration could be a drug of choice. However, further research is needed for a better understanding of the key pathways and mechanisms that could explain the protective effects of baicalin against fluoxetine-induced liver injury, oxidative damage and particularly the anti-inflammatory response.

ARTICLE HIGHLIGHTS

Research background

Fluoxetine is one of the most commonly prescribed drugs for depression and anxiety disorders. Prolonged use of fluoxetine results in hepatic toxicity. Baicalin is a natural compound obtained from the ancient Chinese herb *Scutellaria baicalensis*. Baicalin is known to possess several antioxidant, anti-inflammatory, anticancer, neuroprotective, cardioprotective and hepatoprotective effects.

Research motivation

The hepatotoxic effects of fluoxetine following prolonged treatment have been reported previously. As baicalin has anti-inflammatory and hepatoprotective properties, the aim of this study was to evaluate the hepatoprotective and anti-inflammatory properties of baicalin when co-administered with fluoxetine.

Research objectives

The objective of this study was to assess the protective action of baicalin in fluoxetine-induced liver toxicity and inflammation.

Research methods

Male albino Wistar rats were divided into seven groups. Group 1 was the normal control. Oral fluoxetine was administered at 10 mg/kg body weight to groups 2, 3, 4 and 5. In addition, groups 3 and 4 were also co-administered with oral baicalin (50 mg/kg and 100 mg/kg, respectively) while group 5 received silymarin (100 mg/kg). Groups 6 and 7 were used as positive controls for baicalin (100 mg/kg) and silymarin (100 mg/kg). All treatments were carried out for 28 d. Biomarkers of oxidative stress [superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-S-transferase (GST), advanced oxidation protein products (AOPP), malondialdehyde (MDA)], and liver injury [alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, bilirubin] were studied in serum and tissue using standard protocols and diagnostic kits. Inflammatory markers [tumor necrosis factor (TNF-α), interleukin (IL)-6, IL-10 and interferon (IFN)-γ] in serum were evaluated using ELISA kits. The effect of baicalin on the liver was also analyzed by histopathological examination of tissue sections.

Research results

Fluoxetine-treated rats showed elevated levels of serum liver function markers (total bilirubin, ALT, AST, and ALP) and inflammatory markers (TNF-α, IL-6, IL-10 and IFN-γ), with a decline in total protein and albumin levels. The biochemical markers of oxidative stress such as SOD, CAT, GST, GSH, MDA and AOPP in the liver tissue homogenate were also altered indicating a surge in reactive oxygen species leading to oxidative damage. Histological examination of liver tissue also showed degeneration of hepatocytes. Concurrent administration of baicalin (50 and 100 mg/kg) restored the biomarkers of oxidative stress, inflammation and hepatic damage in serum as well as in liver tissues to near normal levels.

Research conclusions

The results suggested that prolonged fluoxetine treatment leads to oxidative stress *via* the formation of free radicals that consequently cause inflammation and liver damage. Co-administration of baicalin alleviated fluoxetine-induced hepatotoxicity and liver injury by regulating oxidative stress and inflammation.

Research perspectives

Baicalin exhibited considerable hepatoprotective activity at a dose of 100 mg/kg and it was found to be comparable to the standard compound silymarin at the same dose. Therefore, baicalin can be used along with fluoxetine to prevent hepatic toxicity and inflammation. However, further research is needed for a better understanding of the key pathways and mechanisms that could explain the protective effects of baicalin against fluoxetine-induced liver injury, oxidative damage and particularly the anti-inflammatory response.

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FOOTNOTES

Author contributions: Ganguly R performed the research and wrote the manuscript; Ganguly R and Kumar R analyzed the data; Pandey AK designed the research study, critically reviewed and revised the manuscript; and all authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

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Correlation between Fibroscan and laboratory tests in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis patients for assessing liver fibrosis

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), through to advanced fibrosis and cirrhosis. Many patients with NAFLD remain undiagnosed and recognizing those at risk is very crucial. Although liver biopsy is the gold standard method for diagnosing and staging NAFLD, non-invasive imaging and lab modalities are also very promising in diagnosing these diseases.

AIM

To explore some of these non-invasive modalities in this context and assess how they hold up in terms of making a diagnosis while avoiding an invasive procedure like a liver biopsy.

METHODS

This study was conducted on NAFLD/NASH patients (n = 73) who underwent Fibroscan examinations at Saint George Hospital University Medical Center over 17 mo in order to assess liver fibrosis. Obtained Fibroscan results were correlated

to laboratory tests and calculated aspartate transaminase (AST)/alanine transaminase (ALT) ratio, AST platelet ratio index (APRI) score and Fibrosis-4 score.

RESULTS

A significant age difference was observed across fibrosis stages of investigated patients. The mean stiffness score was 9.48 ± 11.77 KPa. A significant negative correlation was observed between ALT, AST, Albumin, gamma-glutamyl transferase, cholesterol, LDL, HDL, triglycerides, and ALP when compared across fibrosis stages. On the other hand, a significant positive correlation was found between Bilirubin, PT INR, partial thromboplastin time, glucose, and Platelet count when compared across fibrosis stages, in addition to AST/ALT ratio, APRI, and Fib-4 scores.

CONCLUSION

This study showed that Ultrasound alone is not efficient in the assessment of advancement of liver disease. Furthermore, the high positive relation between AST/ALT ratio, APRI and Fib-4 scores with fibrosis stages in NAFLD patients suggests that they could be used clinically in combination with Fibroscan to predict significant fibrosis and cirrhosis and to avoid liver biopsy.

Key Words: Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Fibroscan; Cirrhosis; Hepatology

Core Tip: In this paper, we report on the correlation between aspartate transaminase/alanine transaminase ratio, aspartate transaminase platelet ratio index and Fib-4 scores with fibrosis stages in non-alcoholic fatty liver disease patients, suggesting that they could be used in combination with Fibroscan to predict significant fibrosis and cirrhosis. This is significant as this could be helpful in avoiding liver biopsy to assess liver fibrosis.

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INTRODUCTION

The liver is the largest internal solid organ and gland in the human body, its major functions include bile production — consisting of bile salts, cholesterol, bilirubin, and electrolytes, and water, absorbing and metabolizing bilirubin, supporting blood clots, fat and carbohydrates metabolization, vitamin and mineral storage that helps metabolizing proteins for digestion, filtering the blood and removing compounds from the body, being involved in the immune activity and production of albumin. An organ as complex as the liver can experience a range of problems including fatty liver disease, hepatitis, cirrhosis, hepatocellular carcinoma and cholangiocarcinoma[1].

Milder form of liver disease is non-alcoholic fatty liver disease (NAFLD) where excess fat accumulates in the liver of non-alcoholic patients. A small group of NAFLD patients may develop non-alcoholic steatohepatitis (NASH) where fat accumulation is accompanied by hepatocellular inflammation and different degrees of scarring that may lead to severe liver scarring and cirrhosis, causing the liver to lose its proper function[2,3]. Since those with NASH-related cirrhosis generally have worse outcomes, it becomes imperative to identify patients with advanced fibrosis for screening for complications of cirrhosis and receive specific treatments aimed to reverse or prevent progression of fibrosis[4,5].

NAFLD is part of the metabolic syndrome characterized by insulin resistance (diabetes or prediabetes), BMI in the overweight or obese region, abnormal blood lipid levels, and hypertension. Common etiologies of chronic liver disease are alcoholic hepatitis, viral hepatitis alcohol abuse, hemochromatosis, and metabolic disorders that result in hepatocellular injury and consequently liver fibrosis, cirrhosis, and/or hepatocellular carcinoma. Accurate assessment of the disease severity is important treatment planning.

NAFLD patients may have right upper quadrant pain, fatigue, pruritis, and hepatomegaly, but they are often asymptomatic and stigmata of chronic liver disease are uncommon. Risk factors for NASH include age > 45 years, an aspartate transaminase (AST) level > alanine transaminase (ALT) level,

insulin resistance, obesity, and portal hypertension[6].

Liver fibrosis is due to repetitive injury to the liver with the subsequent wound-healing[7]. Following hepatocyte damage (*e.g.*, acute viral hepatitis), parenchymal cells regenerate and replace cells that have undergone apoptosis or necrosis. However, the process is accompanied by an inflammatory response and wound-healing process involving deposition of a limited amount of extracellular matrix (ECM) in the liver parenchyma. If the hepatocellular injury persists or continues, this process of liver regenerations is overwhelmed and fails, and the normal liver parenchyma is substituted with an abundant ECM rich in fibrillar collagen[8]. This ultimately leads to cirrhosis and its associated bad outcomes and high mortality rates. Progression to end-stage liver disease is variable, but typically slow, developing over 2-4 decades in those with chronic liver disease[9].

The gold standard for diagnosis of NAFLD involves a thorough clinical history with pathological correlations. This is then confirmed through the detection of steatosis on liver biopsy and the exclusion of all other causes, including alcohol consumption[10]. Liver biopsy is the most dependable and specific method of detecting and staging fibrosis, diagnosing the cause of fibrosis, and determining whether it had progressed to cirrhosis[11,12].

However, liver biopsy has many limitations including: high cost, sampling error as it only represents 1/50000 of the liver volume and therefore does not accurately reflect the entire liver's architecture and fibrotic changes[13-16]. Consequently, biopsies from different areas depict varying stages of fibrosis and cirrhosis may be missed in up to 30% of patients, resulting in it not being an ideal prognostic indicator. Furthermore, variations of opinion between pathologists may lead to under staging of cirrhosis and this correlates with recounts of inter and intra-observer discrepancies of up to 20% in assessing fibrotic changes[17,18]. Given the prevalence of NAFLD and the invasive nature of a liver biopsy, it is not cost effective or practical to conduct this procedure on all patients at risk of NASH or fibrosis. Lastly, numerous risks and complications may occur with a 1% risk of significant complications post biopsy such as injury to adjacent organs, hemorrhage, bile leak and infection[13].

As a result of these limitations, the use of liver biopsies as a diagnostic tool has greatly reduced and led to the development of novel alternative noninvasive imaging modalities and laboratory tests for assessing liver fibrosis in NAFLD and NASH. These methods include AST/ALT ratio, AST platelet ratio index (APRI), Fibroscan, ultrasonography (US), and Fib-4 score which might be capable of overcoming the limitations of liver biopsy. They have been reported to be highly sensitive and specific in estimating liver fibrosis and predicting outcomes. In addition, they are liver specific, easy to perform, reliable, inexpensive, and are accurate tools for fibrosis staging and disease progression monitoring[16].

To assess the prevalence of fatty liver and detect moderate to severe fatty changes, non-invasive imaging modalities, such as US, are preferred[5,6]. It is recommended that this is the first-line imaging technique, as it is a reliable method for detection of moderate to severe steatosis in the liver. It is inexpensive, non-invasive, and readily available[16].

However, using ultrasound alone is an imperfect measure when staging fibrotic changes in the liver because of a lack of accuracy and reliability. Although ultrasound scoring systems have been proposed that assesses numerous factors to determine fibrosis stage such as evaluating liver edge bluntness, the size of the liver, the coarseness of the parenchyma and the nodularity of the liver surface, these findings are largely dependent on the equipment utilized[13,14]. Previously, clear correlations were not seen between the grayscale ultrasound findings and histological findings. However, recent developments in ultrasound technology have resulted in increased diagnostic accuracy when measuring hepatic fibrosis with the aforementioned ultrasound scoring system[15]. Nonetheless, ultrasound has various drawbacks as it still not able to distinguish NASH from simple steatosis or differentiate between steatosis and fibrosis and its accuracy when staging fibrosis is still questionable due to various influences[19,20].

Ultrasound is subject to intra-observer reproducibility and inter-observer variability that reduces the accuracy and reliability of pathological findings. Furthermore, factors such as patient body habitus may reduce its accuracy[16,17].

Using ultrasound-based techniques, Fibroscan was developed. It is one of the most extensively used noninvasive methods of assessing hepatic fibrosis[18]. It is simple, readily available, inexpensive, performed within a short procedure time (< 15 min), is able to provide accurate and immediate results, and can be performed at the bedside or in an outpatient clinic. This is through the usage of an ultrasound transducer probe, whereby an elastic shear wave is created through mild amplitude and low frequency vibrations that are transmitted through the hepatic tissue. Pulse-echo ultrasound is used to propagate the shear wave in order to measure the velocity (m/s) and provide an accurate liver stiffness measurement (LSM) within a specific volume of liver tissue The LSM is expressed in kilopascal (KPa) that correlate with fibrosis stage[19-21].

In several studies, the Fibroscan showed high sensitivity and specificity levels when predicting hepatic fibrosis and cirrhosis in patients with chronic liver disease[4]. With regards to cirrhosis, the specific and sensitivity of the Fibroscan approaches 90%, however when detecting liver fibrosis the sensitivity and specificity reduces to 70%-80%. Numerous variables can influence the Fibroscan as it utilizes ultrasound technology. For example, adipose tissue and the presence of fluid can alter the velocity of the shear wave. Furthermore, obesity, intercostal wall thickness, liver congestion, elevated portal vein pressure, operator inexperience, heart failure and ascites can all reduce the accuracy of the

Fibroscan[14,18].

Liver function tests are used frequently in clinical settings to assist in diagnosing and monitoring hepatic pathologies and damage through measuring enzyme levels and protein in the blood. Although these investigations vary in range, normal liver function is also tested through its ability to produce protein and clear bilirubin. Other liver function tests measure the enzymes released by hepatic cells in response to damage due to hepatic pathologies or secondary processes. However, liver function tests may produce false positive and negative results and, therefore, do not always indicate disease.

Some common liver function tests include[22]: Alanine transaminase (ALT or SGPT), Aspartate transaminase (AST or SGOT), prothrombin index (PT), International Normalized ratio (INR), partial thromboplastin time (PTT), albumin, bilirubin, gamma-glutamyl transferase (GGT), platelet count, glucose, ALP, Triglycerides, LDL, HDL and Cholesterol are all relevant markers of fibrotic change[23].

Recently, as an alternative to liver biopsy, cost-effective, noninvasive and reliable laboratory assessments for monitoring chronic liver disease have been developed[24]. Although these tests cannot truly distinguish NASH *vs* simple steatosis, patients with significant fibrosis, will by definition also have NASH as simple steatosis is not associated with an acceleration in hepatic fibrosis. Research has emphasized these readily availabe markers in assessing those with more advanced fibrosis. Hence, they may help guide treatment decisions and prediction of cirrhosis complications. However, at this point, no available test or modality can completely take the place of a histological analysis[25]. These include: ALT: one of the first markers of assessing liver disease. Serum ALT has been shown to be highly sensitive and specific, and therefore valuable to measure[25].

AST/ALT ratio is another widely available test. In NAFLD, an AST/ALT ratio > 1 is usually associated with progressive liver fibrosis or cirrhosis[4].

Developed in 2003 by Wai *et al*[13], the APRI in 2003 and is calculated as such:

$$\text{APRI} = \frac{\frac{AST\ Level\ (IU/L)}{AST\ (Upper\ Limit\ of\ Normal)\ (IU/L)}}{Platelet\ Count\ (109/L)} x100$$

Multiple studies had shown that it is highly accurate in predicting advanced fibrosis in different forms of liver disease with a higher correlation coefficient than platelet count, or AST level alone[18]. In a meta-analysis including 40 studies, researchers showed that an APRI score > 1.0 had a 76% sensitivity and 72% specificity in predicting cirrhosis. Furthermore, the investigators concluded that APRI score > 0.7 had a 77% sensitivity and 72% specificity in predicting significant hepatic fibrosis[25].

The FIB-4 score is calculated as such:

$$\text{FIB-4} = \frac{age\ (years)\ x\ AST\ level}{Platelet\ Count\ (109/L) x\ ALT}$$

FIB-4 score less than 1.45 had a negative predictive value of 90% for advanced fibrosis. On the other hand, a FIB-4 score greater than 3.25 had a 97% specificity and 65% positive predictive value for advanced fibrosis[26].

MATERIALS AND METHODS

Study design

This study was performed on 73 patients (45 males and 28 females) diagnosed with NAFLD/NASH based on Fibroscan examination and serum liver enzyme testing. Patients had undergone Fibroscan and Laboratory tests at Saint Georges Hospital University Medical Center, Ashrafieh, Beirut, Lebanon from 24 April 2018 to 6 September 2019. Patients with incomplete data or with ascites were excluded from analyses.

Fibroscans were performed on patients instructed to lay in the dorsal decubitus position along with the right arm put on the head. Estimations were made using a transducer probe starting in the right upper quadrant at the level of the right liver lobe. Up to 10 estimations were performed on every patient with outcomes expressed in KPa.

The following parameters were assessed for each patient: age, gender, total cholesterol, LDL, HDL, triglycerides, ALT, AST, albumin, bilirubin, GGT, PT, PTT, glucose, platelet counts. Relation between laboratory tests, age and gender and Fibroscan stages were analyzed.

APRI is calculated based on the following equation:

$$\text{APRI} = \frac{\frac{AST\ Level\ (IU/L)}{AST\ (Upper\ Limit\ of\ Normal)\ (IU/L)}}{Platelet\ Count\ (109/L)} x100$$

Fibrosis-4 is calculated based on the following equation:

$$\text{Fib-4} = \frac{age\ (years)\ x\ AST\ level}{Platelet\ Count\ (109/L) x\ ALT}$$

Where the laboratory reference normal range of serum ALT is 0-41 U/L. Normal upper serum ALT limits were defined as 40 U/L. The normal range of serum AST is 0-40 U/L, and the normal platelet count reference range is 150-450 × 10^3/mm^3. AST/ALT ratio was also calculated. The results of APRI, Fib-4 and AST/ALT ratio were correlated to Fibroscan results in order to determine the ability of Liver Stiffness measurement to predict fibrosis in patients with chronic liver disease.

Statistical analysis

Statistical analysis was performed using Megastat12, an Excel add-on that enables advanced statistical analyses within an Excel workbook. Means and *P* value were calculated and significance between fibrosis stages, age, gender, Laboratory tests, and scores were assessed. The analyses of the patient characteristics were estimated using one-way ANOVA or chi-square test as appropriate. ANOVA test is a way to compare two means from two independent groups to find out if experiment results are significant (*i.e.*, to reject the null hypothesis or accept the alternate hypothesis) by testing groups to see if there is a difference between them. However, a chi-squared test was used to determine whether a significant difference between expected and observed frequencies in one or more of the categories exists.

RESULTS

A total of 73 patients were identified, 45 males with mean age of 50.24 ± 15.71 and 28 females with mean age of 57.28 ± 15.07. The mean stiffness score was 9.48 ± 11.77 KPa, and the majority of patients did not exhibit fibrosis or advanced liver disease. According to the Metavir score, 29 patients were classified as F0 (Normal), 13 as F0-F1 (Normal-Mild Fibrosis stage), 14 as F2-F3 (Mild-Moderate Fibrosis stage), 5 as F3-F4 (Moderate-Severe Fibrosis stage) and 12 as F4 (Cirrhosis) (Table 1). Female patients (38% of all samples) exhibited higher stiffness scores than male patients (62% of all samples), but this difference was not significant 10.81 ± 15.42 *vs* 8.69 ± 9.02, respectively (*P* value = 0.23) (Figure 1).

The total mean age for our sample was 52.5 years with a 15.5 standard deviation. A significant actual age difference exists across fibrosis stages (*P* value = 0.0036). Furthermore, the ages was classified into two groups (20-49 and > 50 years) in order to study the correlation between specific age groups and fibrosis stages. A significant difference exists across fibrosis stages and the two age groups with a *P* value = 0.0302. Table 2 summarizes the results for the relation between the two age groups and the fibrosis stages where patients above 50 years old are more prone to advanced stages of fibrosis.

Interestingly, a significant positive correlation was observed between Bilirubin, ALP, PT INR, PTT, Glucose, and Platelet count when compared to Fibrosis stages as statistically confirmed using Chi-squared Test (*P* value = 0.0001, 0.033, 0.0011, 0.0054, 0.0063, and 0.0001 respectively). However, a significant negative correlation was observed between ALT, AST, Albumin, GGT, Cholesterol, LDL, HDL and Triglycerides when compared to fibrosis stages as statistically confirmed using Chi-squared Test (*P* value = 0.71, 0.07, 0.44, 0.22, 0.22, 0.07, 0.68, and 0.57 respectively) although it has no clinical significance (Table 3).

On the other hand, a positive correlation was also observed between the AST/ALT ratio, APRI, and Fib-4 scores when compared to Fibrosis stages (Table 4).

DISCUSSION

Data from patients at various stages of NAFLD were obtained in order to investigate if non-invasive biomarkers including AST/ALT ratio, APRI and Fib-4 scores can be used to assess liver fibrosis. The results confirmed that the fibrosis stages increased significantly with elevated AST/ALT ratio, Fib-4 and APRI scores.

Our data showed that based on Fibroscan exams, a high percentage of NAFLD patients had advanced stages of hepatic fibrosis. Furthermore, these findings were also supported by the strong parallel between the Fibroscan results and the FIB-4 scores, AST/ALT ratios, and APRI ratios.

A positive correlation between age and fibrosis stages was noted, while a negative correlation was observed between gender and fibrosis stages. Moreover, there was a negative correlation between both ALT and AST levels and fibrosis stages, which means that it is not effective to rely only on ALT and AST levels when clinically diagnosing patients with NAFLD. Studies conducted on the general population have demonstrated that ALT levels increase with age[27,28]. However, our results did not display a relationship between ALT levels and age. Moreover, studies have shown that a significant amount of NAFLD patients had normal or near-normal liver enzyme levels[29-31].

All patients in this cohort underwent baseline abdominal US that demonstrated steatosis; however as discussed earlier, US alone is not an ideal assessment tool of liver disease advancement. This further

Table 1 Table summarizing the distribution of patients over fibrosis stages and their corresponding percentages

Stage of fibrosis	Number of patients (%)
F0	29 (40)
F0-F1	13 (18)
F2-F3	14 (19)
F3-F4	5 (7)
F4	12 (16)
Total	73 (100)

Table 2 Table summarizing distribution of fibrosis stages over two groups of ages

Patient's age	Fibrosis stages					
	F0	F0-F1	F2-F3	F3-F4	F4	Total
20-49	17	7	7	0	2	33
> 50	12	6	7	5	10	40
Total	29	13	14	5	12	73

Table 3 Table summarizing the *P* value of the studied characteristics

Characteristic	*P* value
Age	0.0036
Gender	0.23
ALT	0.71
AST	0.07
Albumin	0.44
Cholesterol	0.22
GGT	0.22
LDL	0.07
HDL	0.68
ALP	0.033
Triglycerides	0.57
Bilirubin	0.0001
Glucose	0.0063
Platelet count	0.0001
PT INR	0.0011
PTT	0.0054

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; PPT: Partial thromboplastin time.

encourages the use of readily available biomarkers and Fibroscan plus abdominal US in the assessment of NAFLD instead of liver biopsy. Fibroscan is a helpful instrument that was recently developed to assess transient liver elasticity and expresses liver stiffness in KPa. Several studies have shown a direct relationship between liver stiffness on Fibroscan and fibrosis staging with liver biopsy. In a study conducted by Sandrin *et al*[32], the median hepatic elasticity was 4.2 KPa for F0 fibrosis score, 4.5-6.25 KPa for F1 fibrosis score, 5.5-7.8 KPa for F2 fibrosis score, 8.0-13.7 KPa for F3 fibrosis score, and 21–34 KPa for a F4 fibrosis score.

Table 4 Table showing differences in stiffness scores, aspartate transaminase/alanine transaminase ratio, aspartate transaminase platelet ratio index, and Fib-4 scores between patients with mild to moderate fibrosis and those with advanced fibrosis (expressed as mean ± SD)

	< F2 (*n* = 42)	> F2 (*n* = 31)	*P* value
AST/ALT ratio	0.84 ± 0.47	1.31 ± 1.1	0.0029
APRI score	0.35 ± 0.26	1.08 ± 1.2	3.59×10^{-7}
Fib-4 score	1.07 ± 0.73	3.41 ± 3.44	9.11×10^{-9}

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: Aspartate transaminase platelet ratio index.

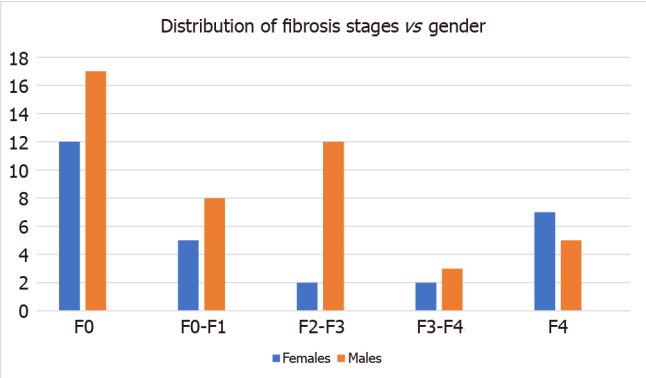

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Figure 1 Graph showing distribution of fibrosis stages over gender.

This study's main finding is that AST/ALT ratio, APRI and Fib-4 score have high positive relation with advanced fibrosis in patients with NAFLD. This suggests that they could be clinically used to avoid liver biopsy. A large numbers of patients with NAFLD are being referred for evaluation, and these non-invasive tests could help reduce the quantity of liver biopsies performed. This would benefit patients in the way of cost staving as well as by directing liver biopsies to patients more likely to be exhibiting advanced disease. Fallatah *et al*[23] showed results that are similar to this study where a strong positive correlation was observed between AST/ALT ratio, APRI and Fib-4 scores and fibrosis scores[26].

Liver enzyme levels in NAFLD patients fluctuate. When they are elevated, the increase is often mild and usually restricted to one or both of AST and ALT. Nevertheless, it is important to emphasize that although elevated ALT is generally associated with histological NASH/NAFLD, a large number of NASH/NAFLD patients had normal or near-normal liver enzyme levels. Therefore, ALT level alone cannot be used to rule out significant liver disease in patients suspected of having NASH/NAFLD.

This study also showed that platelet count, Bilirubin, PT INR, PTT, and Glucose levels are significantly related to fibrosis stages and can be used as independent predictors for fibrosis.

The obvious major advantage of using these simple scoring systems is that the labs they are derived from are readily available. Clearly, AST/ALT ratio is the simplest to calculate. Fib-4 and APRI scores require more complex calculation, but the relevant details can easily be entered onto many of the medical calculator smartphone applications with instantaneous results. Therefore, introduction of the use of these tests into daily practice should be relatively simple and will not result in extra costs.

The limitations of the study were that it took place in a tertiary hospital where there may be selection bias, a higher number of patients will be required for future studies, and no correlation with liver biopsy, the gold standard of NAFLD diagnosis, was possible at that time.

Based on this study, I would recommend the use of Fibroscan in combination with Laboratory tests and non-invasive Laboratory biomarkers including AST/ALT ratio, APRI and Fib-4 scores for assessing liver fibrosis in patients with early fibrosis stages, for their great advantages and cost-effective benefits, and limiting the use of liver biopsy for patients with advanced fibrosis stages.

CONCLUSION

In conclusion, this study showed that Ultrasound alone is not efficient in assessing the advancement of liver disease. Furthermore, the high positive correlation of AST/ALT ratio, APRI and Fib-4 scores from

one side when compared to fibrosis stages in patients with NAFLD suggests that they could be clinically used in combination with Fibroscan to predict significant fibrosis and cirrhosis and to avoid liver biopsy. This benefits to patients with cost savings and less invasive procedures.

As a future perspective, the use of these simple scoring systems that are derived from readily available clinical and laboratory tests, using a pre-designed Excel sheet, can give an instant result, therefore, introducing these tests into daily practice should be rather simple and will not result in extra costs[32].

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), through to advanced fibrosis and cirrhosis. Many patients with NAFLD remain undiagnosed and recognizing those at risk is very crucial. Although liver biopsy is the gold standard method for diagnosing and staging NAFLD, non-invasive imaging and lab modalities are also very promising in diagnosing these diseases.

Research motivation

The main motivation for this research was to objectively assess existing non-invasive modalities alone or in combination and determine whether they could accurately help in diagnosing and staging liver disease, foregoing the need for invasive diagnostics such as liver biopsy.

Research objectives

The objective of this research was to combine clinical, lab, and imaging data and assess their ability to accurately diagnose and stage NAFLD without invasive diagnostics such as liver biopsy.

Research methods

This study was conducted on NAFLD/NASH patients (n = 73) who underwent Fibroscan examinations at Saint George Hospital University Medical Center over 17 mo in order to assess liver fibrosis. Obtained Fibroscan results were correlated to laboratory tests and calculated aspartate transaminase (AST)/alanine transaminase (ALT) ratio, AST platelet ratio index (APRI) score and Fibrosis-4 score.

Research results

A significant age difference was observed across fibrosis stages of investigated patients. The mean stiffness score was 9.48 ± 11.77 KPa. A significant positive correlation was found between Bilirubin, PT INR, partial thromboplastin time, glucose, and platelet count when compared across fibrosis stages, in addition to AST/ALT ratio, APRI, and Fib-4 scores.

Research conclusions

We conclude that ultrasound alone is not efficient in the assessment of the advancement of liver disease. Furthermore, the high positive relation between AST/ALT ratio, APRI and Fib-4 scores with fibrosis stages in NAFLD patients suggests that they could be used clinically in combination with Fibroscan to predict significant fibrosis and cirrhosis and to avoid liver biopsy.

Research perspectives

More research and data is required to make better recommendations. As more and more fields of clinical medicine forego invasive diagnostics in favor of their non-invasive counterparts, the data for such a shift in the diagnosis and staging of NAFLD is encouraging.

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FOOTNOTES

Author contributions: All authors contributed equally to the drafting of the manuscript, development of key ideas, and subsequent revisions.

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ORIGINAL ARTICLE

Retrospective Study

Testosterone therapy reduces hepatic steatosis in men with type 2 diabetes and low serum testosterone concentrations

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is highly prevalent in people with diabetes with no available treatment.

AIM

To explore the effect of testosterone treatment on liver. Testosterone therapy improves insulin resistance and reduces total body fat, but its impact on the liver remains poorly studied.

METHODS

This secondary analysis of a 40 wk, randomised, double-blinded, placebo-controlled trial of intramuscular testosterone undecanoate in men with type 2 diabetes and lowered serum testosterone concentrations evaluated the change in hepatic steatosis as measured by liver fat fraction on magnetic resonance imaging (MRI).

RESULTS

Of 88 patients enrolled in the index study, 39 had liver MRIs of whom 20 received testosterone therapy and 19 received placebo. All patients had > 5% hepatic steatosis at baseline and 38 of 39 patients met diagnostic criteria for NAFLD.

Median liver fat at baseline was 15.0% (IQR 11.5%-21.1%) in the testosterone and 18.4% (15.0%-28.9%) in the placebo group. Median ALT was 34units/L (26-38) in the testosterone and 32units/L (25-52) in the placebo group. At week 40, patients receiving testosterone had a median reduction in absolute liver fat of 3.5% (IQR 2.9%-6.4%) compared with an increase of 1.2% in the placebo arm (between-group difference 4.7% $P < 0.001$). After controlling for baseline liver fat, testosterone therapy was associated with a relative reduction in liver fat of 38.3% (95% confidence interval 25.4%-49.0%, $P < 0.001$).

CONCLUSION

Testosterone therapy was associated with a reduction in hepatic steatosis in men with diabetes and low serum testosterone. Future randomised studies of testosterone therapy in men with NAFLD focusing on liver-related endpoints are therefore justified.

Key Words: Hepatic steatosis; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Testosterone therapy; Testosterone undecanoate; Type 2 diabetes

Core Tip: Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent disease with no current effective treatment. This study demonstrates a reduction in hepatic steatosis in men with type 2 diabetes and low testosterone who received testosterone therapy as part of a randomised controlled trial and provides justification for larger scale studies to assess the effects of testosterone therapy as a treatment for NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined by the presence of hepatic steatosis on imaging or histology in the absence of other secondary causes of hepatic steatosis[1]. It has an estimated overall global prevalence of 25%[2]. Patients with type 2 diabetes are at significantly higher risk of NAFLD due to a bidirectional pathophysiological link between the two disease entities[3,4]. A meta-analysis of 19 observational studies found that patients with NAFLD had a higher incidence of type 2 diabetes than those without, and that the incidence of type 2 diabetes further increased in patients with radiologically higher steatosis scores[5]. Furthermore, type 2 diabetes and features of the metabolic syndrome are known to be independent risk factors for liver fibrosis progression, cirrhosis, hepatocellular carcinoma and death in patients with NAFLD[6,7].

Low serum testosterone has been associated with an increased risk of NAFLD in men after adjustment for other metabolic risk factors[8-10]. A meta-analysis of 13721 men from cross-sectional, cohort and case-control studies reported that serum total testosterone (TT) concentrations were on average 2.8nmol/L lower in men with NAFLD than those without[11]. Subsequently, in a study of 159 men with NAFLD, lower testosterone concentrations were associated with a higher risk for the presence, and increasing severity, of non-alcoholic steatohepatitis, a recognised risk factor for liver disease progression in NAFLD[12].

Testosterone therapy has been shown to ameliorate hepatic steatosis and necroinflammation in animal models of male hypogonadism induced by castration [13,14] Only a small number of studies have examined the effect of exogenous testosterone in men with NAFLD and low testosterone concentrations, and these report mixed results. A placebo controlled study of obese men with severe obstructive sleep apnoea and testosterone concentrations that ranged from low normal to normal demonstrated that testosterone therapy reduced liver fat as measured by computed tomography[15]. A study of 21 men with low serum testosterone concentrations and NAFLD reported that treatment with a novel oral testosterone prodrug improved liver fat as measured by magnetic resonance imaging proton density fat fraction (MRI-PDFF) in 81% of patients[16]. Two small studies of testosterone therapy in men with type 2 diabetes and low testosterone concentrations and one in men with mobility limitation and low testosterone concentrations conversely showed no significant change in hepatic fat compared to placebo as measured by MRI[17-19].

The aim of this study was to evaluate the effect of testosterone therapy on liver fat fraction and to determine other factors associated with changes in liver fat in a population of men with low testosterone concentrations and type 2 diabetes.

MATERIALS AND METHODS

Study design

The study design, eligibility and protocol of the trial is described in full in the original publication[20]. The trial was registered on the Australian and New Zealand Clinical Trials registry (trial number NCT00613782). This was a 40 wk, randomised, double-blind placebo-controlled trial conducted at single tertiary referral centre in Australia. Ethics approval for the study was granted by the Human Research Ethics Committee, Austin Health.

Eligibility

Eligible participants were men aged 35-70 years with a history of type 2 diabetes and a fasting, early morning TT concentration of ≤ 12.0 nmol/L (346 ng/dL), as measured by electrochemiluminescence immunoassay (ECLIA) and averaged across two readings.

Exclusion criteria for the trial were testosterone therapy within five years of randomisation, screening TT concentrations < 5.0 nmol/L (144 ng/dL), established pituitary or testicular disorder, luteinising hormone level > 1.5x upper limit of normal, prostate-specific antigen level > 4 μg/L, a history of urinary obstruction, prostate cancer or breast cancer, haematocrit > 0.50, uncontrolled hypertension (> 160/90 mmHg despite treatment), untreated obstructive sleep apnoea, estimated glomerular filtration rate < 30 mL/min, cardiac insufficiency, active malignancy, unstable psychiatric disease, weight > 135 kg, use of glucagon-like peptide-1 agonist therapy or very low-calorie diet, or an HbA1c level > 8.5% (69 mmol/mol).

Randomisation and study intervention

Eligible participants were randomly assigned in a concealed 1:1 allocation to either testosterone or placebo therapy. Intramuscular testosterone undecanoate 1000 mg or a visually identical placebo was administered at 0, 6, 18 and 30 wk. Participants were followed up for a total of 40 wk.

Outcomes and measurements:

In this post-hoc analysis we assessed as the primary outcome the change in liver fat fraction, as measured using MRI liver in-phase (IP) and opposed-phase (OP) T1 sequences and expressed as percentage fat. The primary outcome measure for the index study was the change across groups and time from baseline in the homeostasis model assessment index of insulin resistance using a computer-based calculation rather than the original linear equation.

Clinical and biochemical variables that were assessed in participants at baseline and 40 wk included body weight, body mass index (BMI), waist circumference, alanine aminotransferase (ALT), gamma-glutamyl transferase, alkaline phosphatase, bilirubin, albumin, international normalised ratio, TT, sex hormone-binding globulin, calculated free testosterone (cFT), fasting glucose, HbA1c and lipid profile. Free testosterone levels were calculated using Vermeulen's formula, as previously described[21]. Additional measurements of total body mass, lean mass and fat mass were assessed by DXA scan (DXA Prodigy, Version 10.51; GE Lunar, Madison, WI) at 0 and 40 wk.

MRI assessment of liver fat

MRI scans of the abdomen were obtained at enrolment and after 40 wk of therapy using a 3 Tesla MRI scanner (Siemens, Erlangen, Germany). As part of the initial study, subcutaneous and visceral adipose tissue volume was calculated for each patient by analysis of five 10-mm slices around the L4 vertebral superior endplate using the SliceOmatic program software (version 4.2; Tomovision, Montreal, Canada) as previously described[20].

MRI images obtained before and after therapy were reanalysed for the present study by an expert liver radiologist who was blinded to treatment allocation. Liver fat fraction before and after therapy was calculated using conventional chemical shift imaging with IP and OP T1 sequences. An averaged signal intensity for IP and OP was obtained from three 5 to 10 cm^2 regions within hepatic parenchyma of each scan. The fat fraction was calculated using the formula (IP - OP)/2 × IP. This technique has been previously validated to accurately estimate liver fat fraction to levels of up to 50%[22-27].

Statistical analysis

Continuous data are displayed as mean (standard deviation) for normally distributed data or median [interquartile range] for skewed data. Categorical data are presented as number (percentage). The Student *t*-test and Mann-Whitney test were used for normal and non-normal data, respectively. Exploratory data analysis included pairwise examination for correlation that may introduce multicollin-

earity into the final regression model. Linear regression modelling was used to identify variables associated with week 40 Liver fat proportion, which was the primary outcome variable, whilst controlling for baseline liver fat proportion (analysis of covariance). The outcome variable was natural log-transformed for these analyses as use of the untransformed values violated model assumptions. Univariate regression with all clinically relevant variables was performed and those with P values < 0.20 were selected for inclusion in the multivariable model. A manual elimination process was undertaken to arrive at the final model. The coefficients in the final model were back transformed to report relative change in liver fat proportion between groups (geometric mean). Standard regression diagnostics were performed to ensure non-violation of the underlying model assumptions. Analyses were performed in R v4.02[28].

RESULTS

Baseline characteristics

A total of 39 men with type 2 diabetes and low serum testosterone were included in our analysis, of whom 20 received testosterone therapy and 19 received placebo. They represent a subset of 88 patients from the original trial who underwent MRI liver scanning both at the start and end of treatment. Remaining study participants did not undertake MRI scans either due to incompatible or unverifiable metal implants, inability to fit in the MRI machine or claustrophobia.

The baseline characteristics of our study participants are shown in Table 1 and are similar between the testosterone and placebo therapy group with the exception of lean mass which was lower in the testosterone group and HDL which was higher in the testosterone group compared to placebo. Compared to the participants in the index study who did not have MRI scans, our cohort had lower baseline BMI, visceral adiposity, waist circumference and higher cholesterol levels, but otherwise had comparable baseline characteristics as shown in Table 2. All patients had hereditary haemochromatosis and other causes of low testosterone excluded and no patients were taking medications were taking steatogenic medications during the study period. Five participants in the testosterone group and three in the placebo group were taking glucagon-like peptide-1 analogues or thiazolidinediones at baseline which continued throughout the study at stable doses. No other patients were taking medications known to directly influence liver fat (Supplementary Table 1). Median alcohol consumption was 3.5 (IQR 0-8.5) standard drinks per week in the testosterone group and 4 (IQR 0.5-7) standard drinks per week in the placebo group. One patient in the testosterone group had heavy alcohol consumption (defined as > 14 units per week), reporting 28 standard drinks per week during the study period. No patients in the placebo group had heavy alcohol consumption.

All patients in the study had significant hepatic steatosis, defined as a liver fat fraction ≥ 5%. All patients but the one patient with heavy alcohol consumption had secondary causes of hepatic steatosis excluded and hence met diagnostic criteria for NAFLD as defined by the American Association for the Study of Liver Diseases[1]. Median liver fat fraction at baseline was 15.0% (IQR 11.5%-21.1%) in the testosterone group and 18.4% (IQR 15.0%-28.9%) in the placebo group (P = 0.14). The median values of all liver function tests were within normal limits for both groups at baseline (Table 1).

Descriptive analysis of change in hepatic steatosis

In the testosterone group, the median absolute reduction in liver fat fraction was 3.5% (IQR 2.9%-6.4%) and median relative reduction was 27.3% (IQR 18.0%-37.6%). Liver fat fraction increased in the placebo group, with a median absolute increase in liver fat fraction of 1.2% (IQR -2.6-3.0%) and median relative increase of 6.8% (IQR -7.3-15.3%). At week 40, the median liver fat fraction was 9.8% (IQR 8.6%-13.5%) in the testosterone group and 19.6% (IQR 17.8%-29.1%) in the placebo group.

The change in absolute liver fat ranged from -15.3% to +3.6% in the testosterone group, with 18 of 20 individuals achieving a reduction in liver fat (Figure 1). The change in absolute liver fat ranged from -5.9% to +27.5% in the placebo group, with eight of 19 individuals achieving a reduction in liver fat (Figure 1). One patient from the placebo group was an outlier who had a 27.5% absolute increase in liver fat associated with a 32% increase in visceral adipose tissue (VAT) and a 7% increase in body weight. At week 40, there were no significant changes in liver function tests in either treatment group.

Factors associated with change in liver fat

Univariate regression analysis found that testosterone therapy, BMI, baseline liver fat and week 40 TT and cFT concetrations were all significantly associated with changes in liver fat. Week 40 cFT and TT concentrations were, however, highly correlated (hence a decision to use only cFT in the development of the multivariate model.

The multivariate model was significant ($F_{(2,35)}$(54.13), R^2 = 0.807, P < 0.001) and included treatment arm controlled for baseline liver fat proportion. In detail, those in the testosterone treatment group had a significantly lower follow-up liver fat proportion than those in the placebo group (P < 0.001). The Beta co-efficient of -0.48 (-0.67, -0.29) is on the log-transformed scale. Back-transformation of the co-efficient and associated 95% confidence interval returned values of -0.383 (-0.490, -0.254). Thus, in this model,

Table 1 Baseline characteristics of study participants

Characteristic	Testosterone group (*n* = 20)	Placebo group (*n* = 19)	*P* value
Age (yr)	62 (58-67)	60.4 (6.5)	0.5
Duration of type 2 diabetes (yr)	8 (6-14)	7 (5-9)	0.2
Insulin therapy, *n* (%)	3 (15)	2 (10.5)	> 0.9
Weight, kg	92 (86-101)	98 (92-105)	0.15
BMI, kg/m^2	30.3 (27.3-31.9)	32.0 (29.7-35.4)	0.14
Waist circumference, cm	106 (102-116)	112 (106-119)	0.4
Fat mass, g	30788 (24875-38117)	31758 (29332-35286)	0.8
Lean mass, g	57090 (51658-62688)	62140 (66226-65844)	0.029
ALT, IU/L	34 (26-38)	32 (25-52)	0.8
ALP, IU/L	72 (60-82)	58 (54-69)	0.057
GGT, IU/L	30 (21-38)	32 (25-45)	0.5
TT, nmol/L (ECLIA)	9.8 (7.2-12.0)	7.8 (6.0-10.8)	0.2
cFT, pmol/L (ECLIA)	216 (151-272)	176 (146-224)	0.3
SHBG, nmol/L	28 (20-34)	28 (23-32)	0.8
LH, IU/L	4.5 (3.4-7.0)	4.3 (3.4-6.0)	0.7
Fasting glucose, mmol/L	8.9 (6.8-10.3)	8.5 (7.7-9.8)	0.6
HbA1c, %	6.6 (6.4-7.2)	7.0 (6.7-7.3)	0.071
Cholesterol, mmol/L	4.4 (4.0-5.0)	4.6 (4.0-4.9)	> 0.9
LDL, mmol/L	2.4 (2.0-3.0)	2.7 (2.0-3.0)	0.7
HDL, mmol/L	1.2 (1.0-1.4)	1.0 (0.7-1.2)	0.03

Data are median (IQR). ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; TT: Total testosterone; cFT: Calculated free testosterone; SHBG: Sex hormone-binding globulin; LH: Luteinising hormone; LDL: Low-density lipoprotein; HDL: High density lipoprotein.

testosterone therapy was associated with a 38.3% relative reduction in liver fat proportion (95% confidence interval 25.4% to 49.0% reduction) after controlling for baseline liver fat proportion compared to those receiving placebo. No other covariates reduced the unexplained residual variance in the model significantly to this estimate of between-group difference.

Changes in testosterone concentrations

Baseline median TT concentrations as measured by ECLIA were 9.8 [7.2-12.00] nmol/L and 7.8 [5.9-10.8] nmol/L in the testosterone and placebo groups, respectively. After 40 wk of therapy, trough TT concentrations increased to 12.9 [11.9-15.0] nmol/L in the testosterone group and 8.8 [7.3-10.8] nmol/L in the placebo group ($P < 0.001$). Baseline median cFT concentrations were 216 [151-272] pmol/L and 176 [146-224] pmol/L in the testosterone and placebo groups respectively. After 40 wk of therapy cFT increased to 322 [231-392] pmol/L in the testosterone group and 193 [167-250] pmol/L in the placebo group ($P < 0.001$).

Changes body weight, glycaemic control, liver function tests and body composition:

At 40 wk patients receiving testosterone therapy had no statistically significant changes in overall body weight, BMI, waist circumference, fasting glucose or HbA1c compared to placebo (Table 3).

At week 40, compared to placebo, patients receiving testosterone therapy had significantly increased lean mass (mean change 2111 g (1148-3073) relative to placebo, $P < 0.001$) and significantly decreased fat mass (mean change -2969 g (-3998 to -1941) relative to placebo, $P < 0.001$). All patients receiving testosterone therapy had reductions in fat mass and 19/20 patients receiving testosterone therapy had increases in lean mass (Supplementary Figure 1). Subcutaneous adipose tissue (SAT) was significantly decreased at week 40 in patients receiving testosterone therapy compared to placebo (mean change -359 cm^3 (-570 to -147) relative to placebo, $P = 0.002$) but VAT was unchanged (Supplementary Figure 2).

Table 2 Baseline characteristics of our participants who had magnetic resonance imaging scans compared to index study participants who did not have magnetic resonance imaging scans

Characteristic	Non-MRI scan group (*n* = 49)	MRI scan group (*n* = 39)	*P* value
Age, yr	62 (58-68)	62 (58-67)	0.6
Duration of type 2 diabetes, yr	9 (4-12)	8 (5-11)	> 0.9
Insulin therapy, *n* (%)	13 (26.5)	5 (12.8)	0.19
Weight, kg	102 (89-111)	95 (90-104)	0.12
BMI, kg/m^2	33.4 (31.2-35.9)	31.5 (28.2-35.3)	0.012
Waist circumference, cm	116 (109-123)	111 (104-118)	0.024
Fat mass, g	32696 (29299-39109)	31832 (26820-37809)	0.2
Lean mass, g	62004 (58056-66170)	60,049 (54278-64848)	0.12
ALT, IU/L	34 (25-50)	32 (25-42)	0.7
ALP, IU/L	71 (56-82)	66 (54-75)	0.2
GGT, IU/L	30 (24-43)	32 (24-43)	0.8
TT, nmol/L (ECLIA)	8.5 (7.3-10.5)	8.1 (6.7-11.4)	0.8
cFT, pmol/L (ECLIA)	176 (151-230)	194 (148-253)	0.6
SHBG, nmol/L	28 (24-32)	29 (22-34)	> 0.9
LH, IU/L	4.5 (3.5-6.2)	4.5 (3.4-6.4)	0.9
Fasting glucose, mmol/L	8.2 (7.0-10.6)	8.6 (7.3-9.9)	> 0.9
HbA1c, %	7.1 (6.6-7.7)	6.9 (6.5-7.3)	0.4
Cholesterol, mmol/L	4.1 (3.5-4.7)	4.6 (4.0-4.9)	0.005
LDL, mmol/L	2.0 (1.6-2.4)	2.7 (2.0-3.1)	< 0.001
HDL, mmol/L	1.0 (0.9-1.2)	1.1 (0.9-1.2)	0.5
SAT, cm^3	4095 (3526-5593)	4661 (3137-5385)	0.7
VAT, cm^3	4786 (3,642-5,617)	3634 (2780-4823)	0.044

Data are median (IQR). MRI: Magnetic resonance imaging; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; TT: Total testosterone; cFT: Calculated free testosterone; SHBG: Sex hormone-binding globulin; LH: Luteinising hormone; LDL: Low-density lipoprotein; HDL: High density lipoprotein; SAT: Subcutaneous adipose tissue; VAT: Visceral adipose tissue.

Adverse events

Testosterone therapy was well-tolerated with rare serious adverse events that were not significantly different compared to placebo as outlined in Table 4 of the index study[20]. Patients receiving testosterone therapy had significant increases in haemoglobin and haematocrit at week 40 compared to placebo ($P < 0.001$ for both).

DISCUSSION

In this study of men with type 2 diabetes and low testosterone concentrations, intramuscular testosterone undecanoate over 40 wk significantly reduced liver fat as measured by MRI in an adjusted model compared to placebo.

Our results are consistent with a recent study in which 32 men with low serum testosterone, of whom 8 (25%) had type 2 diabetes, were given a novel oral testosterone preparation LPCN 1144 for 16 wk. In this study, 21/32 patients met diagnostic criteria for NAFLD, and a mean relative reduction in liver fat of 33% as measured by MRI-PDFF was demonstrated in 17 of these 21 patients[16]. However, in contrast to our study, the LPCN 1144 study did not include a control group. Whilst three previous studies reported no change in liver fat in men with low testosterone concentrations after testosterone treatment [17-19], these all had some limitations including short follow-up time of six months, small sample size [17,18], and one study estimated liver fat changes by a less accurate method of measuring change in liver volume and relaxometry[19]. Additionally, patients in our study received intramuscular

Table 3 Changes in body composition and metabolic parameters

	Testosterone group (*n* = 20)	Placebo group (*n* = 19)	*P* value
Weight, kg			
Baseline	92 (86-101)	98 (91-105)	0.55
40 wk	90 (86-100)	96 (94-100)	
BMI, kg/m^2			
Baseline	30.3 (27.3-31.9)	32.0 (29.7-35.4)	0.64
40 wk	29.3 (27.4-31.8)	31.8 (30.0-34.4)	
Waist circumference, cm			
Baseline	106 (102-116)	112 (106-118)	0.15
40 wk	108 (101-115)	114 (110-118)	
Fasting glucose, mmol/L			
Baseline	8.9 (6.8-10.3)	8.6 (7.7-9.8)	0.93
40 wk	8.3 (6.5-10.5)	9.1 (7.7-9.9)	
HbA1c, %			
Baseline	6.6 (6.4-7.2)	7.0 (6.8-7.4)	0.95
40 wk	7.0 (6.3-7.4)	7.3 (7.0-7.7)	
Fat mass, g			
Baseline	30788 (24875-38117)	31758 (29332-35286)	< 0.001[a]
40 wk	27272 (21948-34558)	32089 (29869-34492)	
Lean mass, g			
Baseline	57090 (51658-62688)	62140 (66226-65844)	< 0.001[a]
40 wk	58236 (55150-63077)	61070 (57408-64833)	
SAT, cm^3			
Baseline	4445 (2928-5669)	4302 (3828-5254)	0.004[a]
40 wk	4140 (2769-5046)	4330 (3741-5179)	
VAT, cm^3			
Baseline	3574 (2460-4711)	3864 (3267-4703)	0.63
40 wk	3248 (2280-4634)	3966 (3462-4699)	
ALT, U/L			
Baseline	34 (26-38)	32 (25-52)	0.27
40 wk	32 (25-36)	44 (29-61)	
GGT, U/L			
Baseline	30 (21-38)	32 (25-45)	0.28
40 wk	28 (20-41)	33 (28-56)	
ALP, U/L			
Baseline	72 (60-82)	58 (54-69)	0.04[a]
40 wk	70 (60-78)	60 (58-72)	
Total cholesterol, mmol/L			
Baseline	4.4 (4.0-5.0)	4.6 (4.0-4.9)	0.008[a]
40 wk	3.7 (3.5-4.5)	4.5 (4.1-5.1)	
LDL, mmol/L			
Baseline	2.4 (2.0-3.0)	2.7 (2.0-3.0)	0.21

40 wk	2.1 (1.8-2.7)	2.4 (1.9-3.2)	
HDL, mmol/L			
Baseline	(1.01-1.40)	1.02 (0.74-1.18)	0.02[a]
40 wk	1.19 (0.93-1.36)	0.98 (0.82-1.09)	
Triglycerides, mmol/L			
Baseline	1.6 (1.3-2.0)	2.0 (1.4-2.4)	0.25
40 wk	1.4 (1.0-1.9)	1.9 (1.6-3.0)	

[a]*P* values are an exploratory univariate analysis of between-group week 40 values after adjusting for baseline. No adjustment for multiple comparisons were made given the exploratory nature. Data are median (IQR). BMI: Body mass index; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; TT: Total testosterone; cFT: Calculated free testosterone; SHBG: Sex hormone-binding globulin; LH: Luteinising hormone; LDL: Low-density lipoprotein; HDL: High density lipoprotein; SAT: Subcutaneous adipose tissue; VAT: Visceral adipose tissue.

Table 4 Univariate analysis table of factors affecting change in hepatic steatosis

Characteristic	*n*	Beta	95%CI	*P* value
Testosterone	38[1]	-0.66	-1.0, -0.37	< 0.001
Age (yr)	38	-0.03	-0.05, 0.00	0.075
BMI (kg/m^2)	38	0.06	0.02, 0.10	0.003
Baseline liver fat proportion	38	4.6	3.3, 6.0	< 0.001
Baseline total testosterone (nmol/L)	38	-0.03	-0.09, 0.03	0.3
Baseline free testosterone (nmol/L)	38	0.00	0.00, 0.00	0.5
Baseline visceral fat (g)	38	0.00	0.00, 0.00	0.4
Follow up total testosterone (nmol/L)	37	-0.05	-0.09, -0.02	0.006
Follow up free testosterone (nmol/L)	37	0.00	0.00, 0.00	0.005
Follow up visceral fat (g)	38	0.00	0.00, 0.00	0.2

[1]One participant did not have follow-up testosterone concentration. BMI: Body mass index.

testosterone administered by study investigators, hence eliminating adherence issues whereas testosterone gel was used in two of the three studies, making adherence difficult to assess[17,19].

Secondary benefits of testosterone therapy in this study included increases in lean muscle mass and reduction in total fat mass, a result consistent with numerous previous testosterone trials across multiple population groups[29,30]. Interestingly, despite a reduction in subcutaneous adiposity, visceral adiposity was unchanged after testosterone therapy suggesting that testosterone may have a direct effect on hepatic steatosis. This is consistent with animal studies showing that the androgen receptor is expressed in the liver and testosterone has biological effects in the liver affecting lipid metabolism and glucose homeostasis[31]. Furthermore, animal models show that testosterone differentially regulates the expression of key targets of lipid and glucose metabolism in a tissue specific manner, with regional differences in action of testosterone on SAT compared to VAT[32]. Total cholesterol levels were also significantly lower in patients treated with testosterone therapy. NAFLD most frequently occurs in the context of the metabolic syndrome, with associated visceral adiposity, dyslipidaemia, and other cardiovascular risk factors[33]. The primary cause of death in such patients is cardiovascular disease, and thus the changes in body composition and lipid profile observed in our study may favourably change cardiovascular risk in patients with NAFLD, but this study was not powered or designed to assess this.

All patients in our study had significant steatosis, and 38/39 patients met diagnostic criteria for a diagnosis of NAFLD. The global prevalence of NAFLD in men with type 2 diabetes is 55%, markedly lower than our cohort's prevalence[34]. This difference is unsurprising as our cohort consisted of older, obese men with a longstanding history of type 2 diabetes and low serum testosterone concentrations[8]. Given concurrent type 2 diabetes is the strongest risk factor for progression of NAFLD to significant fibrosis[35], demonstrating efficacy in this population represents an important finding with potentially important clinical implications.

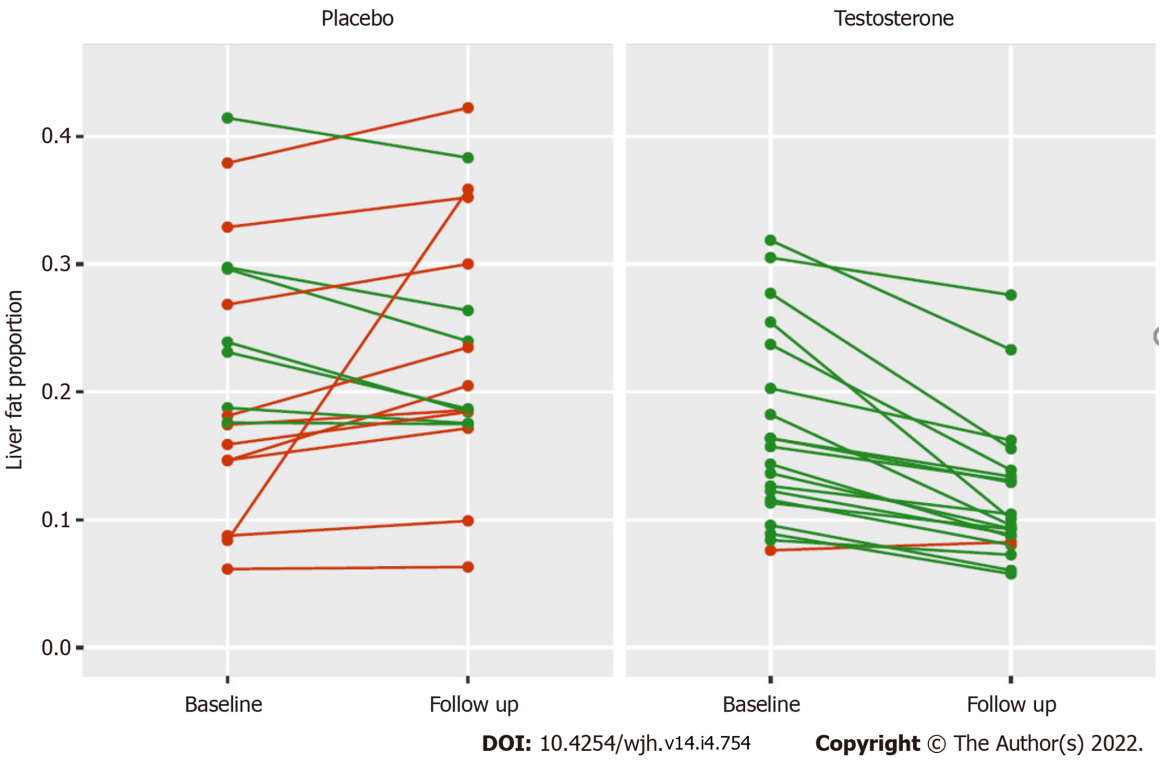

Figure 1 Absolute change in liver fat in all patients receiving placebo and testosterone therapy from baseline to 40 wk follow up, reported as proportion of fat. Lines connect observations from the same participant. Red indicates an increase, green a decrease in liver fat proportion.

Our study has several limitations. This study was a sub-analysis of a randomised controlled trial that was not designed to evaluate liver-related endpoints and not all index cases had liver MRI for fat assessment. However, those included in this study did not appear to significantly differ from those who were not, apart from our cohort having lower weight and waist circumference. Baseline and end of treatment median liver function tests were within the normal ranges for both treatment groups, suggesting that patients in our study did not have significant hepatic inflammation. Finally, fat fraction was assessed using conventional MRI-IP and OP T1 sequences rather than MRI-PDFF, which was not widely available at the time of the index study. Although changes in liver fat MRI-IP and OP T1 sequences do not have the same evidence for correlation with steatohepatitis improvement, it has been shown to accurately quantify liver fat up to 50%, a threshold which was not exceeded in our study[22-24].

Despite these limitations, we identified patients who met diagnostic criteria for NAFLD who had a significant reduction in hepatic steatosis with testosterone treatment while liver fat in most placebo patients increased. The change in hepatic steatosis was measured by MRI scan, which is increasingly recognised as a reasonable surrogate endpoint for histologic response[36]. The two cohorts were well matched with respect to medication use, in particular pertaining to diabetic agents that are known to impact hepatic steatosis and all 39 patients in our study maintained a stable diabetic regimen throughout the trial.

CONCLUSION

This study shows an association between testosterone treatment and a significant reduction in liver fat in men with type 2 diabetes and low testosterone concentrations. These data provide a strong rationale to perform large-scale randomised studies testing the effect of testosterone therapy in high-risk NAFLD patients with low serum testosterone, focusing on liver-specific endpoints including liver inflammation and fibrosis progression.

ARTICLE HIGHLIGHTS

Research background

Testosterone levels are commonly low in men with type 2 diabetes and most men with type 2 diabetes

have hepatic steatosis from non-alcoholic fatty liver disease (NAFLD). Animal models show that low testosterone states from castration results in hepatic steatosis and that testosterone replacement improves hepatic steatosis.

Research motivation

Hepatic steatosis occurs in NAFLD which currently has no readily available effective treatment. This study was conducted to provide rationale for future prospective studies of testosterone therapy for NAFLD.

Research objectives

To evaluate the effect of testosterone therapy on liver fat fraction as measured by magnetic resonance imaging (MRI) in a cohort of diabetic men with lowered testosterone levels. We further aimed to determine other factors associated with changes in liver fat in this population.

Research methods

We performed a secondary analysis of a previous 40 wk, randomised, double-blinded, placebo-controlled trial of intramuscular testosterone undecanoate in men with type 2 diabetes and lowered serum testosterone levels. Liver fat as determined by MRI scan before and after therapy was analyzed in addition to blood tests and body composition scans.

Research results

Patients who received testosterone therapy had an absolute reduction of liver fat fraction by 3.5% and patients who received placebo had an absolute increase in liver fat fraction by 1.2%, with a between group difference of 4.7%, $P < 0.001$. After controlling for baseline liver fat, testosterone therapy was associated with a relative reduction in liver fat of 38.3% ($P < 0.001$).

Research conclusions

Testosterone therapy was associated with a reduction in hepatic steatosis in a cohort of men with type 2 diabetes and lowered serum testosterone levels.

Research perspectives

This study provides rationale for future prospective clinical trials of testosterone therapy for the treatment of NAFLD focusing on liver related endpoints.

FOOTNOTES

Author contributions: Apostolov R, Gow P, Grossmann M and Sinclair M designed the research; Apostolov R, Gianatti E and Kutaiba N were involved in data acquisition; Kutaiba N interpreted and analysed radiological data; Apostolov R, Wong D and Sinclair M drafted the manuscript; Gianatti E, Wong D, Kutaiba N, Gow P, Grossmann M and Sinclair M revised the manuscript for important intellectual content; all authors read and approved the final manuscript.

Institutional review board statement: Human Research Ethics Committee, Research Ethics Unit, Level 8 HSB - Room 8322, Austin Hospital.

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ORIGINAL ARTICLE

Retrospective Study

Impact of liver cirrhosis on ST-elevation myocardial infarction related shock and interventional management, a nationwide analysis

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Abstract

BACKGROUND

Critical care is rapidly evolving with significant innovations to decrease hospital stays and costs. To our knowledge, there is limited data on factors that affect the length of stay and hospital charges in cirrhotic patients who present with ST-elevation myocardial infarction-related cardiogenic shock (SRCS).

AIM

To identify the factors that increase inpatient mortality, length of stay, and total hospital charges in patients with liver cirrhosis (LC) compared to those without LC.

METHODS

This study includes all adults over 18 from the National Inpatient Sample 2017 database. The study consists of two groups of patients, including SRCS with LC and without LC. Inpatient mortality, length of stay, and total hospital charges are the primary outcomes between the two groups. We used STATA 16 to perform statistical analysis. The Pearson's chi-square test compares the categorical variables. Propensity-matched scoring with univariate and multivariate logistic regression generated the odds ratios for inpatient mortality, length of stay, and resource utilization.

RESULTS

This study includes a total of 35798453 weighted hospitalized patients from the 2017 National Inpatient Sample. The two groups are SRCS without LC (n = 758809) and SRCS with LC (n = 11920). The majority of patients were Caucasian in both groups (67% *vs* 72%). The mean number of patients insured with Medicare was lower in the LC group (60% *vs* 56%) compared to the other group, and those who had at least three or more comorbidities (53% *vs* 90%) were significantly higher in the LC group compared to the non-LC group. Inpatient mortality was

also considerably higher in the LC group (28.7% *vs* 10.63%). Length of Stay (LOS) is longer in the LC group compared to the non-LC group (9 *vs* 5.6). Similarly, total hospital charges are higher in patients with LC ($147407.80 *vs* $113069.10, $P \leq 0.05$). Inpatient mortality is lower in the early percutaneous coronary intervention (PCI) group (OR: 0.79 < 0.11), however, it is not statistically significant. Both early Impella (OR: 1.73 < 0.05) and early extracorporeal membrane oxygenation (ECMO) (OR: 3.10 $P < 0.05$) in the LC group were associated with increased mortality. Early PCI (-2.57 $P < 0.05$) and Impella (-3.25 $P < 0.05$) were also both associated with shorter LOS compared to those who did not. Early ECMO does not impact the LOS; however, it does increase total hospital charge (addition of $24717.85, $P < 0.05$).

CONCLUSION

LC is associated with a significantly increased inpatient mortality, length of stay, and total hospital charges in patients who develop SRCS. Rural and Non-teaching hospitals have significantly increased odds of extended hospital stays and higher adjusted total hospital charges. The Association of LC with worse outcomes outlines the essential need to monitor these patients closely and treat them early on with higher acuity care. Patients with early PCI had both shorter LOS and reduced inpatient mortality, while early Impella was associated with increased mortality and shorter LOS. Early ECMO is associated with increased mortality and higher total hospital charges. This finding should affect the decision to follow through with interventional management in this cohort of patients as it is associated with poor outcomes and immense resource utilization.

Key Words: Gastroenterology; Hepatology; Liver; ST-elevation myocardial infarction; Cardiogenic Shock; Percutaneous coronary intervention; Impella; Extracorporeal membrane oxygenation

Core Tip: This paper was written to identify the predictors of mortality and the effect of liver cirrhosis on patients who develop ST-elevation myocardial infarction-related cardiogenic shock requiring interventional management. We reviewed the effect of liver cirrhosis on mortality, length of stay, and total hospital charges. We hope that this article will help build the foundation for future studies that will benefit this population of patients.

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INTRODUCTION

The understanding of Liver cirrhosis (LC) and its relationship with cardiovascular disease (CVD) has evolved over the last half-century. Initial theories suggested an inverse relationship between LC and CVD development[1,2]. These theories are based on the pathophysiology of LC and its effects on known CVD risk factors such as reduced cholesterol and systemic blood pressure. Autopsy studies that showed that plaque burden and myocardial infarction were less prevalent amongst those with LC support this hypothesis[2]. More recent epidemiologic studies refute this notion and portray a positive correlation between LC and CVD risk[3]. This risk elucidates the increasing incidence of obesity and the emergence of nonalcoholic fatty liver disease as the leading cause of cirrhosis. This disease process shares many risk factors (diabetes, obesity, *etc.*) with coronary artery disease[4,5].

Patients with liver cirrhosis develop thrombocytopenia, do not synthesize coagulation factors properly, and have delayed clearance of pro and anti-thrombotic factors, placing them at an increased risk of bleeding and thrombosis[6]. The cornerstone of managing ST-elevation myocardial infarction (STEMI) is revascularization and initiation of antiplatelet agents, and clinical trial data to guide the management of patients with LC and STEMI are lacking as most studies exclude this patient population [7]. To complicate matters further, a potential sequela of STEMI is cardiogenic shock, which requires consideration of mechanical circulatory support such as extracorporeal membrane oxygenation (ECMO) or ventricular offloading devices. Abnormal coagulation makes treating patients with LC who present with STEMI challenging. The bleeding risk cirrhosis poses might dissuade providers from pursuing percutaneous coronary intervention (PCI), an intervention shown to decrease mortality in patients with

STEMI[8]. The optimal strategy, timing, and whether the benefit of such interventions is the same for those patients with LC as the general population is not well studied. Our study aims to identify the predictors of mortality and observe hospitalization outcomes (length of stay (LOS), mortality, and total hospital charges) in patients with LC who develop STEMI-related cardiogenic shock and receive standard revascularization and circulatory support interventions, including PCI, Impella, and ECMO during their hospitalization.

MATERIALS AND METHODS

We conducted a retrospective study using the 2017 National Inpatient Sample (NIS) database, created by The Agency for Healthcare Research and Quality. The NIS is designed as a stratified probability sample to represent all non-federal acute care inpatient hospitalizations in the United States. The population depicted in the database is a randomly selected 20% of the total population from all participating hospitals. Both patient and hospital-level information are collected from these patients and entered into NIS. Information about patients, including demographics, diagnoses, and resource utilization, including length of hospital stay, procedures, and total hospitalization charges, is utilized. NIS does not include unique patient identifiers. Hospitals categorize by bed size, geographical location, teaching status, and urban/rural location. Each hospital discharge is weighted (weight is equal to the total number of patient discharges from all acute care hospitals in the United States divided by the number of discharges included in the 20% sample) to depict a nationally representative sample. Up to 25 discharge diagnoses and 25 procedures are collected using the International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10-CM) coding system. NIS has previously been used to provide reliable estimates of the burden of gastrointestinal diseases[1,9,10].

Study sample

Patients are included in the study if they had a primary ICD-10 diagnosis code indicating STEMI or STEMI related cardiogenic shock and liver cirrhosis. Inclusion criteria were patients above the age of 18 and non-elective admission. In the 2017 NIS, there are a total of 35798453 weighted discharges.

Study variables

The exposure of interest was the presence of STEMI or STEMI related cardiogenic shock in patients with liver cirrhosis. The variable STEMI or STEMI-related cardiogenic shock is a principal diagnosis. The variable LC is a principal or secondary diagnosis. Other variables, including factors to assess the severity of LC (ascites, gastrointestinal bleed, *etc.*), and inpatient outcome measures are also secondary diagnoses.

Patient demographics are provided directly in NIS and consist of age (assessed as a continuous variable), sex, race (Caucasian, African American, Hispanic, Asian or Pacific Islander, Native American, and other), median income in the patient's ZIP code (quartile 1: $1-38999; quartile 2: $39000-47999; quartile 3: $48000-63999; quartile 4: $640001), primary insurance (Medicare, Medicaid, private insurance, and uninsured), and comorbidities measured by the Charlson Comorbidity Index (CCI; categorized as 0, 1 to 2, or over 2), Elixhauser comorbidity index, as well as hospital location (rural *vs* urban), region (Northeast, Midwest, West, or South), teaching status, and size (small, medium, or large).

Outcome

The primary outcome in this study is inpatient mortality in LC patients that developed STEMI or SRCS and underwent an interventional procedure including PCI, Impella placement, or ECMO during the hospitalization. PCI and Impella were defined as "early" if performed within the first 24 h of admission. ECMO was defined as early if it occurred within the first days of admission. Inpatient mortality was compared between the liver cirrhosis patients who received the procedures before and those that received the procedures after the period defined as early. The secondary outcomes included length of stay and total hospital charges between the two cohorts.

Predictors of inpatient mortality [sepsis, acute kidney injury (AKI), AKI on chronic kidney injury, cardiac arrest, any bleed/hemorrhage, electrolyte, and fluid disturbances, all gastrointestinal bleeds, respiratory failure requiring mechanical ventilation] and markers of decompensated cirrhosis [hypoalbuminemia, hepatic encephalopathy (HE), portal vein thrombosis, variceal upper gastrointestinal bleed, ascites, coagulopathy, spontaneous bacterial peritonitis (SBP), and hepatorenal syndrome (HRS)] were generated using secondary diagnosis codes. We cannot use Child-Pugh and model for end-stage-liver-disease (MELD) scoring to stratify the severity of liver cirrhosis as they do not exist as ICD-10 codes. Therefore, previously generated variables by the CCI were utilized (mild liver disease and moderate-severe liver disease) as a placeholder for previously validated scoring systems but cannot be a replacement.

Statistical analysis

Continuous variables were compared using the Student *t*-test, and categorical variables were compared using the chi-square test. Univariate analysis was conducted using logistic regression to identify confounders. Confounders were adjusted for using multivariate logistic regression analysis. The model was constructed by including all statistically significant variables associated with the outcome on univariate analysis with a cutoff *P* value of 0.2 to construct a more accurate model. In addition, all variables considered to be clinically meaningful predictors of the outcomes (based on prior studies' findings) were included in the regression analysis regardless of the *P* value.

All statistical analysis was performed using STATA 16 (StataCorp LP, College Station, TX, United States). Survey (svy) commands were used to account for the stratified sampling design of the NIS. A two-tailed *P* value of 0.05 marks the threshold for significance for all tests. Two different methods control confounders in this study: Propensity matched scoring and multivariate logistic regression. Propensity scores match patients with SRCS with and without liver cirrhosis. A non-parsimonious multivariate logistic regression model was generated using the following variables: Age, sex, race, income in patient's zip code, Charlson's comorbidity index, insurance status, hospital location, hospital teaching status, hospital bed size, and hospital region. This robust double method for controlling confounders was then used to calculate treatment weights. The inverse probability of treatment weights was used to match cases with controls using a generalized linear model. Secondary analysis was computed using multivariate logistic regression using all variables significantly associated with the outcome on univariate analysis (with a *P* value cutoff of 0.2). Any variable believed to be a critical confounder identified from previous literature was added to optimize the model. Logistic regression was utilized to analyze binary outcomes, while linear regression was adopted for continuous variables. Proportions were compared using the Fisher exact test, and continuous variables were compared using the Student *t*-test.

RESULTS

Figure 1 illustrates a flow diagram for study inclusion. From the 35798453 weighted hospitalizations in the United States during 2017, 770730 patients met the inclusion criteria. Table 1 demonstrates the baseline patient characteristics. Patients with liver cirrhosis were younger, with a mean age of 62.7 compared to 66.7 in those without liver cirrhosis. LC patients have a higher percentage of Hispanic patients (13% *vs* 9% $P < 0.05$). The two groups have a similar percentage of females, hospital bed size distribution, hospital region, hospital location, and median household income. Patients with LC have a higher proportion with Medicaid insurance (21% *vs* 10% $P < 0.05$) and a lower percentage of patients with Medicare (56% *vs* 60%). The LC cohort also had more comorbidities reflected by a higher Charlson Comorbidity Index (CCI) (90% *vs* 53%. $P < 0.05$). Figure 2 illustrates baseline comorbidities. Patients with LC have higher percentages of almost all comorbidities listed.

Figure 3 visualizes the odds of mortality of each intervention. The overall inpatient mortality rate was higher for SRCS patients with liver cirrhosis (28.7% *vs* 10.63%. $P < 0.05$). After adjusting for potential confounders, the odds of inpatient mortality were higher at 2.25 (CI 1.98-2.55, $P < 0.05$) for patients with LC compared to those without LC. Patients who had an early PCI had lower odds of mortality (OR: 0.79, CI: 0.6-1.05, $P < 0.11$) than those who had it later, but this was not statistically significant. Patients who had an early Impella (OR: 2.30, CI: 1.73, CI: 1.34-2.23, $P < 0.05$) and early ECMO (3.10: CI 2.43-3.95, $P < 0.05$) had higher odds of mortality compared to those who had it later than the defined period.

Factors of morbidity and decompensated cirrhosis

Through propensity-matched multivariate regression, moderate-severe liver disease (OR: 3.19, CI: 2.83-3.58, $P < 0.05$) shows higher odds of mortality compared to mild-liver disease (OR: 1.38, CI: 1.23-1.57, $P < 0.05$). Table 2 depicts individual markers of decompensated cirrhosis and their respective odds of mortality.

Predictors of mortality

Table 3 depicts propensity-matched adjusted. Sepsis (OR: 2.12, CI: 1.66-2.72, $P < 0.05$), AKI (OR: 2.72, CI: 2.01-3.68, $P < 0.05$), cardiac arrest (OR: 4.87, CI: 3.85-6.67, $P < 0.05$), and any bleed or hemorrhage (OR: 2.44, CI: 1.45-4.10, $P < 0.05$) were associated with statistically significant higher odds of mortality. Interestingly, the cohort of patients with any GIB was associated with lower mortality odds.

Hospital resource utilization

LOS and total hospital charges are markers of hospital resource utilization. LC patients presenting with STEMI or SRCS had a mean LOS of 9 d (CI: 5.5-5.6), while patients without LC had a mean LOS of 5.6 d (CI: 0.1-11.2). Mean LOS of patients with LC who underwent early PCI (6.4 *vs* 9.8 $P < 0.05$) and early Impella (7.9 *vs* 9.2 $P < 0.05$) had a shorter LOS compared to those without LC. After propensity-matched multivariate logistic regression, while adjusting for all confounders, early PCI (-2.57 < 0.05) and early

Table 1 Patient characteristics

Characteristics	SRCS without liver cirrhosis	SRCS with liver cirrhosis	*P* value
	N = 758810 (%)	N = 11920 (%)	
Patient age, mean (SD)	66.7 (66.6-66.8)	62.7 (62.2-63.2)	**< 0.05**
Sex			**< 0.05**
Female	293963 (39)	11920 (34)	
Male	464847 (61)	7901(66)	
Race N (%)			**< 0.05**
White	546570 (72)	7996 (67)	
Black	96975.918 (13)	1676 (14)	
Hispanic	66092 (9)	1525 (13)	
Asian or Pacific Islander	21930 (3)	273 (2)	
Native American	4325 (1)	118 (1)	
Other	22840 (3)	330 (3)	
Insurance, N (%)			**< 0.05**
Medicare	456880 (60)	6728 (56)	
Medicaid	79068 (10)	2495 (21)	
Private	188716 (25)	2145 (18)	
Uninsured	34146 (5)	552 (5)	
Household median income, N (%)			**< 0.05**
1-38999	232348 (31)	4271 (36)	
39000-47999	210114 (28)	3055 (26)	
48000-62900	175513 (23)	2595 (22)	
> 63000	140835 (18)	1998 (17)	
Bed size, N (%)			**< 0.05**
Small	124065 (16)	1665 (14)	
Medium	230602 (30)	3454 (29)	
Large	404218 (53)	6800 (57)	
Hospital region			**< 0.05**
Northeast	134309 (18)	1860 (16)	
Midwest	170201 (22)	2360 (20)	
South	306180 (40)	4915 (41)	
West	148196 (20)	2785 (23)	
Hospital location			**< 0.05**
Rural	51220 (7)	465 (4)	
Urban	707590 (93)	11455 (96)	
Charlson comorbidity index, mean (SD)			**< 0.05**
0	10016 (1)	0	
1	166938 (22)	335 (3)	
2	176043 (23)	830 (7)	
3	405736 (53)	10755 (90)	

SRCS: ST-elevation myocardial infarction-related cardiogenic shock.

Table 2 Propensity matched adjusted odds of decompensated liver cirrhosis markers as predictors of mortality

Markers of decompensated cirrhosis	N (%)	Adjusted odds	Lower limit	Upper limit	*P* value
Hypoalbuminemia	460 (4)	1.38	1.04	1.82	< 0.05
Hepatic encephalopathy	1855 (16)	3.06	2.41	3.9	< 0.05
Portal vein thrombosis	310 (3)	1.13	0.72	1.77	0.6
Variceal upper GI bleed	825 (7)	1.81	1.03	3.19	< 0.05
Ascites	2155 (18)	1.91	1.62	2.27	< 0.05
Coagulopathy	1290 (11)	2.18	1.86	2.56	< 0.05
SBP	295 (2)	2.38	0.69	8.32	0.17
Hepatorenal syndrome	945 (8)	1.69	1.22	2.34	< 0.05

GI: Gastrointestinal; SBP: Spontaneous bacterial peritonitis.

Table 3 Propensity matched adjusted odds of inpatient outcomes as predictors of mortality

In-hospital outcomes	Adjusted odds of mortality propensity matched- needs update	Lower limit	Upper limit	*P* value
Sepsis	2.12	1.66	2.72	< 0.05
AKI on CKD	0.65	0.4	1.06	0.08
AKI	2.72	2.01	3.68	< 0.05
AKI on CKD	2.24	1.84	2.74	< 0.05
Cardiac arrest	4.87	3.85	6.67	< 0.05
Stroke	1.06	0.45	2.5	0.89
Any bleed/hemorrhage	2.44	1.45	4.1	< 0.05
Electrolyte and fluid disturbance	0.95	0.45	1.99	0.89
All GI bleed	0.42	0.25	0.73	< 0.05
Respiratory failure requiring mechanical ventilation	1.14	0.74	1.78	0.55

AKI: Acute kidney injury; CKD: Chronic kidney disease; GI: Gastrointestinal.

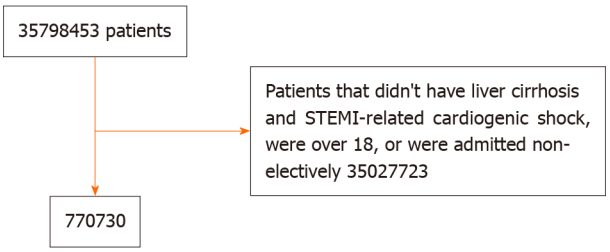

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Figure 1 Illustrates a flow diagram for study inclusion. STEMI: ST-elevation myocardial infarction-related cardiogenic shock.

Impella (-3.25 < 0.05) had a mean average shorter LOS compared to the population without liver cirrhosis. Mean LOS of SRCS patients between patients with and without LC who had an early ECMO is not statistically significant (9 *vs* 8.9, $P = 0.85$) and remains so even after adjusting for confounders (-0.79 < 0.20). Early PCI has lower total hospital charges ($144360.30 *vs* $148362.40), but the difference was not statistically significant (-$11215.00, $P = 0.22$). Early Impella has higher total hospital charges ($152088.30 *vs* $146418.70), with a statistically significant adjusted mean difference (-$26103.57, $P = 0.05$). However, early ECMO increases inpatient total hospital charges ($171334.80 *vs* $133881.8), and after adjustment for confounders with multivariate logistic regression, SRCS patients with LC when compared to those

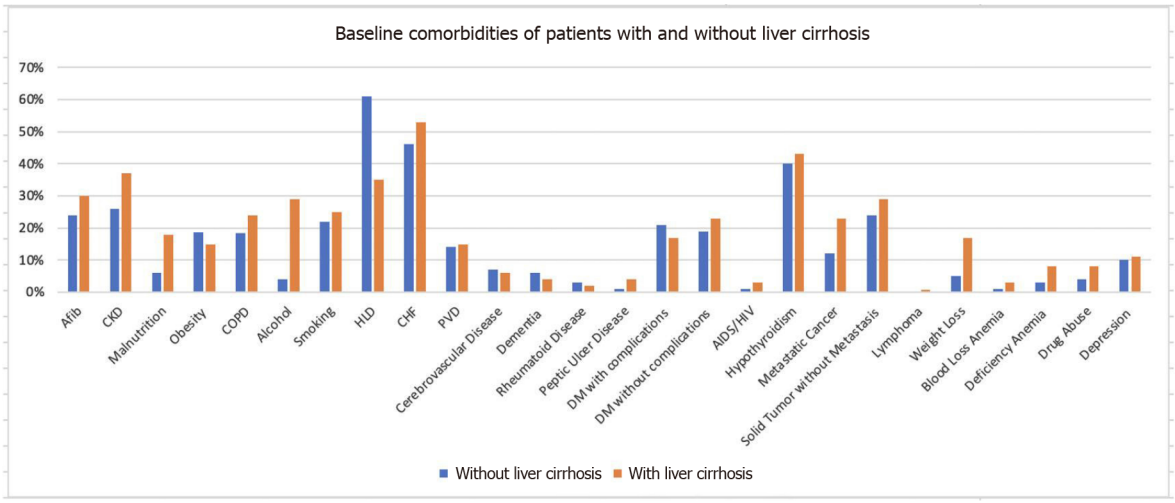

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Figure 2 This bar chart provides a direct visual comparison of the baseline comorbidities between the two groups. AIDS: Acquired immune deficiency syndrome; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; HIV: Human immunodeficiency virus; HLD: Hypomyelinating leukodystrophies; CHF: Chronic heart failure; PVD: Peripheral vascular disease.

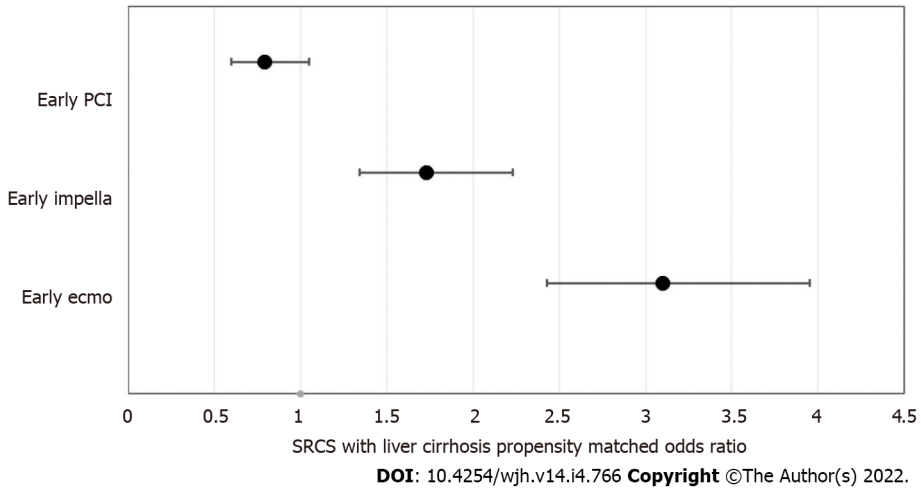

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Figure 3 This Forrest plot reflects the mortality odds ratios for each intervention for cirrhotic patients with ST-elevation myocardial infarction-related cardiogenic shock. SRCS: ST-elevation myocardial infarction-related cardiogenic shock; PCI: Percutaneous coronary intervention.

without LC were found to have an average of $24717.85 ($P < 0.05$) higher total hospital charges than those without liver cirrhosis.

DISCUSSION

In this retrospective study, we evaluate the predictors of mortality and the outcomes of patients with LC who develop SRCS compared to a propensity-matched cohort without LC. We note increased rates of inpatient complications, longer lengths of hospital stay, higher total hospital charges, and an overall higher mortality rate in the cohort of patients with LC.

We find sepsis, AKI, cardiac arrest, any bleed or hemorrhage as predictors of mortality. Despite significantly higher bleeding rates in SRCS patients with LC (23% *vs* 11%), bleeding does not increase the odds of mortality. Not surprisingly, having a cardiac arrest has the highest odds of mortality (OR: 6.67, $P < 0.05$). This relationship has been seen in previous studies portraying patients with LC who undergo cardiac arrest have a significantly higher mortality rate than those without LC[11]. This is likely multifactorial and may in part be due to their higher rates of comorbidities, non-shockable rhythms, and non-cardiac causes of cardiac arrest, including severe electrolyte disturbances, hemorrhage, or infection[11-14]. Patients with LC are particularly prone to infection and sepsis due to cirrhosis-associated immune dysfunction, disruption in gut permeability, increased bacterial translo-

cation, and upregulation of systemic inflammatory mediators, infection, and sepsis[12,15,16]. Moreover, external factors, including the overuse of proton-pump inhibitors, corticosteroids, broad-spectrum antibiotics, and repeated hospital admissions, place these patients at further risk of infections from invasive and multidrug-resistant organisms. In a recent study done in North America, nosocomial infections developed in 15% of hospitalized patients with cirrhosis and were associated with an increased risk of death[16].

Acute kidney injury is also associated with increased mortality and length of hospital stay amongst all hospitalized patients[17,18]. A recent meta-analysis finds that AKI increases the risk of acute coronary syndrome, suggesting that AKI increases the risk of early mortality[19]. Our study suggests AKI, one of the most common complications of cirrhosis, is one of the highest predictors of mortality in the LC cohort, second only to cardiac arrest[1,20]. This is likely due to the underlying disease-specific physiology of cirrhosis. Portal hypertension causes splanchnic and systemic vasodilation leads to a compensatory increase in systemic vasoconstrictors, renal water and salt retention, and ultimately inadequate renal perfusion flow[1]. The underlying physiology behind portal hypertension coupled with intravascular volume depletion secondary to gastrointestinal bleeding, diuretic use, and lactulose-induced diarrhea leads to AKI development in about 20% of hospitalized patients with LC[17,20,21]. These patients develop AKI predominantly secondary to HRS and acute tubular necrosis, both of which tend to occur more commonly in more decompensated LC[19]. A recent systems biologic analysis suggests that the inflammatory state of HRS is similar to that of a chronic non-hepatic inflammatory disease, like systemic lupus erythematosus[22]. Our study's findings align with previous literature highlighting increased morbidity and mortality with the onset of AKI and HRS[17,21].

Patients with LC are also known to have an increased risk of bleeding. Frequent readmissions for gastrointestinal bleeding, chronic thrombocytopenia, and the elevated international normalized ratio of prothrombin time support this notion[23,24]. Recent evidence suggests that cirrhotic patients may have prothrombotic tendencies as well, rather than solely bleeding diathesis[23,24]. Unfortunately, it cannot be temporally determined when bleeding or thrombosis occurred during the hospital admission. Nevertheless, our findings suggest that both coagulopathy and bleeding directly relate to mortality. As of yet, there is no defined correlation between the MELD score and bleeding complications[24,25]. There is limited data on the benefits of transfusion of blood products for a goal INR or platelet count. One study concluded that an elevated INR (> 1.5) was not predictive of post-procedural bleeding in the LC group[26,27]. Another study found that transfusion of fresh frozen plasma (FFP) did not provide any clear benefit in preventing bleeding risk[27]. Platelet transfusions also offer little help in patients with LC due to the short half-life from splenic sequestration[26,27]. Our study indicates that bleeding and coagulopathy increased mortality in patients with SRCS. The dilemma then reverts to using these corrective measures to prevent bleeding and coagulopathic events in this population. Our study results indicate that it may be beneficial to correct red blood cell and platelet counts before interventional procedures; however, further randomized controlled trials are vital to defining what the best method of correction would be and to what extent.

We also found that patients with markers of decompensated cirrhosis had increased odds of mortality. Liver cirrhosis results in a myriad of microvascular structural changes and functional derangements of synthetic liver function, leading to hemostasis abnormalities, portal hypertension, and circulatory dysfunction develop[28,29]. As the severity of the disease progresses, the intensity of the splanchnic vasodilation ultimately contributes to the characteristic hyperdynamic circulation of cirrhosis and its sequelae of decompensation-defining variable (*i.e.*, HRS, HE, ascites)[28,30]. Our study finds that the decompensation-defining factors hypoalbuminemia, hepatic encephalopathy, variceal upper gastrointestinal bleeds, coagulopathy, hepatorenal syndrome, and ascites are associated with the higher odds of mortality of all. At the same time, SBP and portal vein thrombosis do not increase the odds of mortality. Our findings reflect the critical variables utilized in the multiorgan assessment to predict mortality of patients with underlying liver cirrhosis (*i.e.*, Childs-Pugh) CP[31-33]. This study cannot assess disease severity using these scoring systems; however, we can presume that mortality rises as the severity of the disease progresses[24,34,35].

A previous nationwide study identified higher odds of mortality in patients with LC who develop STEMI than those without LC[36]. Another study also identified increased all cause-mortality of liver cirrhosis patients who develop STEMI compared to those without liver cirrhosis[9]. Some studies debate that liver cirrhosis may protect against the development of thrombosis by the shifted balance of coagulation and altered hemostasis[9]. While this remains a debated topic, our study suggests that if a patient with LC progressed to cardiogenic shock after developing a STEMI, they have significantly higher rates and odds of mortality. Although the odds of mortality were lower if PCI was performed within 24 h, it was not statistically significant. However, we find that patients with LC had higher odds of mortality if both Impella and ECMO were performed early (within five days). This finding may be because both Impella and ECMO carry risks of hemolysis, bleeding, and increased need for transfusion. Literature on using percutaneous mechanical circulatory support in patients with LC is scarce; however, our findings are consistent with a previous study that suggests that inpatient and 1-year mortality rates were higher in LC if patients had two or more risk factors (age ≥ 65, respiratory indications for ECMO, hypoalbuminemia, or liver transplantation)[37]. Patients with LC are likely more vulnerable to the effects of ECMO due to the combination of prothrombotic and anti-thrombotic changes that occur in LC

[38]. Known complications of ECMO include thrombosis and bleeding from consumptive coagulation factor deficiency, excessive fibrinolysis, thrombocytopenia, and platelet dysfunction[23,39]. It has been seen in previous studies that multiorgan dysfunction can occur after ECMO, but it is unclear how it alters liver function[40]. Nevertheless, the use of ECMO in LC patients should be done after careful consideration of risks and benefits. More research is vital to evaluate whether hemodynamic and hemostatic optimization lowers mortality rates[34,41,42].

We also noted that the length of hospital stays and total hospital charges were higher amongst patients with liver cirrhosis. The economic burden associated with cirrhosis is significant. In 2004, the US's total direct cost of cirrhosis was approximately $2.5 billion, and the indirect cost was $10.6 billion [10]. Patients with LC had higher odds of adverse inpatient outcomes, which may lead to further intervention, delay in discharge, and higher cost of inpatient stay. In previous studies, the presence of AKI suggests significantly higher inpatient LOS and resource utilization costs[21,43]. Previous studies have also found that infected cirrhotic patients had a higher risk of mortality and associated higher LOS and TOS than those that did not have their hospital course complicated by infection[44]. Interestingly, amongst all patients who received early ECMO, those with LC had significantly higher total hospital charges despite having similar durations of hospital stay. The need for more blood product transfusion and albumin infusion in LC may explain this financial discordance.

CONCLUSION

Over the years, it has become evident that patients with LC have worse outcomes. The severity of their disease correlates with their risk of mortality, inpatient LOS, and total hospital charges[32]. Our study illustrates which underlying comorbidities, hospital complications, and interventions may increase these odds. Our findings suggest that patients with SRCS and decompensated LC who develop AKI or sepsis are at significantly increased odds of mortality and face further increased odds of mortality if they require early invasive mechanical circulatory support with Impella or ECMO than those without LC. Further research is needed amongst this population to establish a standardized assessment criterion for acute risk stratification in SRCS for patients with LC. More research is essential to evaluate whether hemodynamic and hemostatic optimization lowers mortality rates. Lack of standardization and reliance on gestalt poses a significant risk of mortality for patients, contributes to existing enormous financial burdens, and poses a fundamental dilemma to clinicians.

Our study has some limitations inherent to its retrospective database analysis design. First, the data was obtained from an administrative database. This database uses ICD10 codes to identify the clinical diagnosis rather than clinical parameters. Thus, there is a possibility of misclassification or under-coding of a diagnosis. There is no way to code for the severity of liver cirrhosis using Child-Pugh classification or MELD scoring systems. The CCI has a cohort of ICD codes used to define mild liver disease and moderate-severe liver disease, used as a makeshift placeholder for the scoring systems in this study but cannot be used as a replacement. We can assume that increasingly severe liver disease would have worse clinical outcomes, but we cannot definitively assess this in this study. This idea limits further understanding of how the severity of liver cirrhosis may affect clinical outcomes. We expect any potential misclassification to be equal between the LC and the non-LC groups. This study also uses propensity-matched scoring to reduce this bias. Errors do not change the direction or relationship of the variables. Instead, they make it more challenging to establish a clinical significance. Second, the NIS database does not contain variables that allow for information such as medications, including types and dosages, radiological testing, or laboratory values. Instead, similar to previous studies, also utilizing the NIS database, we provided healthcare resource utilization as reflected by the length of stay and total hospital charges. Third, because NIS only captures in-hospital mortality, the rate we report may be slightly underestimated compared to this population's actual calendar year mortality rate. NIS does not include patients who die at home, en route to the hospital, or even in the emergency department. For our particular study group, this may be a small number as most of these patients, if at all, are likely to die at home if they are safely discharged.

Despite these limitations, our study has many strengths. To our knowledge, this is the first study to identify the impact of LC on interventional management of cardiogenic shock and the effect of its timings during the admission on the mortality rate. We use the largest publicly available all-payer inpatient database in the United States, minimizing the likelihood of beta error. Most importantly, the NIS is a nationally representative sample including small, medium, large, teaching, non-teaching, rural, urban, for-profit, non-profit, publicly owned, or private hospitals across 47 states allowing it to encompass 97% of the United States population. This fact makes the study easily generalizable to all parts of the United States. Due to the United States' diverse population, this also allows for a unique perspective on the global population. The impressive sample size allows ascertaining certain risk factors, which would otherwise be difficult in a smaller single-center study. Furthermore, the unique variables in the database allow for exploring hospitalization costs, household income estimates, and other patient and hospital factors that may not be available in single-center studies.

ARTICLE HIGHLIGHTS

Research background

ST-Elevation myocardial infarction (STEMI) remains a significant cause of morbidity and mortality globally. A particularly susceptible population are patients with liver cirrhosis.

Research motivation

This study aims to find what factors predicted morbidity and mortality in patients with liver cirrhosis that may need to undergo interventional management for STEMI related cardiogenic shock.

Research objectives

We aim to identify predictors of morbidity and mortality in patient with liver cirrhosis that undergo interventional management for STEMI related cardiogenic shock. We aim to find the effect of liver cirrhosis on mortality, length of stay, and hospital costs in patients with STEMI related cardiogenic shock.

Research methods

We conducted a retrospective review on the national inpatient sample 2017. Using the student t-test and propensity-matched multivariate logistic regression, we were able to find the *P* value and odds of mortality.

Research results

We find that patients with liver cirrhosis have significantly higher morbidity and mortality rates than those without liver cirrhosis. They are also susceptible to adverse outcomes when undergoing interventional management.

Research conclusions

Physicians must optimize patients with liver cirrhosis before any interventional procedure. Patients with mild cirrhosis seemed to have better outcomes than patients with moderate-severe liver cirrhosis.

Research perspectives

This research will help build the framework for future studies to study this topic further. The goal would be to identify a scoring system that would allow physicians to ascertain which patients would be safely able to undergo interventional management and which would not. As of now, it is mostly under clinical judgment.

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FOOTNOTES

Author contributions: Dar SH decided topic, designed study, completed the analysis, wrote, edited, and finalized paper; Rahim M participated in editing and finalizing the paper; Hoesseini DK and Sarfraz K participated in writing the initial draft.

Institutional review board statement: This is a retrospective review on a national database and did not require IRB approval.

Informed consent statement: This project was done on a large database national inpatient sample and did not require individual patient consent as it is a publicly available database.

Conflict-of-interest statement: There are no known conflicts of interest.

Data sharing statement: No individual patient identifiers are present in the national database. Therefore, no individual can be traced.

Country/Territory of origin: United States

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Observational Study

Gravity assistance enables liver stiffness measurements to detect liver fibrosis under congestive circumstances

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Abstract

BACKGROUND

As survival has been prolonged owing to surgical and medical improvements, liver failure has become a prognostic determinant in patients with congestive heart diseases. Congestive hepatopathy, an abnormal state of the liver as a result of congestion, insidiously proceed toward end-stage liver disease without effective biomarkers evaluating pathological progression. Regular measurements of shear wave elastography cannot qualify liver fibrosis, which is a prognosticator in any type of chronic liver disease, in cases of congestion because congestion makes the liver stiff without fibrosis. We hypothesized that the effects of congestion and fibrosis on liver stiffness can be dissociated by inducing architectural deformation of the liver to expose structural rigidity.

AIM

To establish a strategy measuring liver stiffness as a reflection of architectural rigidity under congestion.

METHODS

Two-dimensional shear wave elastography (2dSWE) was measured in the supine (Sp) and left decubitus (Ld) positions in 298 consecutive cases as they were subjected to an ultrasound study for various liver diseases. Regions of interest were placed at twelve sites, and the median and robust coefficient of variation were calculated. Numerical data were compared using the Mann-Whitney U or Kruskal-Wallis test followed by Dunn's post-hoc multiple comparisons. The inferior vena cava (IVC) diameters at different body positions were compared using the Wilcoxon matched pairs signed rank test. The number of cases with cardiothoracic ratios greater than or not greater than 50% was compared using Fisher's exact test. A correlation of 2dSWE between different body positions was evaluated by calculating Spearman correlation coefficients.

RESULTS

The IVC diameter was significantly reduced in Ld in subjects with higher 2dSWE values in Ld (LdSWE) than in Sp (SpSWE) (P = 0.007, (average ± SD) 13.9 ± 3.6 *vs* 13.1 ± 3.4 mm) but not in those with lower LdSWE values (P = 0.32, 13.3 ± 3.5 *vs* 13.0 ± 3.5 mm). In 81 subjects, SpSWE was increased or decreased in Ld beyond the magnitude of robust coefficient of variation, which suggests that body postural changes induced an alteration of liver stiffness significantly larger than the technical dispersion. Among these subjects, all 37 with normal SpSWE had a higher LdSWE than SpSWE (Normal-to-Hard, SpSWE - LdSWE (Δ2dSWE): (minimum-maximum) -0.74 - -0.08 m/sec), whereas in 44 residual subjects with abnormal SpSWE, LdSWE was higher in 27 subjects (Hard-to-Hard, -0.74 - -0.05 m/sec) and lower in 17 subjects (Hard-to-Soft, 0.04 - 0.52 m/sec) than SpSWE. SpSWE was significantly correlated with Δ2dSWE only in Hard-to-Soft (P < 0.0001). Δ2dSWE was larger in each lobe than in the entire liver. When Hard-to-Hard and Hard-to-Soft values were examined for each lobe, fibrosis-4 or platelet counts were significantly higher or lower only for Hard-to-Soft *vs* Normal-to-Hard cases.

CONCLUSION

Gravity alters the hepatic architecture during body postural changes, causing outflow blockage in hepatic veins. A rigid liver is resistant to structural deformation. Stiff-liver softening in the Ld position suggests a fibrous liver.

Key Words: Shear wave elastography; Inferior vena cava diameter; Congestive hepatopathy; Liver fibrosis; Body positions; Fibrosis-4 index

Core Tip: Medical progress ironically makes the liver a prognostic determinant in patients with congenital heart diseases because there are no effective biomarkers to evaluate pathological progression in congestive hepatopathy. A canonical liver stiffness measurement cannot screen for fibrous liver under congestion because congestion itself makes the liver stiff without fibrosis. Here, we report a simple strategy of liver stiffness measurement to identify clues to liver fibrosis even under congestion. The basic data presented in this report provide insights not only for the clinical application of liver stiffness in patients with congestive heart diseases but also for the physiological components and mechanisms underlying liver stiffness.

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INTRODUCTION

The survival of children and adolescents undergoing the Fontan procedure continues to improve as various modifications of this operation have been applied since 1968[1-3]. In conjunction with technological advancements in the pathophysiological evaluation of the liver, the frequency of encountering the spectrum of liver disease is increasing in patients with heart diseases. The frequency of nonalcoholic

cirrhosis is reported to be greater than 4% among hospital admissions of patients with a single functional ventricle, whereas it is approximately 0.3% of hospitalizations for patients without congenital heart diseases[4]. The pathophysiology is termed congestive hepatopathy, which is not restricted to the postoperative condition of the Fontan procedure but arises from chronically elevated hepatic venous pressures secondary to biventricular or isolated right-sided heart failure. Low cardiac output itself may also accelerate fibrosis pathways by reducing circulating blood flow to the liver. To determine a specific patient's prognosis, screening and management strategies (including candidacy for isolated heart or combined heart-liver transplantation), the detection of fibrous progression in the liver is critical. Unfortunately, there is a growing awareness that fibrosis biomarkers, such as serum tests, fibrosis calculators, and liver stiffness, are not reliable in congestive hepatopathy[5-7]. Even liver biopsy is unlikely to stage fibrosis and predict clinical outcomes accurately because the heterogeneity of fiber deposition is quite large in congestive hepatopathy[5].

Liver stiffness is a useful surrogate marker in viral hepatitis and alcoholic and nonalcoholic fatty liver diseases to assess the degree of fibrous accumulation in the liver[8-11], which is a good prognostic indicator irrespective of the etiologies for chronic liver diseases. Because liver stiffness is directly measured in the liver as a physical property, this value is fundamentally spared from systemic disparity. Based on its noninvasive nature, the value can be repeatedly measured from various sites, especially in shear wave elastography using acoustic radiation force impulse technology or in magnetic resonance elastography. On the other hand, the clinical feasibility may be limited in magnetic resonance imaging, as many patients with congestive hepatopathy have non-magnetic resonance compatible cardiac devices. Furthermore, congestion itself increases liver stiffness and causes overestimation of the amount of fibrosis, as was reported in transient elastography[12].

This study aims to establish a strategy that enables the evaluation of fibrous accumulation in the liver with respect to architectural rigidity under congestive circumstances by measuring shear wave elastography. After assessing the impacts of interstitial tissue pressure on shear wave elastography, the effects of body postural changes on the diameter of the inferior vena cava (IVC) and liver stiffness were evaluated. Based on the different reactions of shear wave elastography upon changing body positions, the patients were hypothetically divided into three groups: normal liver, congestive liver, and congestive liver with fiber accumulation. The Fibrosis-4 Index (FIB4) and its constituents were compared among groups to endorse the significance of hypothetical classification. The possibility of dissociating fibrosis from underlying congestion using a gravity aid to induce architectural deformity of the liver is discussed.

MATERIALS AND METHODS

Patients

Two-dimensional shear wave elastography (2dSWE) was measured in both the supine and left decubitus positions in 298 consecutive patients, who were subjected to 2dSWE measurements for the evaluation of various diseases, including nonalcoholic fatty liver disease (NAFLD). The patients' characteristics are summarized in Table 1. All studies were conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008. Routine blood biochemistry was measured in the clinical laboratories of our hospital, where quality control of each test was regularly performed every day. NAFLD was diagnosed based on the criteria proposed by the Asia-Pacific Working Party on NAFLD [13]. Fatty liver was diagnosed by abdominal US as defined by an increased echogenicity of the liver along with the presence of any two of the following three findings: liver-kidney contrast, vascular blurring, and deep attenuation of echo-beam[14].

To clarify the relationship between liver stiffness and interstitial tissue pressure, virtual touch quantification of point shear wave elastography was measured before and after cardiac surgery in a different cohort consisting of 41 cases (19 males and 22 females, 5.5 (1.7-61.0) years old (median (interquartile range))) with disorders, including 10 valvular and 31 congenital heart diseases. No patients were treated or followed for chronic liver diseases. HBsAg negativity, anti-HCV antibody negativity, and no alcohol abuse were confirmed. Physical properties with respect to cardiac function were evaluated using ultrasound, chest X-ray, and cardiac catheterization. The data are shown in supplementary digital content Figure 1 and referenced in the discussion section.

The review boards of the Uonuma Institute of Community Medicine and Niigata University Medical and Dental Hospital approved the study measuring liver stiffness in our main cohort consisting of 298 cases with various diseases in two body positions and another cohort of 41 patients undergoing cardiac surgery. These studies did not require informed consent because they were retrospective studies using only medical records or noninvasive imaging examinations.

Shear wave elastography measurements

Shear wave elastography (SWE) evoked by acoustic radiation force impulse was measured as point shear wave elastography using an ACUSON S2000 ultrasound system (Siemens Healthcare, Eriangen, Germany) or as 2dSWE using an Aplio 500 (Canon Medical System Corporation, Ohtawara, Japan).

Table 1 Patients' characteristics

Background			
Sex (F:M)	142:156		
Age	62.3[1]	years old	49.6-71.4[2]
BMI	22.9[1]	kg/m^2	20.8-25.5[2]
Liver diseases			
Alcoholic liver disease	28		
HBV	38		
HCV	40		
Nonalcoholic fatty liver diseases	56		
Hepatocellular carcinoma	12		
Other chronic liver dysfunction	69		
Miscellaneous	55	Total	298
Shear wave elastography			
2dSWE (supine)	1.52 (6.93)[1]	m/sec (kPa)	1.43-1.67[2] (6.13-8.37)
2dSWE (left decubitus)	1.57 (7.39)[1]	m/sec (kPa)	1.46-1.74[2] (6.39-9.08)
			Wilcoxon matched-pairs signed rank test, $P < 0.0001$
%CVRsup	9.7[3]	%	5.7[4]
%CVRsup ≤ Δ2dSWE%	81 (27.2%)		

[1]Median.
[2]Interquartile range.
[3]Average.
[4]Standard deviation. F: Female; M: Male; BMI: Body mass index; 2dSWE: Two-dimensional shear wave elastography; %CVRsup: Robust coefficient of variation on supine position in percentage against the median value; Δ2dSWE%: The difference of 2dSWEs between supine and left decubitus positions in percentage against the median value.

SWE was measured thrice in each segment (posterior, anterior, medial, and lateral) with a transient breath hold at a neutral cycle after one-night of fasting followed by a 30 min or longer rest while the patient was in the supine position. A region of interest (ROI) was set between 1 and 5 cm beneath the liver capsule. In the case of 2dSWE measurements, the size of the ROI was approximately 30 mm × 30 mm square, and 3 measurements were achieved in each ROI by placing an acquisition circle 2 mm in diameter after confirming the proper propagation of shear waves in a "wavefront" style display. When 2dSWE was measured at two body positions, the measurements were performed again in the liver at 12 sites in the left decubitus position. SWE was measured in the cohort consisting of 298 or 41 cases by 7 ultrasonographers or 2 medical doctors, respectively, who had conducted SWE measurements every day for more than 2 years or ultrasonography of the abdomen for more than 2 decades and SWE measurements for more than 3 years.

To define the cutoff value of 2dSWE suggesting the least fiber accumulation in the liver, 2dSWE was measured in 480 voluntary annual medical checkup visitors who had been diagnosed with NAFLD one year prior. Because median 2dSWE values in the 480 visitors fit well on a Gaussian distribution represented by an average of 1.324 m/sec (5.26 kPa) with a standard deviation of 0.0847 m/sec (0.022 kPa, $r^2 = 0.98$), a cutoff value to distinguish the liver with fiber accumulation was statistically defined and reported as the average plus standard deviation of 1.41 m/sec (5.96 kPa) [15].

Statistical analysis

A robust counterpart to the standard deviation was calculated as follows. First, the median absolute deviation was calculated as the median of the difference in the absolute values between each SWE and the median of 12 measurements; thereafter, a constant factor of 1.4826 was multiplied. Finally, the robust coefficient of variation (CVR) was calculated by dividing the robust standard deviation by the median and expressed as a percentage. The inter- or intraobserver variation was not evaluated.

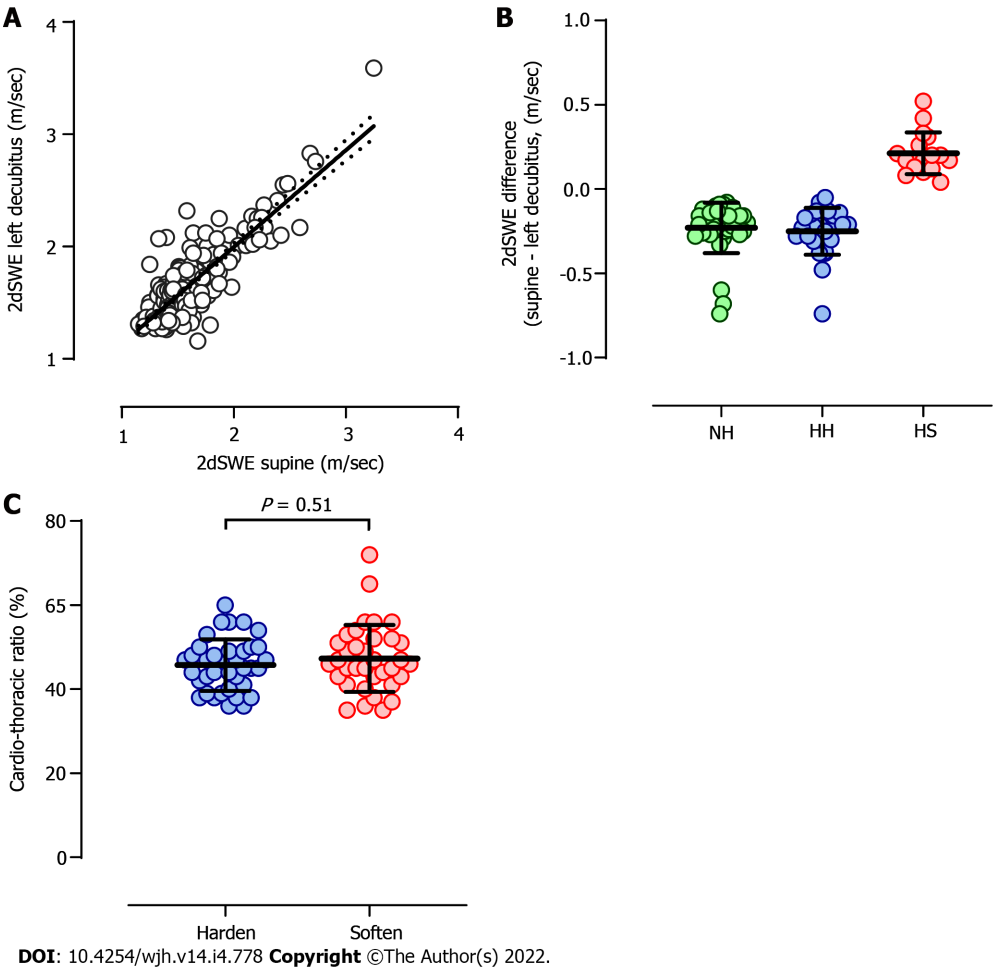

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Figure 1 Body position effects on liver stiffness. A: Two-dimensional shear wave elastography (2dSWE) values that were measured in the supine and left decubitus positions revealed a significant positive correlation ($P < 0.0001$, $r = 0.68$). The black continuous and dotted lines reveal the best hit and 95% confidence band in the equation of least squares; B: The cases in which 2dSWE increased or decreased in association with changing body positions beyond the magnitude of robust coefficient of variation can be classified into 3 groups: normal 2dSWE (Normal-to-Hard: NH) or abnormal 2dSWE that increased (Hard-to-Hard: HH) or decreased (Hard-to-Soft: HS) in the left decubitus position. The difference in 2dSWE between supine and left decubitus positions (supine - left decubitus) was negative in NH (-0.23 ± 0.15 m/sec) and HH (-0.25 ± 0.14 m/sec) but was positive in HS (0.21 ± 0.12 m/sec); C: The cardiothoracic ratio was not significantly different between the patients with abnormal 2dSWE in the supine position that further hardened or softened in the left decubitus position ($P = 0.51$, 47.3 ± 8.0 *vs* 45.7 ± 6.1%). The horizontal bars in B and C indicate the average ("Bold") and standard deviation. 2dSWE: Two-dimensional shear wave elastography; HH: Hard-to-Hard; NH: Normal-to-Hard; HS: Hard-to-Soft.

Numerical data from independent cases were compared using the Mann-Whitney U or Kruskal-Wallis test followed by Dunn's post-hoc multiple comparisons between two groups or among three groups, respectively. IVC diameters at different body positions in each case were compared using the Wilcoxon matched pairs signed rank test. A correlation of 2dSWE between different body positions was evaluated by calculating Spearman correlation coefficients. The number of cases with cardiothoracic ratios greater than or not greater than 50% was compared using Fisher's exact test. The statistical methods of this study were reviewed by Professor Kohei Akazawa from the Department of Medical Informatics, Niigata University Medical and Dental Hospital. All statistical analyses were conducted with GraphPad Prism version 7.0 (GraphPad Software Inc., La Jolla, CA, USA), and two-sided P values less than 0.05 were considered statistically significant.

RESULTS

Livers with normal stiffness in the supine position harden in the left decubitus position, whereas stiff livers harden or soften

When 2dSWE was measured for both supine (SpSWE) and left decubitus (LdSWE) positions, the values revealed a significant positive correlation, as shown in Figure 1A ($P < 0.0001$, $r = 0.68$). Because 12 values of 2dSWE in each liver were dispersed on a case-by-case basis, it is reasonable to assume that 2dSWE is substantially affected by changing body positions only when the difference between SpSWE and

LdSWE (Δ2dSWE; SpSWE - LdSWE) is greater than the dispersion of SpSWE, which is a robust coefficient of variation (CVR). Among 298 cases, LdSWE increased or decreased from SpSWE over the magnitude of CVR in 81 cases (27.2%). These 81 cases can be classified into four groups based on SpSWE normality and positive/negative Δ2dSWE values. For 37 cases in which SpSWE was lower than the upper normal limit of 1.41 m/sec (5.96 kPa, see Methods), Δ2dSWE was negative in all the cases (Normal-to-Hard: NH), as shown in Figure 1B. On the other hand, in 44 cases with stiff livers in the supine position, Δ2dSWE was negative (Hard-to-Hard: HH) or positive (Hard-to-Soft: HS) in 27 and 17 cases, respectively. The 2dSWE values in each group at different body positions are summarized in Table 2.

To assess the possibility that Δ2dSWE is determined by cardiac function, the cardiothorax ratio was compared between cases with negative and positive Δ2dSWE. As shown in Figure 1C, the cardiothorax ratio was not significantly different between the two groups (P = 0.51). The number of cases showing a cardiothoracic ratio larger than 50% was 11 out of 35 Δ2dSWE-positive cases and 6 out of 37 Δ2dSWE-negative cases and was not significantly different between the two groups (P = 0.17).

IVC shrinks in the left decubitus position as the liver hardens but not as the liver softens

Next, the effects of body position on IVC diameter were evaluated irrespective of whether the Δ2dSWE scale was beyond or within the CVR. In the results, the diameter of the IVC in the left decubitus position was significantly reduced compared with that in the supine position in the cases showing normal liver stiffness in the supine position, as shown in the left panel of Figure 2A (P = 0.013). Consistently, the IVC diameter was also shortened in the cases with a stiff liver in the supine position that hardened further in the left decubitus position (Figure 2A middle panel, P = 0.0070). On the other hand, the IVC diameters in the supine and left decubitus positions were not significantly different in the cases with a stiff liver in the supine position that softened in the left decubitus position (Figure 2A right panel, P = 0.32).

Liver stiffness is tightly associated with body postural change in cases in which a stiff liver softens in the left decubitus position, especially in the right lobe

To understand the implications of the pressure connection between the liver and IVC, the correlation between SpSWE and Δ2dSWE was evaluated. As shown in Figure 2B, a significant correlation was not observed in the cases showing normal liver stiffness in the supine position (P = 0.56) or the cases with a stiff liver in the supine position that hardened farther in the left decubitus position (P = 0.88). In contrast, SpSWE and Δ2dSWE revealed a significant positive correlation in the cases with a stiff liver in the supine position that softened in the left decubitus position (P < 0.0001, r = 0.38), suggesting a direct connection between the IVC pressure and the interstitial pressure of the liver. When the same relation was separately evaluated in the right or left lobe, as shown in Figure 2C, the correlation was clearly tighter in the right lobe (P < 0.0001, r = 0.48) than in the left lobe (P < 0.0001, r = 0.31).

Gravity unevenly impacts the liver architecture between the right and left lobes

The paradoxical increment/shrinkage of LdSWE/IVC in the left decubitus position indicates that pressure thresholds exist between the hepatic veins and IVC, where outflow blocks would be built under architectural deformation of the liver during postural changes. Given that postural changes may not evenly impact the liver architecture, Δ2dSWE was separately evaluated in the right and left lobes. As shown in Figure 3, larger differences in Δ2dSWE were noted between the right and left lobes in cases with positive or negative Δ2dSWE values in the entire liver. When Δ2dSWE is positive or negative in the entire liver, Δ2dSWE in a single lobe is reciprocally negative or positive, respectively, suggesting that the impact of postural change on liver architecture would be detected much more easily in a single lobe than in the entire liver.

Softening of the stiff liver in the left decubitus position suggests fibrous progression of the liver

To infer the relationship between pathological differences of the liver and Δ2dSWE, FIB4 and its constituents, platelet count, age, and alanine aminotransferase, were compared among Normal-to-Hard, Hard-to-Hard, and Hard-to-Soft cases. As shown in Figure 4, FIB4 and platelet counts revealed significantly higher and lower values, respectively, in Hard-to-Soft than in Normal-to-Hard cases, especially when a Hard-to-Soft texture was not judged in the entire liver but in a single lobe on the right or left (judged in the entire liver, right lobe, left lobe; (FIB4) P = 0.04, P = 0.006, P = 0.01; (platelet counts) P = 0.29, P = 0.05, P = 0.05, respectively). In terms of age and alanine aminotransferase, no significant differences were noted between Hard-to-Soft and Normal-to-Hard cases even when Hard-to-Soft values were determined in each lobe. No significant differences were noted between the Normal-to-Hard and Hard-to-Hard groups in terms of FIB4, platelet counts, age, or alanine aminotransferase levels.

DISCUSSION

It has been reported that the IVC diameter and area decrease significantly from the right lateral to the

Table 2 Summary of shear wave elastography

	Shear wave elastography		
Group (*n*)	Supine		
	Median		Inter quartile range
Normal-to-Hard (37)	1.35 (5.47)	m/sec (kPa)	1.30 (5.07)-1.38 (5.71)
Hard-to-Hard (27)	1.56 (7.30)	m/sec (kPa)	1.47 (6.48)-1.64 (8.07)
Hard-to-Soft (17)	1.62 (7.87)	m/sec (kPa)	1.57 (7.39)-1.84 (10.16)
	Left decubitus		
	Median		Inter quartile range
Normal-to-Hard (37)	1.53 (7.02)	m/sec (kPa)	1.46 (6.39)-1.63 (7.97)
Hard-to-Hard (27)	1.79 (9.61)	m/sec (kPa)	1.68 (8.47)-1.99 (11.88)
Hard-to-Soft (17)	1.52 (6.93)	m/sec (kPa)	1.33 (5.31)-1.64 (8.07)

supine position and further to the left lateral position in a healthy population[16]. The height of the IVC relative to the right ventricle, compression of the IVC between the liver and spine, different levels of venous return and/or splanchnic blood pooling are thought to cause postural differences in IVC size[16, 17]. Consistently, the IVC diameter was significantly reduced in cases with normal liver stiffness when the body positions were changed from supine to left decubitus in our cohort. Liver stiffness is clearly correlated with IVC pressure/diameter in the supine position, as shown in Supplementary Figure 1A and B. Thus, if the pressure is equilibrated between the IVC and hepatic veins during body position changes, liver stiffness should be reduced in the left decubitus position. However, our study clearly revealed that IVC diameter and liver stiffness exhibited paradoxical changes. The liver hardened, whereas the IVC diameter was reduced. These findings suggest that a pressure threshold exists between the IVC and hepatic veins in the left decubitus position in livers with normal stiffness. Given that intra-abdominal organs relocate along with postural change[18], it is reasonable to assume that the hepatic veins are vented and twisted against the IVC in the left decubitus position, establishing an outflow block. Furthermore, it is anticipated that a rigid liver is less deformed after a body position change. A minimal outflow block keeps the efflux from the liver to the IVC and obviates the shrinkage of the IVC. Therefore, we hypothesized that a stiff liver in the supine position would soften in the left decubitus position if substantial fiber accumulation was present. Otherwise, the liver will further harden (Supplementary Figure 2).

Because IVC pressure strikingly affects liver stiffness[12], as shown in Supplementary Figure 1A and B, the correlation of liver stiffness before and after changing of the IVC pressure strongly indicates a direct connection between the IVC and hepatic veins (Supplementary Figure 1C). Along with the body position changes from the supine to left decubitus position, a significant correlation between SpSWE and Δ2dSWE was only observed in cases with a liver that softened in the left decubitus position. These results strongly support the notion that pressure thresholds generally exist between the IVC and hepatic veins in the left decubitus position, but fewer pressure differences are noted between the IVC and hepatic veins in cases with a stiff liver that softens in the left decubitus position. Furthermore, the correlation coefficients were substantially different between the lobes. In addition, Δ2dSWE revealed large differences between the right and left lobes. These values are reciprocally negative and positive, suggesting that poor venous drainage in the left decubitus position heterogeneously occurs in the liver and is compensated through the area where gravity generates less impact. It is well known that if the flow volume is reduced from the portal vein, the arterial flow instantly compensates, and *vice versa*[19]. In a similar way, if venous drainage is hindered in a certain area, congestion is avoided by opening latent vascular connections toward the outside of the burden area, as noted in the case of Budd-Chiari syndrome[20].

The different anatomical connections between the IVC and hepatic veins are one reason for the uneven impacts of gravity on the lobes among cases[21]. Given that liver stiffness is measured in two different body positions, it is assumed that a separate evaluation of each lobe should have a higher probability of detecting the different architectural rigidities. In fact, higher probabilities were calculated when the groups for the comparison of FIB4 were assessed in each lobe. One limitation of our study is the relatively smaller number of cases and selection bias. The limited number of enrollments may have caused inadequate assessment of the biological variability. In particular, the efficacy as a prognostic indicator of liver stiffness measurements in supine and left decubitus postures has to be validated in a cohort of congestive heart diseases to guide decisions with respect to the burden of liver diseases. Although the significance of our hypothesis was supported by FIB4 and platelet counts of surrogates for liver fibrosis, there is no standardized indicator for liver fibrosis in congestive hepatopathy referred to in a validation study. A longitudinal observation would be necessary. Furthermore, the gravitational

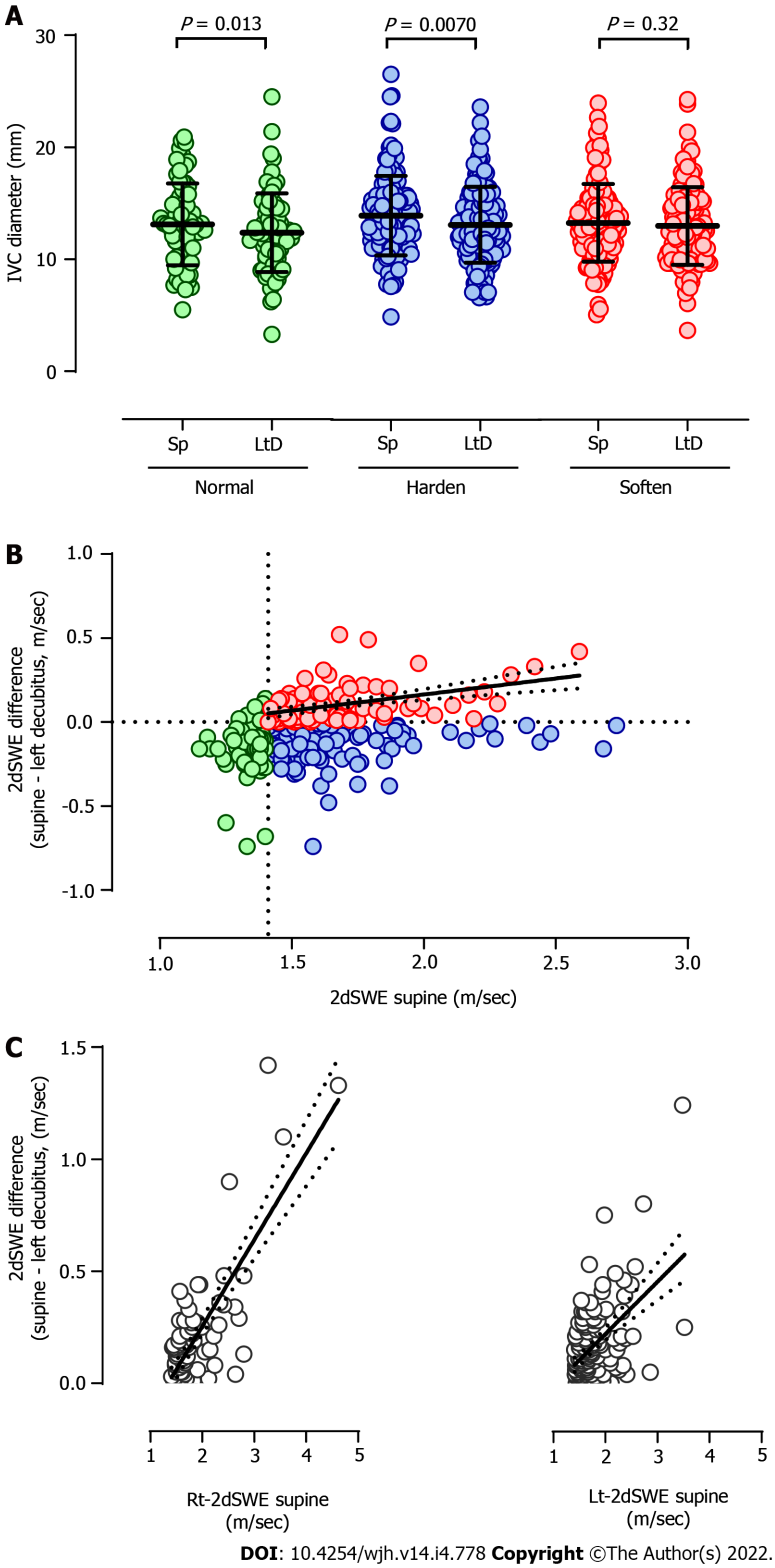

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Figure 2 Alteration of the inferior vena cava diameter and liver stiffness after changing body positions. A: The inferior vena cava diameter was significantly reduced in patients with normal two-dimensional shear wave elastography (2dSWE) values in the supine position (Normal, $P = 0.013$, 13.1 ± 3.7 *vs* 12.4 ± 3.5 mm) or patients with abnormal 2dSWE in the supine position that further hardened in the left decubitus position (Harden, $P = 0.0070$, 13.9 ± 3.6 *vs* 13.1 ± 3.4 mm). However, the diameter was not reduced in patients with abnormal 2dSWE in the supine position that softened in the left decubitus position (Soften, $P = 0.32$, 13.3 ± 3.5 *vs* 13.0 ± 3.5 mm). The horizontal bars in each plot indicate the average ("Bold") and standard deviation. Supine and LtD indicate the supine and left decubitus positions, respectively; B: 2dSWE in the supine position revealed a significant positive correlation with the difference in 2dSWE between the two body positions only in the Soften group (red) ($P < 0.0001$, $r = 0.38$) but not in the Normal (green) and Harden (blue) groups; C: In the Soften group, 2dSWE values in the supine position were plotted against the difference in 2dSWE between the supine and left decubitus positions in the right or left lobe. A Spearman's correlation coefficient of 0.48 in the right lobe was higher than 0.31 in the left lobe. The black continuous and dotted lines reveal the best hit and 95% confidence band in the equation of least squares between 2dSWE values in the supine position and the difference in 2dSWE for two body positions in B and C. IVC: Inferior vena cava; Sp:

Supine; 2dSWE: Two-dimensional shear wave elastography.

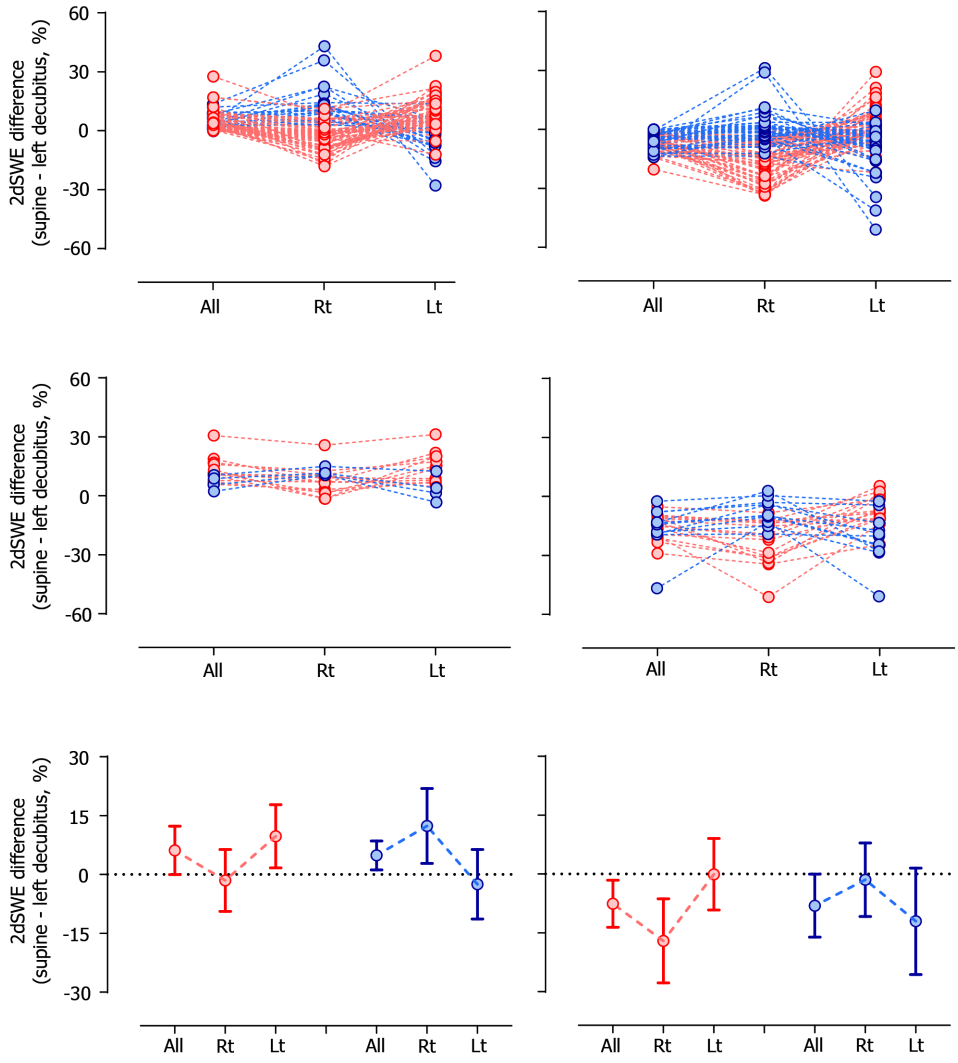

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Figure 3 Reciprocal variation in liver stiffness difference between lobes. In both cases, with positive (left column) or negative (right column) differences of two-dimensional shear wave elastography (2dSWE) values between two body positions in the entire liver (All), the difference varies less (positive: -1.52 ± 7.91 m/sec, negative: -17.04 ± 10.71 m/sec) or more (positive: 12.38 ± 9.55 m/sec, negative: -1.46 ± 9.39 m/sec) in the right lobe compared with the entire liver (red or blue, respectively, positive: 6.13 ± 6.18 m/sec, negative: -7.57 ± 5.98 m/sec) and reciprocally more (positive: 9.74 ± 8.03 m/sec, negative: -0.08 ± 9.11 m/sec) and less (positive: -2.51 ± 8.85 m/sec, negative: -12.05 ± 13.56 m/sec) in the left lobe compared with the entire liver (positive: 4.86 ± 3.68 m/sec, negative: -8.06 ± 8.02 m/sec). The upper and middle panels represent cases where the difference in 2dSWE for the entire liver is within or beyond the magnitude of robust coefficient of variation, respectively. In the bottom panel, the results in the upper and middle panels are combined. The circle and horizontal bars in each plot indicate the average and standard deviation, respectively. Lt: Left lobe; Rt: Right lobe; 2dSWE: Two-dimensional shear wave elastography.

effects on the liver architecture were proposed but not visualized or quantified in this study. To obtain direct evidence, SWE should be measured at two body positions coupled with a quantitative evaluation of structural deformation of the liver.

CONCLUSION

In this report, a strategy was proposed for measuring shear wave elastography that enables evaluation of architectural deformity under congestive circumstances. With the help of gravity, the impacts on architectural rigidity and interstitial tissue pressure are dissociated when measuring liver stiffness. The basic data presented in this report provide insights not only for the clinical application of liver stiffness in patients with congestive heart diseases but also for the physiological components and mechanisms defining liver stiffness.

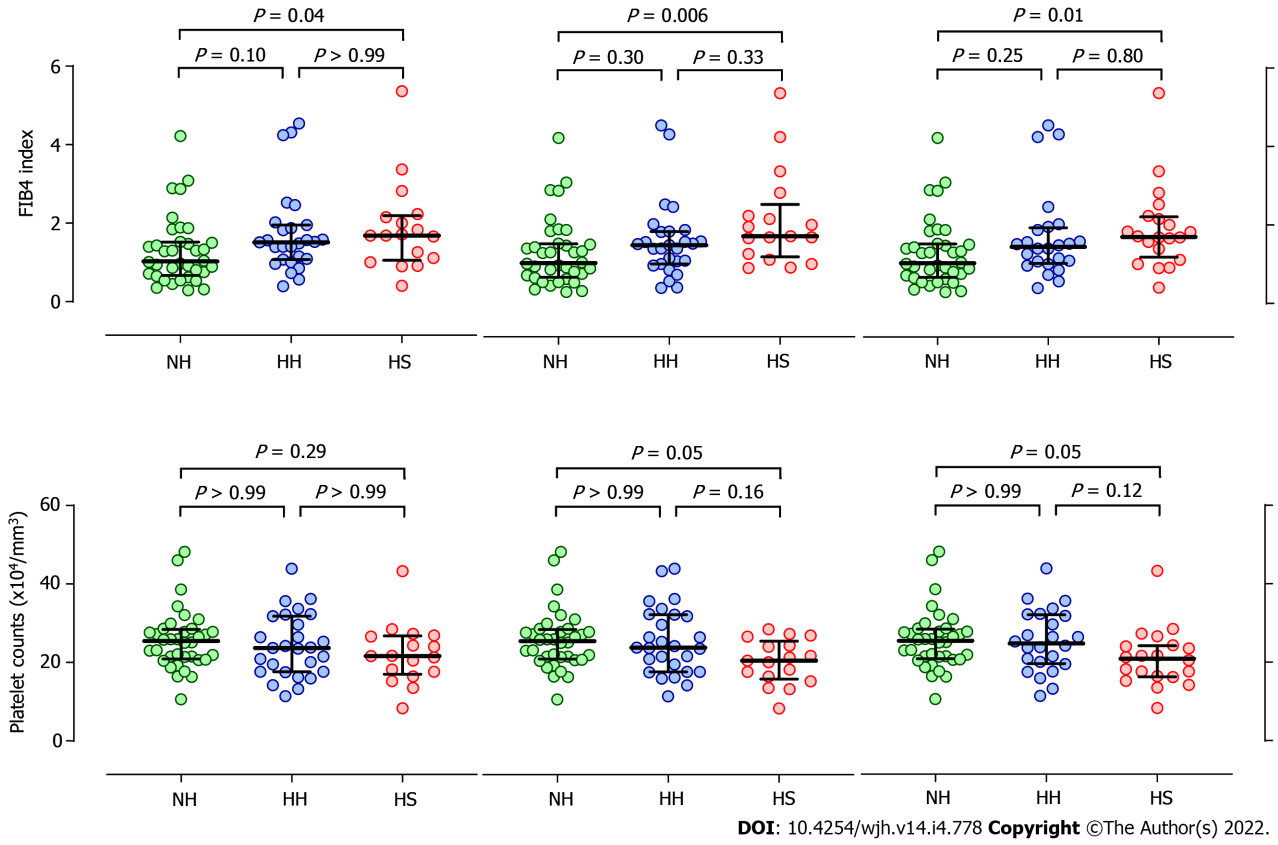

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Figure 4 Fibrous progression of the liver was suggested in the soften group. Fibrosis-4 (FIB4, top panel) and its constituent of platelet counts (bottom panel) were compared among 3 groups in which two-dimensional shear wave elastography (2dSWE) values increased or decreased in association with changing body positions beyond the magnitude of robust coefficient of variation; normal in the supine position (Normal-to-Hard: NH), abnormal and increased (Hard-to-Hard: HH) or decreased (Hard-to-Soft: HS) in the left decubitus position. The group was classified based on the difference in 2dSWE values in the entire liver (left column) or each lobe of right (middle column) or left (right column). A significant difference in FIB4 (entire: $P = 0.04$, 1.29 ± 0.87 *vs* 1.89 ± 1.16, right: $P = 0.006$, 1.29 ± 0.87 *vs* 2.12 ± 1.22, left: $P = 0.01$, 1.29 ± 0.87 *vs* 1.91 ± 1.07) and platelet counts (entire: $P = 0.29$, 25.7 ± 7.6 *vs* 22.1 ± 7.7 x10^4/mm^3, right: $P = 0.05$, 25.7 ± 7.6 *vs* 20.2 ± 5.7 x10^4/mm^3, left: $P = 0.05$, 25.7 ± 7.6 *vs* 21.0 ± 7.3 x10^4/mm^3) was observed between Normal-to-Hard and Hard-to-Soft. The probabilities were higher when the group was determined in each lobe. The horizontal bars in each plot indicate an average ("Bold") and standard deviation. HH: Hard-to-Hard; NH: Normal-to-Hard; HS: Hard-to-Soft.

ARTICLE HIGHLIGHTS

Research background

Congestive hepatopathy, an abnormal state of the liver as a result of congestion, has become a prognostic determinant by insidiously proceeding toward end-stage liver disease without effective biomarkers in patients with congestive heart diseases as survival has been prolonged owing to surgical and medical improvements. Although liver stiffness is generally a useful surrogate marker for liver fibrosis, which is a universal prognosticator in any type of chronic liver disease, regular measurements of shear wave elastography cannot qualify liver fibrosis in cases of congestion because congestion makes the liver stiff without fibrosis. A noninvasive biomarker is demanded for the managements of patients with congestive heart diseases.

Research motivation

When it is difficult to clearly visualize some area of the liver in ultrasound study, we ask patients to change body postures from supine to such as left decubitus position. At that time, we realized that shear wave elastography values substantially changed in some case. We hypothesized that the effects of congestion and fibrosis on liver stiffness may be dissociated by measuring shear wave elastography in different body positions.

Research objectives

To establish a strategy that enables the evaluation of fibrous accumulation in the liver with respect to architectural rigidity under congestive circumstances by measuring shear wave elastography.

Research methods

Two-dimensional shear wave elastography was measured in the supine and left decubitus positions in 298 consecutive cases as they were subjected to an ultrasound study for various liver diseases. To clarify the relationship between liver stiffness and interstitial tissue pressure, virtual touch quantification of point shear wave elastography was measured before and after cardiac surgery in a different cohort consisting of 41 cases. Regions of interest were placed at twelve sites, and the median and robust coefficient of variation were calculated. The liver stiffness values and clinicopathological data such as cardiothoracic ratio and the Fibrosis-4 Index were statistically analyzed.

Research results

The inferior vena cava diameter was significantly reduced in left decubitus (Ld) position in subjects with higher 2-dimensional shear wave elastography (2dSWE) value in Ld (LdSWE) than the 2dSWE value (SpSWE) in supine (Sp) ($P = 0.007$) but not in those with lower LdSWE values ($P = 0.32$). Among 81 patients, in whom SpSWE was increased or decreased in Ld beyond the magnitude of robust coefficient of variation, all 37 with normal SpSWE had a higher LdSWE than SpSWE (Normal-to-Hard), whereas in 44 residual subjects with abnormal SpSWE, LdSWE was higher in 27 subjects (Hard-to-Hard) and lower in 17 subjects (Hard-to-Soft) than SpSWE. SpSWE was significantly correlated with the difference between 2dSWE values in Sp and Ld (Δ2dSWE) only in Hard-to-Soft ($P < 0.0001$). Δ2dSWE was larger in each lobe than in the entire liver. When Hard-to-Hard and Hard-to-Soft values were examined for each lobe, fibrosis-4 or platelet counts were significantly higher or lower only for Hard-to-Soft *vs* Normal-to-Hard cases.

Research conclusions

With the help of gravity during body postural changes, the impacts on architectural rigidity and interstitial tissue pressure are dissociated when measuring liver stiffness. Because a rigid liver is resistant to structural deformation, stiff-liver softening in left decubitus position suggests fiber accumulation of the liver. In this report, a simple strategy of liver stiffness measurement is proposed to identify clues to liver fibrosis even under congestive circumstances.

Research perspectives

Because there is no standardized indicator for liver fibrosis in congestive hepatopathy, a longitudinal observation would be only the way to validate the efficacy of liver stiffness measurements in supine and left decubitus postures as a decision guidance strategy with respect to the burden of liver diseases in a cohort of congestive heart diseases. Furthermore, synergistic studies that measure shear wave elastography and quantify structural deformation of the liver in different body positions will help understand the physiological components and mechanisms defining liver stiffen.

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FOOTNOTES

Author contributions: Suda T established the study concept, designed the research; Hoshi T, Abe S, Morita S and Takahashi M acquired the data; Suda T, Sugimoto A, Kanefuji T and Yokoo T analyzed and interpretated the data; Abe A supported the materials and performed statistical analyses; Suda T wrote the paper; Yagi K supervised the study; Abe A and Takahashi M critically revised the manuscript for important intellectual content; Terai S administratively supervised.

Institutional review board statement: In this study, two different cohorts were employed for liver stiffness measurements: cohort #1 (298 cases with various liver diseases) and cohort #2 (41 cases receiving cardiac surgery). The review boards of the Uonuma Institute of Community Medicine and Niigata University Medical and Dental Hospital approved both studies.

Informed consent statement: The review boards of Uonuma Institute of Community Medicine and Niigata University Medical and Dental Hospital did not require informed consent in the studies for cohorts #1 and #2 because these studies were retrospective studies using medical records and no additional invasive examinations were conducted for the study.

Conflict-of-interest statement: Takeshi Suda, Ai Sugimoto, Atsushi Abe, Tsutomu Kanefuji, Takahiro Hoshi, Satoshi

Abe, Shinichi Morita, Takeshi Yokoo, Kazuyoshi Yagi, Masashi Takahashi, and Shuji Terai declare that they have no conflicts of interest. There is no relationship that should be disclosed in association with this study. The authors have nothing to disclose in relation to this manuscript.

Data sharing statement: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

STROBE statement: The guidelines of the STROBE Statement have been adopted.

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ORIGINAL ARTICLE

Observational Study

Total cholesterol to high-density lipoprotein ratio and nonalcoholic fatty liver disease in a population with chronic hepatitis B

Yu-Ge Zhou, Ning Tian, Wei-Ning Xie

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Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is characterized by hypertriglyceridemia, increased low-density lipoprotein cholesterol levels, and reduced high-density lipoprotein cholesterol (HDL-C) particles. Previous studies have shown that the total cholesterol to high-density lipoprotein cholesterol ratio (TC/HDL-C) was superior to other lipid metabolism biomarkers for predicting NAFLD risk and could be a new indicator of NAFLD. However, the association between TC/HDL-C and NAFLD in patients with hepatitis B virus (HBV) has not yet been determined.

AIM

To investigate the association between TC/HDL-C and NAFLD in a population with chronic hepatitis B (CHB).

METHODS

In this study, 183 HBV-infected patients were enrolled. All participants underwent blood chemistry examinations and abdominal ultrasound. Univariate and multivariate logistic regression models, curve fitting analysis, and threshold calculation were used to assess the relationship between TC/HDL-C and NAFLD.

RESULTS

The overall prevalence of NAFLD was 17.49% (n = 32) in the 183 CHB par-

ticipants. The TC/HDL-C of non-NAFLD and NAFLD patients were 3.83 ± 0.75 and 4.44 ± 0.77, respectively ($P < 0.01$). Logistic regression analysis showed that TC/HDL-C was not associated with NAFLD after adjusting for other pertinent clinical variables. However, at an optimal cutoff point of 4.9, a non-linear correlation between TC/HDL-C and NAFLD was detected. The effect size of the left and right sides of the inflection point were 5.4 (95% confidence interval: 2.3-12.6, $P < 0.01$) and 0.5 (95% confidence interval: 0.1-2.2, $P = 0.39$), respectively. On the left side of the inflection point, TC/HDL-C was positively associated with NAFLD. However, no significant association was observed on the right side of the inflection point.

CONCLUSION

This study demonstrated a non-linear correlation between TC/HDL-C and NAFLD in a population with CHB. TC/HDL-C was positively associated with NAFLD when TC/HDL-C was less than 4.9 but not when TC/HDL-C was more than 4.9.

Key Words: Cholesterol; Lipoprotein cholesterol ratio; Nonalcoholic fatty liver disease; Chronic hepatitis B population; Correlation

Core Tip: Nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis B (CHB) are both common chronic liver diseases. In this observational cross-sectional study, we explored the association between NAFLD and a lipid metabolism biomarker [the total cholesterol to high-density lipoprotein cholesterol ratio, total cholesterol to high-density lipoprotein cholesterol ratio (TC/HDL-C)] in a population with CHB. Our findings showed a non-linear correlation between TC/HDL-C and NAFLD. TC/HDL-C was positively associated with NAFLD when TC/HDL-C was less than 4.9 but not when TC/HDL-C was more than 4.9.

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INTRODUCTION

Chronic hepatitis B (CHB) is a common disease threatening public health and is a leading cause of multitudinous liver-related morbidity and mortality[1]. In 2016, about 257 million people were affected by hepatitis B virus (HBV) infection worldwide, with an estimated prevalence of 3.5%[2]. Over the past decades, with the implementation of nucleoside analogs (NAs) and hepatitis B vaccine, the risk of liver cirrhosis complications and hepatocellular carcinoma (HCC) have been substantially reduced in CHB patients[3]. However, since approximately 25% of the CHB population with nonalcoholic fatty liver disease (NAFLD) have hepatic steatosis, the effects of CHB on hepatosteatosis have recently been garnering attention[4].

NAFLD is a common chronic hepatic disease worldwide that is closely associated with cardiovascular disease, metabolic disorders, and end-stage liver diseases such as cirrhosis and HCC[5]. The prevalence of NAFLD has been increasing, and in the past few years it has reached alarming proportions (29.1%) in China due to changes in lifestyle habits and rapid socio-economic growth[6]; therefore, increased awareness to recognize NAFLD as a chronic liver disease is urgently needed.

Due to the growing prevalence of NAFLD, the coexistence of HBV infection and NAFLD is commonly observed around the world. However, a clear association between these two diseases remains questionable. Previous studies indicated that NAFLD could be inversely associated with the levels of HBV seromarkers[7,8], but interestingly, there is substantial evidence indicating an association between HBV infection and reduced incidence of hyperlipidemia or NAFLD[9,10].

Alterations in lipid metabolism are central drivers of disease progression, for instance, the progression of hepatic steatosis to nonalcoholic steatohepatitis (NASH) and hepatic fibrosis[11]. Therefore, deciphering the lipid metabolism characteristic of NAFLD is the crucial for disease treatment and prevention. NAFLD is characterized by hypertriglyceridemia, increased low-density lipoprotein cholesterol (LDL-C) levels, and reduced high-density lipoprotein cholesterol (HDL-C) particles[12,13]. A recent study showed that the total cholesterol to high-density lipoprotein cholesterol ratio (TC/HDL-C) was better at predicting NAFLD risk than other markers such as total cholesterol (TC), HDL-C, and the

ratio of apolipoprotein B (ApoB) to apolipoprotein A1 (ApoA1) and might be a new indicator of NAFLD [14]. However, the association between TC/HDL-C and NAFLD in an HBV-infected population has not yet been investigated. Therefore, the objective of this study was to assess the correlation between TC/HDL-C and NAFLD in a population with CHB.

MATERIALS AND METHODS

Study population and criteria

This was a retrospective, observational study comprising of HBV-infected patients who were treated at the Integrated Traditional Chinese and Western Medicine Hospital of Foshan (Guangdong, China) from January 2019 to December 2020. The study flow chart is illustrated in Figure 1. Chronic HBV infection was defined by hepatitis B surface antigen (HBsAg) positive for more than 6 mo[1]. All participants underwent abdominal ultrasonography for NAFLD and blood tests for assessing lipid metabolism and hepatic and renal function. Each participant completed a detailed questionnaire concerning information on their sex, age, alcohol consumption history, disease history, and medication history. Patients were excluded if they had (1) a daily alcohol intake ≥ 30g (for men) or 20 g (for women); (2) history of cancer; (3) history of chronic renal insufficiency; (4) history of hepatobiliary surgery; and (5) missing data on the key clinical variables required for study analysis.

Laboratory investigations

Blood samples were collected from all patients after an overnight fasting of at least 8 h. Peripheral venous blood was drawn from their cubital vein. Blood test parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT), creatinine (CRE), uric acid (UA), triglyceride (TG), TC, HDL-C, LDL-C, ApoA1, and ApoB, were measured using an Olympus AU-640 autoanalyzer (Olympus, Tokyo, Japan). Platelets (PLT) were measured using the Sysmex 2100 whole blood cell analyzer (Sysmex, Kobe, Japan). Hepatitis B serum examinations included the detection of HBV-DNA level, HBsAg, and hepatitis B e antigen (HBeAg) using polymerase chain reaction. HBV-DNA(+) was defined as a level of serum HBV-DNA over 100 IU/mL. TC/HDL-C was defined as TC divided by HDL-C. The patient's height and weight were measured while wearing light clothing without shoes. Body mass index (BMI) was calculated by dividing a person's weight in kilograms by the square of their height in meters. Abdominal ultrasound was used to detect the presence of NAFLD. NAs therapy was defined as the use of oral NAs antiviral drugs for more than 3 mo. All the above data were obtained from the Clinical Laboratory of the Guangdong Provincial Hospital of Integrated Traditional Chinese and Western Medicine.

Ethics and consent

The research project was submitted to and approved by the Ethics Committee of Guangdong Provincial Hospital of Integrated Traditional Chinese and Western Medicine (Approval number: 2018-1254). No informed consent was required because this was a retrospective observational study.

Definition of NAFLD based on abdominal ultrasonography

Ultrasonography was the most commonly used examination for fatty liver screening due to its noninvasiveness, low cost, and easy operability[15]. Abdominal ultrasonography was performed on all enrollees by two trained ultrasound physicians using the ACUSON X150 ultrasound system (Siemens, Munich, Germany). The presence of at least two of the following criteria was required for considering fatty liver: (1) More than 5% of hepatocytes had excessive hepatic fat accumulation and steatosis[16]; (2) Diffuse echo enhancement of the liver relative to the kidney; (3) Occurrence of ultrasonic beam attenuation; and (4) Poor visualization of intrahepatic structures. After excluding alcohol abuse and other hepatic diseases, NAFLD was then formally diagnosed by abdominal ultrasonography[17].

Statistical analysis

All statistical analyses were conducted using SPSS (version 23.0, IBM, Armonk, NY, United States) and EmpowerStats (http://www.empowerstats.com, X&Y solutions, Inc. Boston, MA, United States) software. Normal distribution continuous variables are expressed as mean ± standard deviation, and *t*-test was used for group comparison. Non-normal distribution variables are described as median [interquartile range (IQR)], and the Mann-Whitney *U* test was used to compare the groups. Categorical variables were presented as their corresponding number and percentage (*n*, %) and compared using the chi-squared test. All enrollees were stratified into two groups based on the presence or absence of NAFLD on ultrasonography. Then, the demographic characteristics of the study participants of the two groups were assessed. Univariate analyses of all variables were conducted using a binary logistic regression analysis model. Based on their TC/HDL-C, the patients were also divided into three groups according to TC/HDL-C tertiles: TC/HDL-C ≤ 3.5, 3.5 < TC/HDL-C ≤ 5, and TC/HDL-C > 5. Multivariable models were constructed as follows: Model 1 was not adjusted for other pertinent clinical

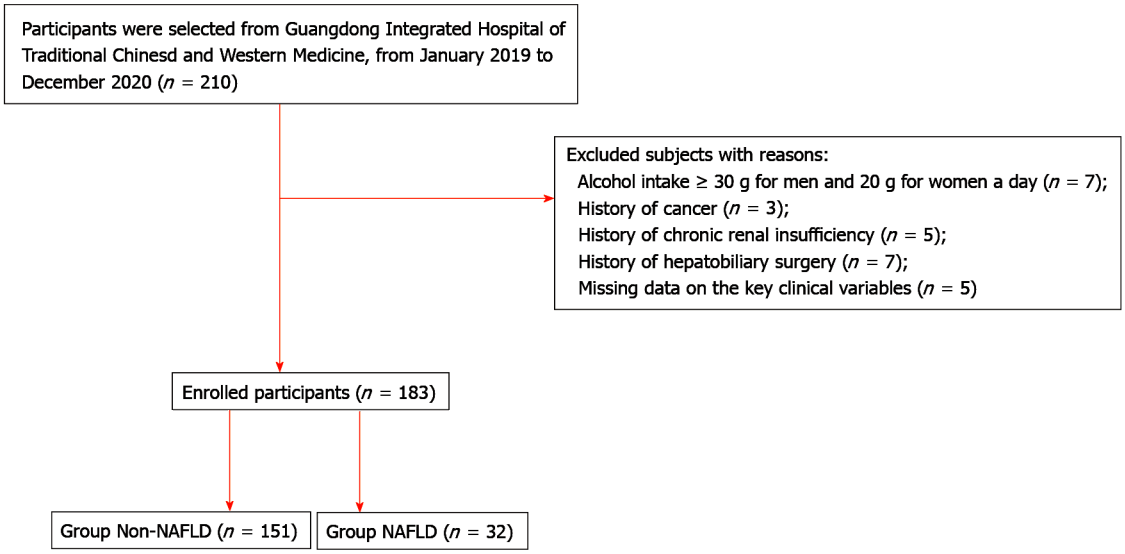

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Figure 1 Flow chart of participant selection. NAFLD: Nonalcoholic fatty liver disease.

variables; Model 2 was adjusted for sex and age; Model 3 was adjusted for sex, age, BMI, AST, ALT, γ-GT, PLT, HBsAg, CRE, UA, TG, TC, HDL-C, LDL-C, ApoA1, ApoB, HBV-DNA (+), HBeAg(+), and NAs. Lastly, a non-linear relationship between TC/HDL-C and NAFLD was investigated, and smooth curve fitting was also used. *P* values (two-tailed) less than 0.05 were considered statistically significant.

RESULTS

Demographic characteristics

The demographic characteristics of the study participants with and without NAFLD are shown in Table 1. In the whole study population, the overall prevalence of NAFLD was 17.49% (n = 32). A total of 183 patients (70.5% males) were included in this study. Their mean age was 45.41 ± 11.59 years, and their average BMI was 23.14 ± 2.63 kg/m^2. The TC/HDL-C of the non-NAFLD and NAFLD groups were 3.83 ± 0.75 and 4.44 ± 0.77, respectively (P < 0.01). Compared with the non-NAFLD group, patients from the NAFLD group had higher levels of BMI, ALT, γ-GT, PLT, UA, TG, TC, LDL-C, ApoB, and TC/HDL-C (P < 0.05). Conversely, age, HBV-DNA (+) levels, and usage of NAs were significantly lower in the NAFLD group than in the non-NAFLD group (P < 0.01). However, there was no statistically significant difference between the two groups in terms of sex and levels of AST, HBsAg, CRE, HDL-C, ApoA1, and HBeAg (+) (P > 0.05).

Univariate analysis

Binary logistic regression of independent risk factors of NAFLD is shown in Table 2. Univariate analysis indicated that age, BMI, ALT, γ-GT, PLT, UA, TG, LDL-C, ApoB, TC/HDL-C, and HBV-DNA (+) were significantly positively correlated with NAFLD (P < 0.05), whereas age and NAs were negatively correlated with NAFLD. However, no significant association between NAFLD and sex, AST, HBsAg, CRE, TC, HDL-C, ApoA1, and HBeAg(+) were observed (P > 0.05).

Association between TC/HDL-C and NAFLD using logistic regression model

Logistic regression model was used to evaluate the association between TC/HDL-C and NAFLD. The unadjusted and adjusted models are shown in Table 3. In model 1, TC/HDL-C was positively correlated with NAFLD [odds ratio (OR) = 0.94, 95% confidence interval (CI): 1.55-4.19, P < 0.01). In model 2 (adjusted for sex and age), the relationship between TC/HDL-C and NAFLD were significant (OR = 0.96, 95%CI: 1.52-4.51, P < 0.01). However, this association was not detected in model 3 (OR = -2.27, 95%CI: 0.0001-79.91, P = 0.50). The same trend was observed for TC/HDL-C from 3.5 to 5 in model 1 and model 2 (P < 0.05).

Analysis of non-linear relationships between TC/HDL-C and NAFLD

Since TC/HDL-C was a continuous variable, we analyzed the non-linear relationship with NAFLD. After adjusting for all variables and conducting smooth curve fitting, we found that the relationship between TC/HDL-C and NAFLD was non-linear (Figure 2). The inflection point was calculated as 4.9 by a piecewise linear regression model. On the left side of the inflection point, TC/HDL-C was found to

Table 1 Demographic characteristics of the study participants with and without nonalcoholic fatty liver disease

Parameters	Total, *n* = 183	Non-NAFLD, *n* = 151	NAFLD, *n* = 32	*P* value
Sex (male)	129 (70.5%)	103 (68.2%)	26 (81.3%)	0.14
Age (yr)	45.41 ± 11.59	46.29 ± 12.01	41.25 ± 8.30	< 0.01
BMI (kg/m^2)	23.14 ± 2.63	22.73 ± 2.40	25.05 ± 2.82	< 0.01
AST (U/L)	25 (21-32)	25 (21-31)	25.5 (19.75-34.53)	0.75
ALT (U/L)	28 (19-41)	26 (19-38)	39.5 (27-59.5)	< 0.01
γ-GT (U/L)	25 (19-37)	24 (18-32)	39 (25-55.5)	< 0.01
PLT (× 10^9/L)	208.37 ± 61.13	203.76 ± 63.49	230.09 ± 42.90	0.03
HBsAg (IU/mL)				0.09
≤ 1500	105 (57.4%)	91 (60.3%)	14 (43.8%)	
> 1500	78 (42.6%)	60 (39.7%)	18 (56.3%)	
CRE (μmol/L)	77.73 ± 17.96	77.14 ± 18.09	80.49 ± 17.28	0.34
UA (μmol/L)	345.20 ± 92.53	332.61 ± 85.18	404.56 ± 103.65	< 0.01
TG (μmol/L)	1.22 ± 0.63	1.08 ± 0.42	1.85 ± 0.99	< 0.01
TC (μmol/L)	4.59 ± 0.98	4.52 ± 0.99	4.89 ± 0.82	0.05
HDL-C (μmol/l)	1.18 ± 0.25	1.19 ± 0.26	1.11 ± 0.14	0.08
LDL-C (μmol/L)	2.72 ± 0.68	2.66 ± 0.65	2.98 ± 0.73	0.02
ApoA1 (g/L)	1.36 ± 0.19	1.36 ± 0.19	1.35 ± 0.15	0.85
ApoB (g/L)	0.91 ± 0.18	0.89 ± 0.17	0.97 ± 0.18	0.02
TC/HDL-C	3.94 ± 0.79	3.83 ± 0.75	4.44 ± 0.77	< 0.01
HBV-DNA (+)	131 (71.6%)	116 (76.8%)	15 (46.9%)	< 0.01
HBeAg (+)	49 (26.8%)	39 (25.8%)	10 (31.3%)	0.52
NAs	168 (91.8%)	144 (95.4%)	24 (75%)	< 0.01

TC/HDL-C: the ratio of TC to HDL-C; NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GT: γ-glutamyl transpeptidase; PLT: Platelets; HBsAg: Hepatitis B virus surface antigen; CRE: Creatinine; UA: Uric acid; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; ApoA1: Apolipoprotein A1; ApoB: Apolipoprotein B; HBV-DNA (+): Hepatitis B virus DNA > 100 IU/mL; HBeAg (+): Hepatitis B e antigen positive; NAs: Nucleoside analogues.

be positively correlated with NAFLD (β = 5.4, 95%CI: 2.3-12.6, P < 0.01). However, no significant correlation was observed between TC/HDL-C and NAFLD on the right side of the inflection point (β = 0.5 95%CI: 0.1-2.2, P = 0.39) (Table 4).

DISCUSSION

In epidemiologic studies, the highest incidences of NAFLD were reported in the Middle East (32%) and South America (31%), followed by Asia (27%) and the United States (24%)[18]. However, in a study involving 810 northern Japanese children, the prevalence of fatty liver was observed to be only 2.6% based on ultrasonographic criteria[19], but the prevalence of NAFLD was increased to 77% among obese children[20]. In our previous study, we found that the incidence rate of NAFLD in the general population was 35.92%[21]. The above studies only focused on the prevalence of NAFLD in the general population. In this present study, the overall prevalence of NAFLD in the investigated CHB population was 17.49%. Consistent with our findings, previous studies indicated that the prevalence of NAFLD with and without HBsAg positivity was 14.3% and 28.6%, respectively (P < 0.01)[22]. A prior study observed a low incidence of NAFLD in their investigated CHB population and hypothesized that such could be mainly because HBV infection influences the secretion of a variety of adipokines and alterations in lipid profiles[23].

Substantial evidence indicated an association between HBV infection and reduced incidence of hyperlipidemia or NAFLD risk[9,10]. In a large cross-sectional study, the researchers observed that HBsAg-positive subjects had a significantly lower risk of NAFLD (OR = 0.42)[22]. Adiponectin may be

Table 2 Binary logistic regression of independent risk factors of nonalcoholic fatty liver disease

Parameters	Statistics	OR	(95%CI)	*P* value
Gender (male)	129 (70.5%)	2.02	(0.78-5.23)	0.15
Age	45.41 ± 11.59	0.96	(0.92-1.00)	0.03
BMI	23.14 ± 2.63	1.40	(1.20-1.63)	< 0.01
AST	25 (21–32)	0.99	(0.98-1.02)	0.99
ALT	28 (19–41)	1.01	(1.00-1.02)	0.04
γ-GT	25 (19–37)	1.02	(1.01-1.04)	< 0.01
PLT	208.37 ± 61.13	1.01	(1.00-1.01)	0.03
HBsAg (IU/mL)				0.09
≤ 1500	105 (57.4%)	0.51	(0.24-1.11)	
> 1500	78 (42.6%)	1.95	(0.90-4.22)	
CRE	77.73 ± 17.96	1.01	(0.99-1.03)	0.34
UA	345.20 ± 92.53	1.01	(1.00-1.01)	< 0.01
TG	1.22 ± 0.63	6.72	(3.07-14.71)	< 0.01
TC	4.59 ± 0.98	1.46	(1.00-2.15)	0.05
HDL-C	1.18 ± 0.25	0.19	(0.03-1.16)	0.07
LDL-C	2.72 ± 0.68	1.94	(1.11-3.39)	0.02
ApoA1	1.36 ± 0.19	0.81	(0.10-6.44)	0.84
ApoB	0.91 ± 0.18	11.86	(1.37-102.86)	0.03
TC/HDL-C	3.94 ± 0.79	2.55	(1.55-4.19)	< 0.01
HBV-DNA (+)	131 (71.6%)	3.74	(1.70-8.28)	< 0.01
HBeAg (+)	49 (26.8%)	1.31	(0.57-2.00)	0.53
NAs	168 (91.8%)	0.15	(0.05-0.44)	< 0.01

TC/HDL-C: Cholesterol to high-density lipoprotein cholesterol; NAFLD: Non-alcoholic fatty liver disease; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GT: γ-glutamyl transpeptidase; PLT: Platelets; HBsAg: Hepatitis B virus surface antigen; CRE: Creatinine; UA: Uric acid; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; ApoA1: Apolipoprotein A1; ApoB: Apolipoprotein B; HBV-DNA(+): Hepatitis B virus DNA > 100 IU/mL; HBeAg(+): Hepatitis B e antigen positive; NAs: Nucleoside analogues.

central to this observed association. Adipokine may attenuate hepatic steatosis and the degree of its decline was shown to correlate with the severity of NAFLD[24]. Moreover, adiponectin levels were also shown to be positively correlated with HBV-DNA viral load in CHB patients[24,25].

However, the cross-talk between CHB and NAFLD remained controversial. There are studies indicating that NAFLD was inversely associated with the levels of HBV seromarkers[7,8]. In this present study, the proportion of HBV-DNA positivity in the NAFLD group (n = 15, 46.9%) was significantly lower than that in the non-NAFLD group (n = 116, 76.8%). Further, a large cohort study demonstrated that HBsAg clearance was significantly higher in CHB patients with hepatic steatosis than in those without[26], and these results were in agreement with animal experiments[27,28].

In regards to treatment, long-term oral use of NAs drugs such as entecavir and tenofovir were the main anti-HBV treatment as they are simple and safe to use, which is recognized all over the world[1]. Thus, oral NAs therapy alone was the first option for patients with CHB. However, CHB patients with NAFLD needed additional treatment besides antiviral drugs. Lifestyle intervention was a basic method for losing weight. For severe cases, pharmacological treatment was required to regulate the patients' lipid metabolism disorders[29].

Metabolic alterations in NAFLD may directly or indirectly affect the HBV-DNA levels of CHB patients[30]. Due to the common immune pathways of NAFLD and CHB, NAFLD-related metabolic stress may activate the suppressed innate immunity to restore the production of antiviral substances, which ultimately accelerates the clearance of HBV-DNA and HBsAg[31,32].

Metabolic syndrome is a highly prevalent concern in patients with NAFLD[17,33]. The typical characteristics of NAFLD are abnormal lipid accumulation in hepatocytes, hypertriglyceridemia, increased

Table 3 Correlation between cholesterol to high-density lipoprotein cholesterol and nonalcoholic fatty liver disease in different models

Variable	Model 1			Model 2			Model 3		
	OR	95%CI	*P* value	OR	95%CI	*P* value	OR	95%CI	*P* value
TC/HDL-C	0.94	1.55-4.19)	< 0.01	0.96	(1.52-4.51)	< 0.01	-2.27	(0.01-79.91)	0.5
TC/HDL-C									
≤ 3.5	Reference			Reference			Reference		
3.5-5	-2.53	(0.01-0.48)	0.01	1.59	(1.10-21.94)	0.04	-0.64	(0.04-6.77)	0.63
> 5	-0.77	(0.14-1.50)	0.2	2.29	(1.60-61.40)	0.01	-1.74	(0.01-14.45)	0.44
P for trend	0.02			0.05			0.73		

Model 1 was not adjusted for other pertinent clinical variables.
Model 2 was adjusted according to gender and age.
Model 3 was adjusted according to gender, age, body mass index, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, platelets, Hepatitis B virus surface antigen, creatinine, uric acid, triglyceride, total cholesterol, High-density lipoprotein cholesterol, Low-density lipoprotein cholesterol, Apolipoprotein A1, Apolipoprotein B, Hepatitis B virus DNA > 100 IU/mL, Hepatitis B e antigen positive, nucleoside analogues.
CI: Confidence interval; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol.

Table 4 The independent correlation between cholesterol to high-density lipoprotein cholesterol and nonalcoholic fatty liver disease by multivariate piecewise linear regression

TC/HDL-C	β	95%CI	*P* value
< 4.9	5.4	(2.3-12.6)	< 0.01
≥ 4.9	0.5	(0.1-2.2)	0.39

Effect: nonalcoholic fatty liver disease, cause: Total cholesterol High-density lipoprotein cholesterol.
Adjusted: gender, age, Body mass index, aspartate aminotransferase, Alanine aminotransferase, γ-glutamyl transpeptidase, Platelets, Hepatitis B virus surface antigen, Creatinine, Uric acid, Triglyceride, Total cholesterol, High-density lipoprotein cholesterol, Low-density lipoprotein cholesterol, Apolipoprotein A1, Apolipoprotein B, Hepatitis B virus DNA > 100 IU/mL, Hepatitis B e antigen positive, Nucleoside analogues. CI: Confidence interval; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol.

LDL-C levels, and reduced HDL-C particles. Metabolic perturbations promote liver injury and inflammation, which can lead to increased risk for hepatic fibrosis[34]. A cohort study of Chinese people with normal lipid metabolism indicated that a low-density lipoprotein to high-density lipoprotein (LDL/HDL) ratio was superior to other lipoproteins in identifying people at risk of NAFLD[35]. Studies from the Framingham Cardiovascular Institute also showed that a ratio of TC/HDL-C greater than 4 was a major risk factor for cardiovascular thrombosis[36]. In this present study, our results showed that TG, TC, LDL-C, ApoB, and TC/HDL-C had a significant increment in CHB patients combined with NAFLD. Concordant with the results of previous studies, we observed that although the levels of HDL-C and ApoA1 were decreased, no significant statistical difference was observed[12].

In our study, TC/HDL-C was a positive risk factor for NAFLD ($P < 0.01$) in univariate analysis. Previous studies suggested that there was a linear relationship between TC/HDL-C and NAFLD in the general population[14]. However, in this study, curve fitting analysis model showed that the association between TC/HDL-C and NAFLD was non-linear in the CHB population for an inflection point of 4.9. Thus, we speculated that TC/HDL-C was positively associated with NAFLD when the ratio of TC/HDL-C was less than 4.9 in the CHB population.

There were some limitations observed in this study. First, the investigated population was relatively small and therefore, large-scale studies are needed to validate our findings. Second, the assessment of NAFLD was based on hepatic ultrasonography rather than liver biopsy, which was the traditional gold standard for the assessment of NAFLD[37]. Patients could be reluctant to undergo liver biopsy because of its high cost, invasiveness and risk of complications[38]. Furthermore, fibrosis indices such as hyaluronic acid, laminin, procollagen III peptide, collagen type IV, and transient elastography were not included in the analyses due to missing data on fibrosis indices and could have been conducive to evaluating the relationship between NAFLD and different stages of CHB. In future studies, we will assess the relationship between fibrosis indices and TC/HDL-C. Lastly, this cross-sectional study only explored the relationship between the TC/HDL-C and NAFLD and was unable to reveal the causal and effect relationship between them.

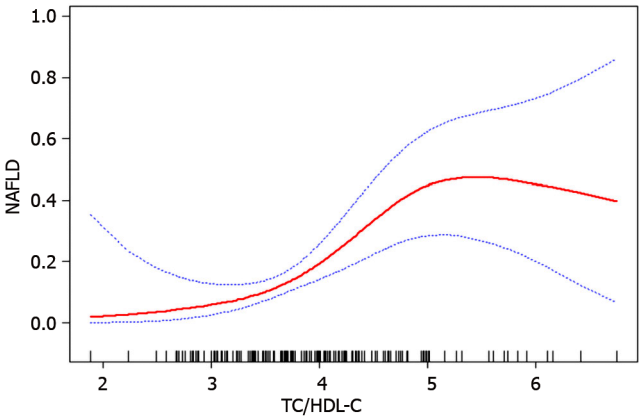

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Figure 2 Relationship between total cholesterol to high-density lipoprotein cholesterol ratio and nonalcoholic fatty liver disease by smooth curve fitting. Adjustment variables: Sex, age, body mass index, aminotransferase, aminotransferase, γ-glutamyl transpeptidase, platelets, hepatitis B surface antigen, creatinine, uric acid, triglyceride, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, apolipoprotein A1, apolipoprotein B, hepatitis B virus DNA (+), Hepatitis B e antigen (+), nucleoside analogs. TC/HDL-C: Total cholesterol to high-density lipoprotein cholesterol ratio; NAFLD: Nonalcoholic fatty liver disease.

CONCLUSION

In conclusion, the study demonstrated that the relationship between TC/HDL-C and NAFLD was non-linear in the CHB population. TC/HDL-C was positively correlated with NAFLD when TC/HDL-C was less than 4.9, but no such trend could be observed when the ratio of TC/HDL-C was more than 4.9.

ARTICLE HIGHLIGHTS

Research background

Due to the growing prevalence of nonalcoholic fatty liver disease (NAFLD), the coexistence of hepatitis B virus (HBV) infection and NAFLD is commonly observed around the world. However, the cross-talk between these two diseases remained questionable.

Research motivation

Previous studies showed that the total cholesterol to high-density lipoprotein cholesterol ratio (TC/HDL-C) was a better predictor of NAFLD than other lipid metabolism biomarkers and might be a new indicator of NAFLD. However, the association between TC/HDL-C and NAFLD in an HBV-infected population has not been previously investigated.

Research objectives

To investigate the association between TC/HDL-C and NAFLD in a CHB population.

Research methods

Univariate and multivariate logistic regression models, curve fitting analysis and threshold calculations were used to assess the relationship between TC/HDL-C and NAFLD.

Research results

A non-linear association was detected between TC/HDL-C and NAFLD in the CHB population at an inflection point of 4.9. The effect size on the left and right sides of inflection point were 5.4 (95%CI: 2.3-12.6, $P < 0.01$) and 0.5 (95%CI: 0.1-2.2, $P = 0.39$), respectively.

Research conclusions

In the CHB population, the relationship between TC/HDL-C and NAFLD was non-linear. TC/HDL-C was positively correlated with NAFLD when TC/HDL-C was less than 4.9.

Research perspectives

Further large-scale cohort studies are needed to validate whether TC/HDL-C is indeed a better predictor of NAFLD than other lipid metabolism biomarkers in the CHB population.

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FOOTNOTES

Author contributions: Xie WN designed the study, constructed the research ideas and interpreted the data; Zhou YG participated in the acquisition and analysis of the data and drafted the initial manuscript; Tian N supervised the project process and revised the article critically for important intellectual content.

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ORIGINAL ARTICLE

Observational Study

Assessment of resting energy expenditure in patients with cirrhosis

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Abstract

BACKGROUND

Malnutrition affects 20% to 50% of patients with cirrhosis. It may be associated with serious complications and has a direct impact on prognosis. Resting energy expenditure (REE) is an important parameter to guide the optimization of therapy and recovery of nutritional status in patients with cirrhosis. However, the REE of patients with cirrhosis is still unclear, casting doubt upon the optimal nutritional management approach.

AIM

To identify the best method that predicts the REE of cirrhotic patients, using indirect calorimetry (IC) as the gold standard.

METHODS

An observational study was performed on 90 patients with cirrhosis. REE was assessed by IC, bioelectrical impedance analysis (BIA), and predictive formulas, which were compared using Bland-Altman plots and the Student's *t*-test.

RESULTS

REE values measured by IC (1607.72 ± 257.4 kcal) differed significantly from those determined by all other methods (BIA: 1790.48 ± 352.1 kcal; Harris & Benedict equation: 2373.54 ± 254.9 kcal; IOM equation: 1648.95 ± 185.6 kcal; Cunningham equation: 1764.29 ± 246.2 kcal), except the Food and Agriculture Organization of the United Nations, World Health Organization, and United Nations University

(FAO/WHO/UNU) (1616.07 ± 214.6 kcal) and McArdle (1611.30 ± 241.8 kcal) equations. We found no significant association when comparing IC and 24-h dietary recall among different Child-Pugh classes of cirrhosis.

CONCLUSION

The IOM and FAO/WHO/UNU equations have the best agreement with the CI. These results indicate a possibility of different tools for the clinical practice on cirrhotic patients.

Key Words: Liver cirrhosis; Calorimetry; Indirect; Energy metabolism; Malnutrition

Core Tip: Patients with cirrhosis usually have a poor nutritional status, associated with complications of liver disease, which is an independent factor for mortality. Identifying the metabolic energy expenditure of these patients is the main guide for a more assertive nutritional and clinical application. The objective of this study was to recognize the best method for estimating resting energy expenditure between bioelectrical impedance analysis and predictive formulas, compared to the gold standard, indirect calorimetry (IC). Ninety cirrhotic patients were included. The Food and Agriculture Organization of the United Nations, World Health Organization and United Nations University (FAO/WHO/UNU) equation showed the best agreement with the IC. These results indicate a possibility of different tools for the clinical practice on cirrhotic patients.

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INTRODUCTION

The liver plays a key role in maintaining homeostasis and is the fundamental site of the metabolism of nutrients and other exogenous substances. Liver cirrhosis is the final stage of a chronic disease characterized by a process of disorganization in the lobular and vascular architecture of the liver, with fibrosis and diffuse nodular formation[1]. Importantly, it is estimated that there are 1.5 billion people diagnosed with chronic liver diseases, with an age-standardized incidence rate of 27.7/100000 for cirrhosis in these patients[2]. Patients with cirrhosis, regardless of etiology, commonly present malnutrition, resulting in a significant imbalance in energy metabolism that negatively impacts their prognosis and quality of life[3-5]. In this context, it is well established that cirrhotic patients benefit from improvements in dietary habits and nutritional interventions, and adequate dietary prescription depends on the precision of the protocols for energy requirement estimation.

The resting metabolic rate (RMR) reflects the energy required to maintain physiological processes, representing approximately 60% to 70% of the total daily energy requirement, whilst hepatic tissue metabolism accounts for almost 20% of the RMR in most patients[6,7]. RMR is influenced by different aspects of body composition, which could be drastically changed in the cirrhotic patient, due to hypercatabolism which is proportional to the disease progression[8]. Different studies show that protein degradation is measured by increased oxygen consumption through indirect calorimetry (IC), where an increase in resting energy expenditure (REE) is observed in 35% of people with cirrhosis compared to the healthy population[9-11]. This conflict could be explained by several confounding factors, such as the use of medication, the patient's body composition, and the presence of comorbidities[12]. However, the current literature is still conflicting regarding the relationship between cirrhosis progression and RMR alterations. Some studies have reported an increase in REE compared to the healthy population[13,14] while others have reported a decrease in REE[15,16]. Therefore, since the nutritional prescription is crucial to mitigate the progression of liver malfunction and/or alleviate complications characteristic of cirrhosis, appropriate estimation of patients' energy requirements is vital.

IC is the most reliable method to estimate the RMR, but it is expensive and time-consuming, and requires trained personnel and specific apparatus[17]. Alternatively, several predictive equations were developed to estimate the REE using specific individual characteristics[18]. Although most of the equations were developed in different populations, their accuracy in clinical practice is widely variable. It is a feasible method for RMR estimation when the proper equation for the individual is applied[18].

Currently, there is still no predictive equation considered the most accurate for cirrhotic patients. So much so that in the meta-analysis by Eslamparast *et al*[19], when analyzing 17 articles on the estimation of RMR in cirrhotic patients, which compared IC with different predictive formulas, they observed that the RMR values are underestimated, especially in males and in the Western population. Furthermore, there are insufficient data regarding the value of RMR according to the severity of chronic liver disease.

Noteworthy, miscalculation of REE in patients with cirrhosis can lead to inaccurate or inappropriate therapeutic management and worsening symptoms such as anorexia, dysgeusia, early satiety, nausea, and vomiting (especially in the presence of hepatic encephalopathy), and may potentiate adverse drug reactions[20,21]. In this context, the objective of the present study was to determine the REE of patients with cirrhosis by IC and compare the values thus obtained to those estimated by bioelectrical impedance analysis (BIA) and common predictive equations, in order to identify a reliable method for calculating energy expenditure applicable in clinical practice.

MATERIALS AND METHODS

This was an observational study. We included 90 patients who were receiving clinical management of liver cirrhosis at the Outpatient Gastroenterology and Liver Transplantation Clinics of Santa Casa de Misericórdia de Porto Alegre, Rio Grande do Sul, Brazil from March 2017 to July 2018. All patients included in this study agreed to participate and provided written informed consent. The study protocol was approved by the Research Ethics Committees of Santa Casa de Misericórdia de Porto Alegre (No. 2.387.800). Sample size calculation was based on a previous study by Teramoto *et al*[22] which compared measured and predicted energy expenditure in patients with cirrhosis. Considering a statistical power of 80% and a significance level of 5%, the minimum sample size was estimated at 90 patients.

Adult patients (age 18 years or older) of both sexes with cirrhosis of the liver were eligible for inclusion. Patients on enteral feeding were excluded, as were those with amputation of any limb and those unable to complete the proposed evaluations (*e.g.*, those who reported discomfort during IC, who could not remain in position, or who had a pacemaker which precluded BIA). Data from the electronic medical records of the patients, related to the diagnosis, staging by the Child-Pugh score, age, and sex of the participants, were collected. The diagnosis of cirrhosis was made by clinical, laboratory, imaging, and/or, eventually, liver biopsy in accordance with the hospital liver transplant group standards[12].

Current body weight was measured on a calibrated Filizola anthropometric scale (precision 0.1 kg). Height was measured with a wall-mounted stadiometer, with the patient standing upright and barefoot. Body mass index (BMI) was calculated as {BMI = weight (kg)/[height (cm)]^2} and classified according to the World Health Organization curves[23].

BIA was performed as described elsewhere using a Biodynamics model 450 BIA device (current 800 μA, frequency 50 kHz), with electrodes placed on the hand/wrist and foot/ankle.

IC was performed by the same investigator, using a Korr MetaCheck calorimeter. The assessment was begun after a minimum of 4 h and a 30-min rest. Measurement was performed with the patient perfectly still in the supine position, for 10 to 30 min, wearing a rigid face mask. The formula described by Weir (14) was used to calculate REE during the most stable period of analysis, based on O_2 consumption (VO_2), CO_2 output (VCO_2), and urine urea nitrogen, as follows: REE = [3.9 (VO_2)] + [1.1 (VCO_2)][24].

Table 1 describes the energy expenditure predictive equations used in the study: BIA - Based on Grande & Keys[25]; Cunningham[26]; Harris and Bennedict[27]; Food and Agriculture Organization of the United Nations, World Health Organization and United Nations University [Food and Agriculture Organization of the United Nations, World Health Organization and United Nations University (FAO/WHO/UNU)][23]; Institute of Medicine[28]; McArdle[29]; and Mifflin[30].

Statistical analysis

Quantitative variables are expressed as the mean and standard deviation, and categorical variables, as absolute and relative frequencies. The equations were compared with IC using the Bland-Altman method[31], and also the Student's *t*-test for paired samples. The Student's *t*-test for paired samples was also used for comparison between IC and 24 h dietary recall findings. The correlation between BMI and IC was assessed by Pearson's correlation coefficient. Analysis of variance (ANOVA) with Tukey's post-hoc test was used for comparison of mean 24-h dietary recall and REE-IC according to Child-Pugh class. The significance level was set at 5% ($P < 0.05$). All analyses were performed with PASW Statistics, Version 18.0.

RESULTS

Ninety patients, with a mean age of 57.1 (± 9.3) years, were assessed. Of these, 52 (57.8%) were male. The clinical profile of the sample is described in Table 2.

Table 1 Predictive equations for derivation of energy expenditure, all values obtained in kilocalories

BIA[25]	
Women and men	31.2 × fat-free mass in kilograms
Cunningham[26]	
Women and men	22 × fat-free mass in kilograms + 500
Harris and Benedict[27]	
Women	655 + 9.56 × weight + 1.85 × height − 4.68 × age
Men	66.5 + 13.75 × weight + 5.0 × height − 6.78 × age
FAO/WHO/UNU[23]	
Women	Age 30–60 years: 8.7 × weight + 829
	Age > 60 years: 10.5 × weight + 596
Men	Age 30–60 years: 11.6 × weight + 879
	Age > 60 years: 13.5 × weight + 487
IOM[28]	
Women	[247 − (2.67 × age) + (401.5 × height)] + [8.6 × weight]
Men	[293 − (3.8 × age) + (456.4 × height)] + [10.12 × weight]
McArdle *et al*[29]	
Women and men	[(lean body mass in kilograms) × 21.6] + 370
Mifflin *et al*[30]	
Women	10 × weight in kilograms + 6.25 × height − 5 × age -161
Men	10 × weight in kilograms + 6.25 × height − 5 × age +5

BIA: Bioelectrical impedance analysis; FAO: Food and Agriculture Organization of the United Nations; WHO: World Health Organization; UNU: United Nations University.

Table 3 shows the values of REE in kilocalories, measured by IC and predictive methods. The mean REE measured by IC was 1607.72 ± 257.4. A correlation between REE measured by IC and muscle mass in kilograms (R^2 = 0.353, P = 0.001) was found. Also, the IC values were not different between patients classified in groups in accordance with their Child-Pugh scores (P = 0.885). Although the IC values showed a positive correlation with predictive methods, the IC values were significantly different when compared to predictive methods, except for the McArdle and FAO/WHO/UNU predictive equations.

As shown in Figure 1, we found differences in agreement between IC and the predictive methods. The best agreement was found between IC and the IOM equation, followed by FAO/WHO/UNU and McArdle equations. The agreement between IC and BIA was below 10% of the mean difference. The Harris and Benedict and the Mifflin equations showed less agreement with the IC values. The ANOVA analysis showed no differences of IC or REE estimated by different methods when patients were grouped by their Child-Pugh scores (data not showed).

DISCUSSION

The present study aimed to determine the REE of patients with cirrhosis by IC and compare the values thus obtained to those estimated by BIA and common predictive equations. The IOM and FAO/WHO/UNU equations showed the best agreement with IC, whilst the McArdle equation and BIA could also be considered appropriate for REE estimation.

The present study evaluated 90 patients, with a mean age of 57 (± 9.3) years, which is close to that previously reported[32] whilst the male predominance of the sample is also consistent with prior work by Tajika *et al*[5] and Wilkens Knudsen *et al*[33]. Regarding Child-Pugh classification, our sample was homogeneous, with 33 patients in class A, 36 in class B, and 21 identified as having class C; this proportion differs from that reported by Qing-Hua Meng, where 60% of patients had Child-Pugh A and only eight had class C[34]. Regarding nutritional status, the mean BMI of patients in our study was 28.6 ± 5.6 kg/m^2, which would classify them as overweight[23]. This result is in line with Brazilian studies of patients with cirrhosis which confirmed the same classification[3,35]. Like Fernandes *et al*[3], we did not

Table 2 Sample characteristics

Characteristic	*n* = 90
Female	38 (42.2)
Male	52 (57.8)
Age (years), mean ± SD	57.1 ± 9.3
BMI (kg/m^2), mean ± SD	28.6 ± 5.6
Child-Pugh, *n* (%)	
A	33 (36.7)
B	36 (40.0)
C	21 (23.3)
Hepatic encephalopathy, *n* (%)	5 (5.5)
Ascites, *n* (%)	17 (18.8)
Edema, *n* (%)	8 (8.8)
Etiology of cirrhosis, *n* (%)	
HCV	28 (31.1)
Alcohol	21 (23.3)
Cryptogenic	7 (7.8)
NASH	17 (18.9)
HCC	5 (5.5)
HBV	4 (4.4)
Other	8 (8.8)

BMI: Body mass index; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; NASH: Nonalcoholic steatohepatitis; HBV: Hepatitis B virus; REE: Resting energy expenditure; IC: Indirect calorimetry; SD: Standard deviation.

Table 3 Comparisons and correlations between resting energy expenditure measured by indirect calorimetry and different predictive methods

Variable	mean ± SD (kcal)	Difference (95%CI)	*P* value	R^2 (*P*)
Indirect calorimetry	1607.72 ± 257.4	-	-	-
Bioelectrical impedance	1790.48 ± 352.1	-182.8 (-217.3 to -148.1)	< 0.001	0.899 (< 0.001)
Cunningham	1764.29 ± 246.2	-156.5 (-180.3 to -132.7)	< 0.001	0.899 (< 0.001)
Harris & Benedict	2373.54 ± 254.9	-765.8 (-802.4 to -729.1)	< 0.001	0.767 (< 0.001)
FAO/WHO	1616.07 ± 214.6	-8.35 (-61.8 to 45.1)	0.757	0.457 (< 0.001)
IOM	1648.95 ± 185.6	-41.23 (-61.6 to -20.8)	< 0.001	0.955 (< 0.001)
McArdle *et al*	1611.30 ± 241.8	-3.58 (−27.3 to -20.1)	0.765	0.899 (< 0.001)
Mifflin *et al*	1558.71 ± 201.0	49.00 (30.4 to 67.58)	< 0.001	0.955 (< 0.001)

FAO: Food and Agriculture Organization of the United Nations; WHO: World Health Organization; UNU: United Nations University.

find BMI to be a reliable method of estimating nutritional status in this population, due to the distortion of body weight inherent to the underlying disorder. Strikingly, we did not identify a correlation between BMI and REE, albeit we report a correlation between REE and muscle mass (in kilograms).

IC is considered by many researchers as the gold standard for measuring REE. It is a non-invasive method, capable of measuring basal energy expenditure by means of gas exchange, thus ensuring greater precision in measurement[35-37]. In our study, the average REE-IC was 1522 ± 271 kcal, very close to the result reported by Pinto *et al*[36] of 1534 ± 300 kcal in a sample of 45 patients waitlisted for liver transplantation, which corroborates the expectation of accuracy of caloric prediction by this

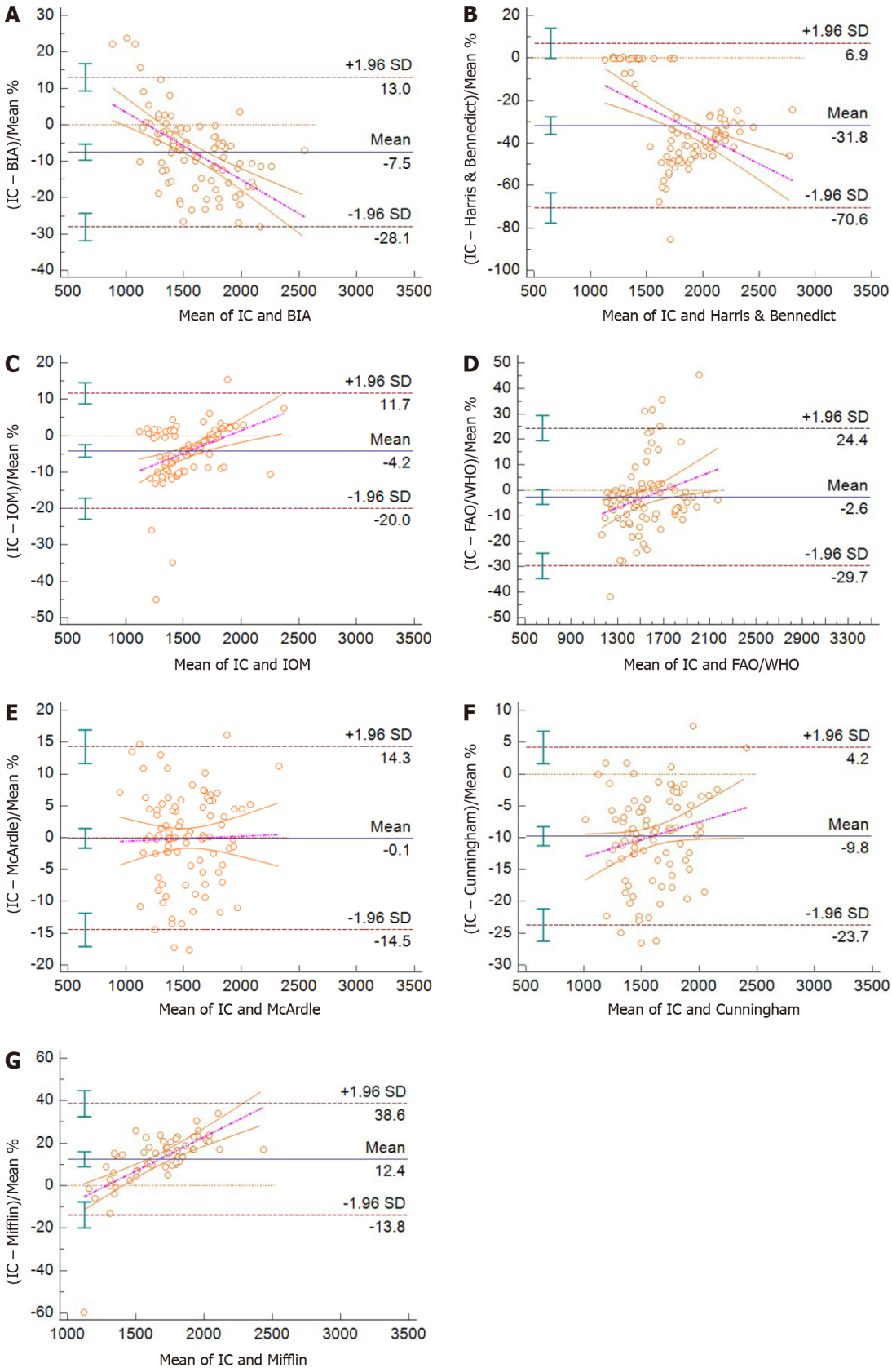

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Figure 1 Bland-Altman plots comparing indirect calorimetry with predictive methods. A: Mean of indirect calorimetry (IC) and bioelectrical impedance analysis (BIA); B: Mean of IC and Harris & Bennedict; C: Mean of IC and IOM; D: Mean of IC and FAO/WHO; E: Mean of IC and McArdle; F: Mean of IC and Cunningham; G: Mean of IC and Mifflin. BIA: Bioelectrical impedance analysis; FAO: Food and Agriculture indirect calorimetry Organization of the United Nations; IC: Indirect calorimetry; WHO: World Health Organization.

method.

Comparison of REE-IC values with those calculated by the Harris and Benedict (HB) equation revealed super estimated values. Consistent with other studies[22,34,35], our findings suggest that common predictive equations for estimation of REE could be clinically inaccurate in cirrhotic patients, since they are usually based on body weight, a parameter that can be altered by several factors—such as ascites and fluid retention—and thus directly affect the energy expenditure estimated by the equation [33]. Thus, even considering their low cost and applicability, using predictive equation should consider the aforementioned aspects, since overestimation of REE has been reported in many previously published comparisons[22,34,35]. Corroborating our line of thought, Meng *et al*[34] found a reduced REE in 53% of their sample of 153 patients with liver cirrhosis when REE measured by IC as compared to REE estimated by the HB equation. Likewise, Teramoto *et al*[22] evaluated 488 patients and found that the estimated REE was 1256 kcal by IC *vs* 1279 kcal by the HB formula.

Boullata *et al*[38] aimed to compare the accuracy of seven predictive equations, including the Harris-Benedict and the Mifflin equations, against measured REE in hospitalized patients, including patients with obesity and critical illness. The authors concluded that no predictive method was accurate when considering accuracy as 90% to 110% of the value obtained by IC. In our study, most of the evaluated predictive methods resulted in an error below 10%. Further, based on our findings, in circumstances where IC is not available, the FAO/WHO/UNU or McArdle *et al*[29] equations can be used to accurately estimate REE, since they may yield values closer to those of IC in patients with cirrhosis. Also, the IOM equation could be used, since it also showed good agreement in the Bland-Altman analysis, albeit it was significantly different in the *t*-test. Noteworthy, a previous study including patients with portal hypertension reported that the Zanella *et al*[39] equation was one of the predictive methods that differed most in REE estimates in the study population. As in our study, IC yielded a higher value than all other methods. The authors noted that all other methods underestimated the predicted REE by more than 200 kcal when compared to IC, except Cunningham's predictive equation. Therefore, it bears stressing that the same method of assessment in different populations can present different correlations with the available predictive equations.

Although we have not found prior publications supporting the use of BIA to determine REE as a means of extrapolating energy expenditure in patients with cirrhosis, this method was used in a study by Strain *et al*[40] of morbidly obese patients. There was no significant difference between the value predicted by BIA (which was based on the HB equation) and IC, which could support the indication of BIA as a good predictor of energy expenditure in this population[40]. Our study also found that the BIA equipment was able to predict REE, albeit different BIA equipment applies different equations to predict REE using body composition parameters, and users should observe which equation is being applied.

We found no significant differences in IC between patients classified as Child-Pugh A and those classified as Child-Pugh B. In this respect, our findings corroborate those of Teramoto *et al*[22] and Meng *et al*[34], who found no statistically significant difference in IC when comparing the three Child-Pugh prognostic classes. Moreover, Belarmino *et al*[32] reported that the dietary intake of patients with cirrhosis in their sample was 1.4 times greater than that predicted by IC, while in our study, it was 1.14 times greater. Teramoto *et al*[22] found that most patients in their sample had adequate dietary intake and there was no statistically significant difference between Child-Pugh classes, corroborating the findings in the present study. Meng *et al*[34] highlighted that dietary intake can be impaired by factors such as anorexia, weakness, fatigue, low-grade encephalopathy, and restrictions on sodium, protein, and fluid intake. These data, in addition to the insufficient energy intake in 48% of the patients studied by Nunes *et al*[41], who evaluated a sample of 25 cirrhotic patients and found an average of 2012 ± 720 kcal, highlight the importance of adequate estimation of REE in these patients, to prevent malnutrition and improve prognosis and outcomes.

Limitations of the present study include the absence of a healthy control group for comparison and the possibility of recall bias interfering with the 24 h dietary recall, despite this being a validated method.

CONCLUSION

The present study aimed to determine the REE of patients with cirrhosis by IC and compare the values thus obtained to those estimated by BIA and common predictive equations. The McArdle and FAO/WHO/UNU equations showed the best agreement with IC, whilst the IOM equation and BIA could also be considered appropriate for REE estimation. Further studies in different populations of patients with cirrhosis, including different severity profiles, are needed to determine the best methods for REE estimation in clinical practice.

ARTICLE HIGHLIGHTS

Research background

Patients with cirrhosis commonly present malnutrition, resulting in a significant imbalance in energy metabolism that negatively impacts their prognosis and quality of life. However, adequate dietary prescription depends on the precision of the protocols for energy requirement estimation, and the current literature is still conflicting regarding the relationship between cirrhosis progression and resting metabolic rate alterations.

Research motivation

Reliable calculation of resting energy expenditure (REE) in patients with cirrhosis is pivotal to appropriate therapeutic management. However, there is still a need to evaluate which of the predictive equations is more effective in the clinical setting.

Research objectives

The objective of the present study was to determine the REE of patients with cirrhosis by indirect calorimetry (IC) and compare the values thus obtained to those estimated by bioelectrical impedance analysis (BIA) and common predictive equations.

Research methods

This was an observational study performed at the Outpatient Gastroenterology and Liver Transplantation Clinics of Santa Casa de Misericórdia de Porto Alegre, Rio Grande do Sul, Brazil. Data from the electronic medical records of the patients, related to the diagnosis, staging by the Child-Pugh score, age, and sex of the participants, were collected. The diagnosis of cirrhosis was made by clinical, laboratory, imaging, and/or, eventually, liver biopsy in accordance with the hospital liver transplant group standards. BIA and IC were performed and the results were compared to energy expenditure predictive equations using the Bland-Altman method, and also the Student's *t*-test for paired samples.

Research results

Ninety patients, with a mean age of 57.1 years, were assessed. The mean REE measured by IC was 1607.72 and there were no differences in REE when comparing groups with different Child-Pugh scores. The IC values were significantly different when compared to predictive methods, except for the McArdle and Food and Agriculture Organization of the United Nations, World Health Organization and United Nations University (FAO/WHO/UNU) predictive equations. The best agreement was found between IC and the IOM equation, followed by the FAO/WHO/UNU and McArdle equations. The agreement between IC and BIA was below 10% of the mean difference. The Harris and Benedict and the Mifflin equations showed less agreement with the IC values.

Research conclusions

The present study determined the REE of patients with cirrhosis, indicating that the McArdle and FAO/WHO/UNU equations showed the best agreement with IC, whilst the IOM and BIA could also be considered appropriate for REE estimation.

Research perspectives

Further studies in different populations of patients with cirrhosis, including different severity profiles, are needed to determine the best methods for REE estimation in clinical practice.

FOOTNOTES

Author contributions: Ferreira S contributed to the conception and design of the study, data collection, statistical analysis and writing of the manuscript; Marroni CA contributed to the conception and design of the study and writing of the manuscript; Stein JT, Henz AC and Rayn RG collected the data; Schmidt NP contributed to the conception and design of the study, data collection; Carteri RB statistical analysis and manuscript writing; Fernandes SA manuscript writing and critical review.

Institutional review board statement: This study was approved by the Research Ethics Committee of Irmandade Santa Casa de Misericórdia de Porto Alegre (No. 2.387.800).

Informed consent statement: Patients who agreed to participate in the study signed the Free and Informed Consent Form.

Conflict-of-interest statement: All authors declare that there are no conflicts of interest related to this article.

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ORIGINAL ARTICLE

Prospective Study

Prognostic value of von-Willebrand factor in patients with liver cirrhosis and its relation to other prognostic indicators

Elena Curakova Ristovska, Magdalena Genadieva-Dimitrova

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Abstract

BACKGROUND

Von-Willebrand factor (vWF) disposes certain prognostic value in patients with liver cirrhosis, but its relation to other prognostic indicators has not been fully investigated.

AIM

To analyze the relation between vWF and other prognostic indicators in cirrhotic patients and to evaluate its prognostic value for mortality.

METHODS

This analytic prospective study was carried out in a tertiary center and initially enrolled 71 patients with liver cirrhosis and portal hypertension. It analyzed the relation between vWF and the stage of the disease and several inflammatory and prognostic indicators. The prospective analysis, performed on a sample of 63 patients, evaluated the association between the selected variables [vWF, Model for End-stage Liver Disease (MELD) score, C-reactive protein (CRP), ferritin, vitamin D, activated partial thromboplastin time, thrombin time, D-dimer concentration] and the survival time as well as their predictive value in terms of 3-mo, 6-mo and 1-year mortality.

RESULTS

vWF was significantly higher in patients with higher Child-Turcotte-Pugh class (P = 0.0045), MELD group (P = 0.0057), ferritin group (P = 0.0278), and D-dimer concentration (P = 0.0232). vWF significantly correlated with D-dimer concen-

tration, ferritin, CRP, International Normalized Ratio, and MELD, Child-Turcotte-Pugh, Sequential Organ Failure Assessment, and CLIF-consortium organ failure (CLIF-C OF) scores. vWF, MELD score, and CRP were significantly associated with death and were significant predictors of 3-mo, 6-mo, and 1-year mortality. Each vWF unit significantly increased the probability for 3-mo mortality by 1.005 times (P = 0.008), for 6-mo mortality by 1.006 times (P = 0.005), and for 1-year mortality by 1.007 times (P = 0.002). There was no significant difference between the diagnostic performance of vWF and MELD score and also between vWF and CRP regarding the 3-mo, 6-mo, and 1-year mortality.

CONCLUSION

In patients with liver cirrhosis, vWF is significantly related to other prognostic indicators and is a significant predictor of 3-mo, 6-mo, and 1-year mortality similar to MELD score and CRP.

Key Words: von-Willebrand factor; Cirrhosis; Mortality; C-reactive protein; D-dimer

Core Tip: The prognostic value of von-Willebrand factor (vWF) in cirrhotic patients has been previously evaluated, but its relation to other inflammatory and prognostic indicators has not been fully investigated. The study confirmed that vWF was significantly associated with the stage of liver disease, D-dimer concentration, ferritin, and survival and that vWF was a significant predictor of 3-mo, 6-mo, and 1-year mortality similar to Model for End-stage Liver Disease score and C-reactive protein. These data reflect the important prognostic role of the complex and dynamic interaction between endothelial dysfunction, systemic inflammation, and cirrhosis-related coagulopathy in cirrhotic patients.

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INTRODUCTION

The high mortality rate in cirrhotic patients and the shortage of organs worldwide are constantly raising the issue of mortality prediction in patients with end-stage liver disease in terms of differentiating the most critically ill patients with the highest short-term mortality[1]. This leads to continuous and intense research towards defining new biological variables that possess certain prognostic potential in cirrhotic patients. Chronic liver disease is inevitably related to portal hypertension (PH), an entity that closely accompanies and often defines the natural course of the disease. Its prognostic significance derives from the fact that PH is closely related to several severe, life-threatening complications that are associated with high morbidity and mortality[2]. PH is diagnosed and quantified with the hepatic venous pressure gradient, but since it is an invasive, expensive, and not widely available procedure, there is a need for alternative relevant and noninvasive indicators of PH.

Recent research emphasizes the significant contributing role of endothelial dysfunction (ED) in the pathogenesis and progression of PH and its relation to poor prognosis in cirrhotic patients[3]. Intrahepatic ED is considered a major determinant of the increased hepatic vascular tone of the cirrhotic liver, and systemic ED due to endotoxemia is the cause of increased nitric oxide production, the major determinant of the hyperdynamic circulation [3]. Considering its important contributing role in the pathogenesis of PH, von-Willebrand factor (vWF) has recently gained some attention as a prognostic indicator in cirrhotic patients. The increased vWF production due to ED favors hypercoagulable state, formation of platelet-induced thromboses in the hepatic microcirculation, and gradual thrombotic vascular obliteration[4,5]. It is considered that the increased vWF concentration and the cirrhosis-related procoagulant imbalance are the two crucial predisposing events responsible for the progressive vascular occlusion of the portal circulation[4,5]. Also, ED is the major cause of many complex hemostatic abnormalities that occur in cirrhotic patients. The imbalance in the secretion of pro-coagulant, anticoagulant, fibrinolytic, and antifibrinolytic substances due to ED in different clinical settings may have a different hemostatic phenotype. On the other hand, short-term prognosis in cirrhotic patients largely depends on the accompanying liver-related events that temporarily worsen the liver function[6]. Recent data also emphasize the important contributing role of systemic inflammation (SI) in the pathogenesis of the majority of the acute events in cirrhotic patients. It has been established that SI is common and almost a persistent state, especially in advanced liver disease, that it has a crucial role in the course of

the disease, and that SI is related to adverse outcomes in cirrhotic patients[6-8].

It seems that ED, SI, and liver-related coagulopathy have an important role in the natural course of chronic liver disease[7,9-11]. The involvement of vWF as an indicator of ED plays a substantial role in the progression of PH, which explains the significant and relevant prognostic potential of vWF in cirrhotic patients. Still, the relation between vWF and other inflammatory and prognostic indicators has not been completely investigated. The aim of the study was to evaluate the relation between vWF and liver cirrhosis, its relation to other relevant prognostic indicators in cirrhotic patients, and the prognostic value of vWF in terms of 3-mo, 6-mo, and, 1-year mortality.

MATERIALS AND METHODS

Study design

This analytic monocentric prospective study initially enrolled 71 patients with liver cirrhosis and PH. Data regarding demographic and clinical characteristics of patients (age, gender, etiology, disease duration, data regarding previous complications, related diagnostic/therapeutic interventions) were collected, and a number of imaging and laboratory investigations were performed in order to determine the stage of the disease, to register the present complications of PH, and to assess the mortality risk. Besides the basic biochemical and hemostatic analyses, the concentration of vWF was also measured. Afterward, by using the American Society of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) criteria, the presence of systemic inflammatory response syndrome (SIRS) was determined, and the Child-Turcotte-Pugh (CTP) score, Model for End-stage Liver Disease (MELD) score, Sequential Organ Failure Assessment (SOFA) score, CLIF-consortium organ failure (CLIF-C OF) scores, and Simplified Acute Physiology Score II (SAPS II) were calculated. After enrollment, patients were prospectively monitored for 1 year, and every 3 mo a regular control examination was performed. During every regular visit clinical examination (physical and abdomen examination), vital parameters measurement (blood pressure, heart rate, respiratory rate, blood oxygenation, body temperature), abdominal ultrasound examination with color Doppler of the portal vein, complete blood count, and biochemical analysis of blood sample and urine sediment was performed. In case of some clinical deterioration during the follow-up, an additional investigation was performed (patients were provided with phone communication with the study doctor), after which the patients went back to the regular study protocol. During the follow-up period, 8 patients dropped out (occurrence of some of the exclusion criteria, transplanted or noncompliant patients). Within the prospective analysis performed in 63 patients, the predictive value of vWF and several parameters of interest [MELD score, C-reactive protein (CRP), ferritin, vitamin D, activated partial thromboplastin time (aPTT), thrombin time (TT), D-dimer concentration] were analyzed in terms of 3-mo, 6-mo, and 1-year mortality. All patients signed an informed consent form for participation in the study. The research and the study protocol were in line with the ethical principles of the Helsinki declaration.

Patients

The study enrolled patients with clinically evident liver cirrhosis and portal hypertension with no significant preexisting comorbidities (systemic, infective, cardiovascular, metabolic, or neoplastic disease) and without active alcohol consumption, previous thrombotic event, blood transfusion, or interferon, antiplatelet, or anticoagulant therapy. Some patients were enrolled after hospitalization at the University Clinic for Gastroenterohepatology in Skopje, and some were enrolled during the outpatient follow-up.

Biochemical analysis, hemostatic analysis, and vWF assay

At enrollment and during every regular visit, a complete blood count and biochemical blood analysis [glucose, blood urea nitrogen, creatinine, bilirubin, protein profile (albumin, globulin), sodium, potassium, calcium, iron, total iron-binding capacity, lipid profile (cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides), CRP, ferritin, vitamin D, urinary sediment, alpha fetoprotein], hemostasis [prothrombin time (PT), International Normalized Ratio (INR), aPTT, TT], D-dimer concentration, urinary sediment, and gas analyses from capillary blood sample were performed. The vWF concentration was measured in platelet-rich plasma aliquoted after centrifugation of sodium citrate blood sample. The analysis was performed by using the immunoturbidimetric method (vWF Ag Test Kit, Siemens, Munich, Germany) on an automatic coagulometer (BCS XP System-Siemens Healthiness Global device). The normal range of vWF was 50%-150%. According to the obtained score values, patients were classified in three CTP classes (Class A, B and C), in three MELD groups [group 1 (MELD ≤ 9), group 2 (MELD 10-19), and group 3 (MELD ≥ 20)], in three serum ferritin (SF) groups [group 1 (SF < 200 ng/mL), group 2 (SF 200-400 ng/mL), and group 3 (SF > 400 ng/mL)], in two D-dimer groups (below/above 500 μg/mL), and in two vitamin D groups (below/above 20 ng/mL).

Statistical analysis

The statistical analysis was performed by using the SPSS software package, version 22.0 for Windows (IBM Corp., Armonk, NY, United States). Descriptive statistics were provided as mean ± standard deviation, median, and interquartile range (IQR). Mann Whitney *U* test and Kruskal-Wallis *H* test were used to test the significance of the difference between the numeric variables without normal distribution. Correlation between vWF and other variables was analyzed by Spearman's correlation. Univariate Cox proportional model was used in order to evaluate the association between the selected variables and the survival time, and the univariate logistic regression analysis was used to determine the significant predictors of mortality. The diagnostic performance of the significant mortality predictors was assessed by the receiver operating characteristic (ROC) analysis and the area under the curve (AUC) values of two independent ROC curves that were compared using the *Z* test. *P* values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics and stage of disease

The mean age in the group was 58.8 ± 10.7 years [95% confidence interval (CI): 54.4-59.1], and there was a significant male predominance [56 (78.87%) men and 15 (21.13%) women; (gender ratio 3.73:1)]. Regarding etiology, alcoholic liver disease was the most prevalent entity (36 patients, 50%). According to the CTP classification, most patients were in class C (28, 39.40%), 25 patients (35.20%) in class B, and 18 patients (25.30%) in class A [CTP score 8.9 ± 2.9 (5-15); IQR = 9 (6-11)]. MELD score was 19.7 ± 9.9 (6-59); IQR = 18 (11-25). The CRP concentration was 21.1 ± 27.5 mg/L and SIRS was registered in 43 (60.60%) patients. The ferritin concentration was 290.45 ± 354.33 ng/mL [SF < 200 ng/mL in 39 (62.9%) patients, SF 200-400 ng/mL in 5 (8.1%), and SF > 400 ng/mL in 18 (29.0%) patients]. The vitamin D concentration was 17.65 ± 13.31 ng/mL, and the prevalence of vitamin D deficiency was 48.9% (Table 1).

vWF concentration and its relation to stage of disease and inflammatory and prognostic indicators

The mean vWF concentration in the group was 346.18 ± 155.97% (150-850), IQR = 318.40% (214.00-410.10) (Table 1). The analysis confirmed significantly higher vWF values in higher CTP class [Kruskal-Wallis *H* test: Chi-square (2) = 10.8177; *P* = 0.0045], MELD group [Kruskal-Wallis *H* test: Chi-square (2) = 10.3357; *P* = 0.0057], and SF group [Kruskal-Wallis H test: Chi-square (2) = 7.1653; *P* = 0.0278] and in patients with elevated (> 500 μg/mL) D-dimer concentration (Mann-Whitney *U* test: *Z* = 2.6407; *P* = 0.0083) (Table 2). The analysis did not show a significant difference between the vWF values regarding vitamin D deficiency (Mann-Whitney *U* test: Z = -1.6916; *P* = 0.0907) and platelet count [(above/below 100×10^9/L), (Mann-Whitney *U* test: Z = -0.02898; *P* = 0.9769)], (Table 2). The correlation analysis confirmed a strong significant positive linear correlation only between vWF and D-dimer concentration (*r* = 0.407) and a moderate significant positive linear correlation between vWF and CTP score, MELD score, SOFA score, CLIF-C OF score, CRP, ferritin, and INR (*r* = 0.348, 0.387, 0.327, 0.328, 0.315, 0.360 and 0.262, respectively) (Figure 1). The correlation between vWF and the other selected parameters (SAPS II score, vitamin D, Alveolar-Arterial Oxygen Gradient, hemoglobin, platelet count, WBC, PT, aPTT, TT) was weak or not significant (Figure 2).

Association between vWF and other variables with death and their predictive value for mortality

The Cox proportional model and the univariate logistic regression analysis showed that vWF, MELD score, and CRP were significantly associated with the event (death) and significant predictors of mortality in all three follow-up periods. The Cox proportional model showed that vWF, MELD score, CRP, and aPTT were significantly associated with 3-mo survival; that vWF, MELD score, CRP, and vitamin D were significantly associated with 6-mo survival; and that vWF, MELD score, CRP, vitamin D, ferritin, and aPTT were significantly associated with 1-year survival (Table 3). The univariate logistic regression analysis showed that vWF, MELD score, CRP, and D-dimer concentration were significant predictors of 3-mo mortality; that vWF, MELD score, CRP, and vitamin D were significant predictors of 6-mo mortality, and that vWF, MELD score, CRP, and ferritin were significant predictors of 1-year mortality (Table 3).

Regarding the association between vWF and survival in cirrhotic patients, the analysis showed that vWF was significantly associated with survival in all three follow-up periods and that each vWF unit significantly increased the daily association with death by 0.4% regarding 3-mo [Exp(B) hazard ratio (HR) = 1.004], by 0.6% regarding 6-mo [Exp(B) (HR) = 1.006], and by 0.4% [Exp(B) (HR) = 1.004] regarding 1-year survival. Regarding mortality, the analysis confirmed that each vWF unit significantly increased the probability for 3-mo mortality by 1.005 (*P* = 0.008) times, for 6-mo mortality by 1.006 (p = 0.005) times, and for 1-year mortality by 1.007 (*P* = 0.002) times (Table 3).

Regarding the association between CRP and survival in cirrhotic patients, the analysis showed that CRP was significantly associated with survival in all three follow-up periods and that each CRP unit significantly increased the daily association with the event (death) by 2.9% [Exp(B) (HR) = 1.029]

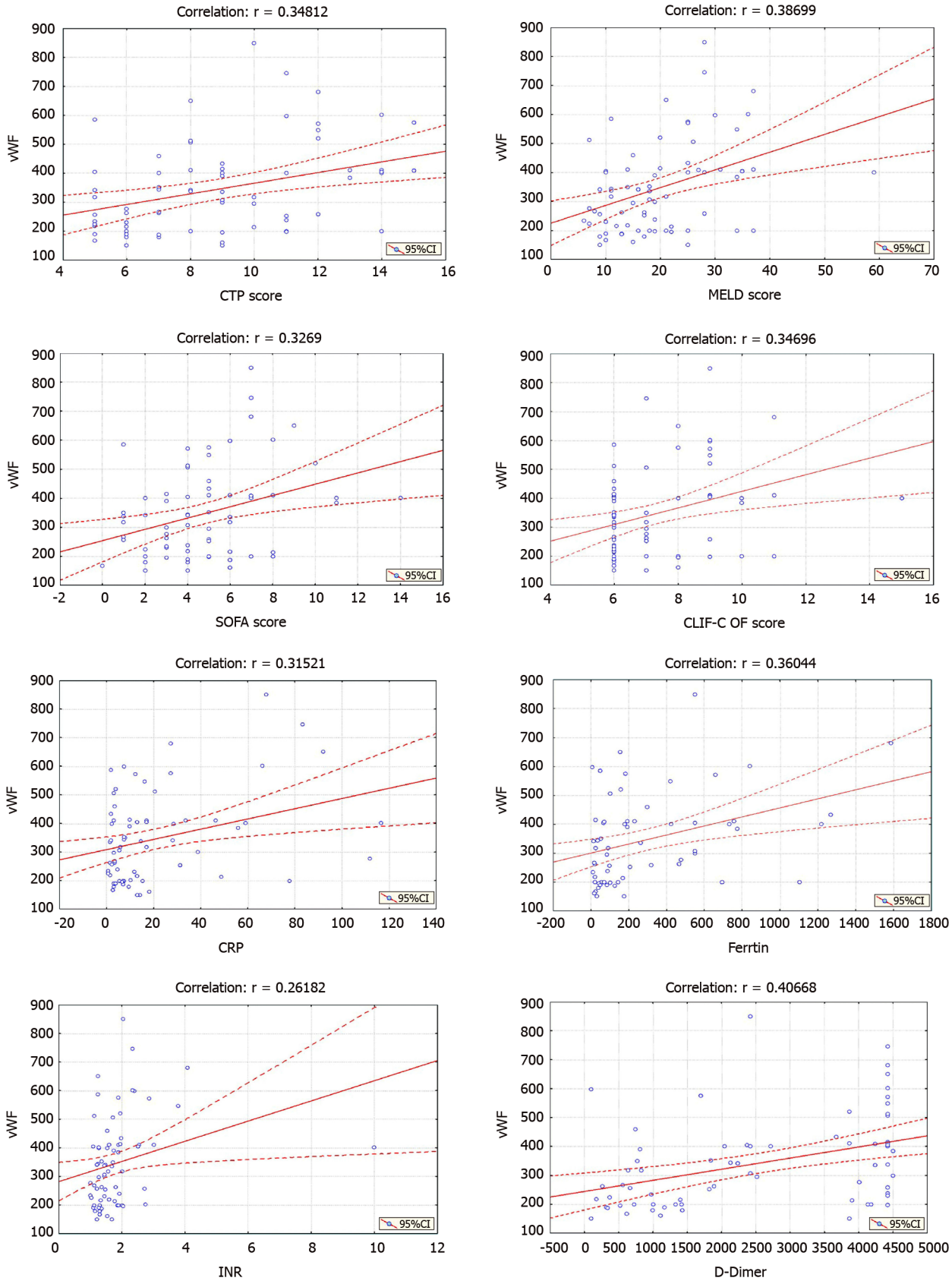

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Figure 1 Significant positive linear correlation between von-Willebrand factor and C-reactive protein score, model for end-stage liver disease score, sequential organ failure assessment score, CLIF-consortium organ failure score, ferritin, C-reactive protein, international normalized ratio, and D-dimer level. vWF: von-Willebrand factor; CTP: Child-Turcotte-Pugh; MELD: Model for End-stage Liver Disease; CRP: C-reactive protein; INR: International normalized ratio.

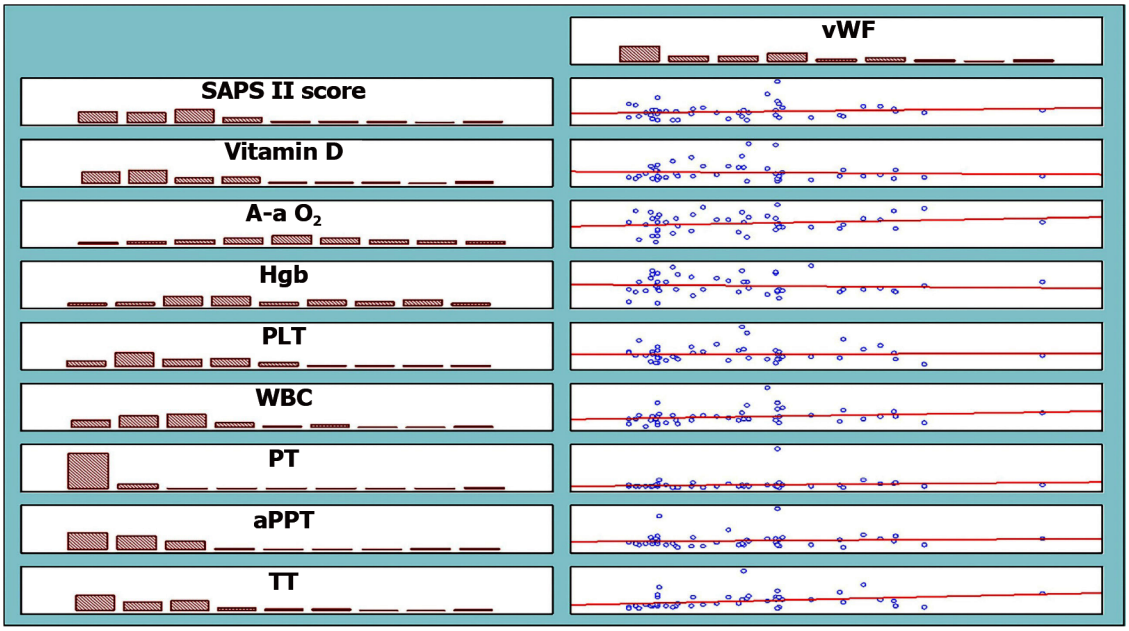

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Figure 2 Weak or insignificant correlation between von-Willebrand factor and Simplified Acute Physiology Score II score, vitamin D, A-a O_2, hemoglobin, platelets, white blood cell, prothrombin time, activated partial thromboplastinin time, and thrombin time. vWF: von-Willebrand factor; SAPS: Simplified Acute Physiology Score; A-a O_2: Alveolar-arterial oxygen gradient; PLT: Platelets; WBC: White blood cell; PT: Prothrombin time; aPTT: Activated partial thromboplastinin time; TT: Thrombin time.

regarding the 3-mo, by 4.4% [Exp(B) (HR) = 1.044] regarding the 6-mo, and by 2.5% [Exp(B) (HR) = 1.025] regarding the 1-year survival. More importantly, we confirmed that CRP was a significant predictor of mortality in patients with liver cirrhosis and that each CRP unit significantly increased the probability for 3-mo mortality by 1.044 (P = 0.001) times, for 6-mo mortality by 1.044 (P = 0.001) times, and for 1-year mortality by 1.046 (P = 0.002) times (Table 3).

Diagnostic performance of vWF, MELD score, and CRP for mortality

The ROC analysis did not confirm a statistically significant difference between the AUC values of the ROC curves for vWF and MELD score (Z = 1.459; P = 0.1444) and between the AUC values for vWF and CRP (Z = 1.063; P = 0.2876) regarding the 3-mo mortality [vWF-AUC = 0.734 (95%CI: 0.571-0.897), P = 0.008; MELD score-AUC = 0.884 (95%CI: 0.803-0.966), P = 0.000 and CRP-AUC = 0.848 (95%CI: 0.744-0.953), P = 0.000], between the AUC values for vWF and MELD score (Z = 1.385;P = 0.1662) and between the AUC values for vWF and CRP (Z = 1.601; P = 0.547) regarding the 6-mo mortality [vWF-AUC = 0.700 (95%CI: 0.544-0.856), P = 0.011; MELD score- AUC = 0.833 (95%CI: 0.725-0.940), P = 0.000 and CRP-AUC = 0.851 (95%CI: 0.758-0.943), P = 0.000], and between the AUC values for vWF and MELD score (Z = 1.276; P = 0.20192) and between the AUC values for vWF and CRP (Z = 1.366; P = 0.1718) regarding the 1-year mortality [vWF – AUC = 0.729 (95%CI: 0.590-0.868), P = 0.002; MELD score – AUC = 0.841 (95%CI: 0.742-0.941), P = 0.000 and CRP- AUC = 0.848 (95%CI: 0.747-0.948), P = 0.000] (Figure 3). According to the ROC curve, we received vWF cut off = 207.15; sensitivity = 85.5%; specificity = 71.4%, Youden index = 0.569 for 3-mo mortality, vWF cut off = 199.85; sensitivity = 80.0%; specificity = 74.4%, Youden index = 0.544 for 6-mo mortality, and vWF cut off = 199.35; sensitivity = 87.5%; specificity = 79.5%; Youden index = 0.67 for 1-year mortality.

DISCUSSION

Our study confirmed a significant relation between vWF and liver cirrhosis, CRP, ferritin, and D-dimer concentration. The study also confirmed that in patients with liver cirrhosis vWF, MELD score and CRP were significantly related to death and significant predictors of 3-mo, 6-mo, and 1-year mortality. Regarding mortality, our study did not confirm a significant difference between the diagnostic performance of vWF and MELD score and between the diagnostic performance of vWF and CRP.

Regarding its pronounced ability for short-term outcomes prediction, MELD score is the most widely accepted and currently the most useful indicator of liver function. Its wide scale provides high precision and good discriminating ability in assessing the death risk in cirrhotic patients[12]. However, the variability due to different laboratory methodologies[13], the low ability for prediction of post-

Table 1 Classification scores, von-Willebrand factor concentration, and biochemical and hemostatic parameters[1]

Parameter	*n*	mean ± SD	Min	Max	Percentiles		
					25th	50th	75th
CTP score	71	8.94 ± 2.95	5.00	15.00	6.00	9.00	11.00
MELD score	71	19.72 ± 9.86	6.00	59.00	11.00	18.00	25.00
vWF (%)	71	346.18 ± 155.97	150	850	214.00	318.40	410.10
CRP (mg/L)	71	21.15 ± 27.50	0.60	116.50	3.30	9.70	27.40
Ferritin (ng/mL)	62	290.45 ± 354.33	7.20	1586.10	47.50	149.40	464.30
Vitamin D (ng/mL)	46	17.65 ± 13.31	3.00	62.24	9.12	11.89	24.82
D-dimer (μg/mL)	69	2558.4 ± 1645.1	99.00	4500.00	969.90	2420.70	4427.00
PT (s)	71	20.50 ± 14.71	11.60	133.20	14.70	17.57	21.26
aPTT (s)	70	43.99 ± 14.32	23.56	120.00	35.53	41.94	48.07
TT (s)	70	24.10 ± 7.08	16.00	59.00	19.22	22.94	22.93
PLT (10^9/L)	71	105.51 ± 60.52	18.00	311.00	62.00	91.00	127.00
WBC (10^9/L)	71	6.62 ± 3.43	1.34	23.20	4.70	6.20	7.50
Bilirubin (μg/mL)	71	84.73 ± 119.64	8.00	611.00	25.30	39.30	83.00
Albumin (μg/mL)	71	29.68 ± 7.88	12.00	46.00	24.00	29.00	35.00
Sodium (μg/mL)	71	135.65 ± 4.77	117.00	141.00	134.00	137.00	138.00
Creatinine (μg/mL)	71	106.02 ± 96.15	41.00	530.00	61.30	72.00	105.40

[1]The values are expressed as mean ± standard deviation (SD).

CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; vWF: von-Willebrand factor; CRP: C-reactive protein; PT: Prothrombin time; aPTT: Activated partial thromboplastin time; TT: Thrombin time; PLT: Platelets; WBC: White blood cell.

transplant outcomes[14], the lower discriminating power of the lower MELD values[15], and its poor usefulness in compensated disease and in acute decompensation are the most pointed limitations of MELD score. Considering these facts many studies investigated the predictive value of other potential prognostic indicators in cirrhotic patients and compared it to the prognostic value of MELD score.

Most studies that evaluated the predictive value of vWF in cirrhotic patients confirmed significantly higher vWF values in patients with an advanced stage of disease[5,16-18] and in uncensored patients[18, 19] and also a significant predictive value of vWF for mortality[18]. We also confirmed a significantly higher vWF level in patients with higher CTP class and in higher MELD group. Still, considering the fact that vWF does not always adequately correlate with the indicators of liver dysfunction, it seems that higher vWF concentration in advanced disease is probably more directly related to the degree of PH than with the level of liver dysfunction. One of the most important findings of our research was that along with MELD score and CRP, vWF was significantly associated with death and that vWF was a significant predictor of mortality in all follow-up periods.

Since MELD score is currently the most reliable short-term mortality predictor in cirrhotic patients, we compared the diagnostic efficacy of vWF to the diagnostic efficacy of MELD score. The ROC analysis in the study did not confirm a significant difference between the diagnostic performances of the two parameters, suggesting that the predictive value of vWF for mortality is similar to the predictive value of MELD score. Most studies in the literature that compared the diagnostic performance of the two parameters for mortality came across similar results[16,18]. The study of Kalambokis *et al*[17] demonstrated that the predictive performance of vWF for new-onset ascites and for variceal bleeding was stronger than that of MELD score, suggesting that in terms of liver disease complications, the procoagulant state could be a stronger determining factor than the severity of the liver disease. Previous research investigating the prognostic role of vWF defined cut-off values with the best sensitivity and specificity discriminating patients with significantly different prognoses[16-18]. Ferlitsch *et al*[18] defined a vWF cut-off value of 315% that stratifies patients with completely different survival. La Mura *et al*[16] defined vWF value of 216 U/dL, differencing two groups of patients with significantly different probability of survival without the occurrence of clinical events related to death and transplantation. Kalambokis *et al*[17] defined a vWF cut-off value of 392%, indicating significantly higher 3-year mortality in patients with liver cirrhosis. According to the ROC curves, we also defined cut-off values for mortality (207.15% for 3-mo, 199.85% for 6-mo, and 199.35% for 1-year mortality) that did not differ much between each other.

Table 2 The relation between von-Willebrand factor and stage of disease, systemic inflammatory response syndrome, and other inflammatory and prognostic indicators[1]

Parameter	vWF							*P* value
	n	mean ± SD	Min	Max	Percentiles			
					25th	50th	75th	
CTP score								
Class A	18	258.0 ± 104.2	150	586	190.0	228.0	276.0	Kruskal-Wallis *H* test: Chi-square (2) = 10.8177; *P* = 0.0045[a]
Class B	25	336.5 ± 122.5	150	650	262.0	341.0	400.0	
Class C	28	411.5 ± 182.2	198	850	246.0	402.8	560.0	
Class A/B = Mann-Whitney *U* test: *Z* = -2.191; *P* = 0.028[a]								
Class A/C = Mann-Whitney *U* test: *Z* = -3.028; *P* = 0.002[a]								
Class B/C = Mann-Whitney *U* test: *Z* = -1.639; *P* = 0.101								
MELD score								
Group 1	9	271.5 ± 106.3	150	513	225.0	257.0	276.0	Kruskal-Wallis *H* test: Chi-square (2) = 10.3357; *P* = 0.0057[a]
Group 2	33	301.0 ± 115.9	161	650	200.0	296.0	350.0	
Group 3	29	420.8 ± 179.7	150	850	258.0	409.0	548.0	
Group 1/2 = Mann-Whitney *U* test: *Z* = -0.690; *P* = 0.507								
Group 1/3 = Mann-Whitney *U* test: *Z* = -0.031; *P* = 0.029[a]								
Group 2/3 = Mann-Whitney *U* test: *Z* = -2.942; *P* = 0.003[a]								
SIRS score								
SIRS (+)	43	339.2 ± 142.0	150	680	200.0	336.5	409.0	Mann-Whitney *U* test: *Z* = -0.3529; *P* = 0.7241
SIRS (-)	28	356.9 ± 177.5	150	850	216.0	309.2	410.8	
Ferritin (ng/mL)								
< 200	39	310.0 ± 140.7	150.0	650.0	198.0	262.0	405.0	Kruskal-Wallis *H* test: Chi-square (2) = 7.1653; *P* = 0.0278[a]
200-400	5	343.3 ± 91.2	253.0	458.9	258.0	336.5	410.1	
> 400	18	423.9 ± 171.0	199.7	850.0	300.0	400.5	548.0	
< 200/200-400 = Mann-Whitney *U* test: *Z* = -0.292; *P* = 0.311								
< 200/> 400 = Mann-Whitney *U* test: *Z* = -2.584; *P* = 0.010[a]								
200-400/> 400 = Mann-Whitney *U* test: *Z* = -0.820; *P* = 0.446								
Vitamin D (ng/mL)								
≤ 20	22	279.8 ± 108.9	150.0	513.0	199.7	241.0	344.0	Mann-Whitney *U* test: *Z* = -1.6916; *P* = 0.0907
> 20	23	372.0 ± 179.8	161.0	850.0	214.0	385.0	458.9	
PLT (10^9/ L)								
≤ 100	40	350.8 ± 171.4	161	850	207.2	312.7	412.1	Mann-Whitney *U* test: *Z* = -0.02898; *P* = 0.9769
> 100	31	340.2 ± 136.0	150	598	239.0	337.0	405.0	
D-dimer (μg/mL)								
≤ 500	6	205.2 ± 38.5	150	262	187.2	203.5	225.0	Mann-Whitney *U* test: *Z* = 2.6407; *P* = 0.0083[a]
> 500	63	355.9 ± 156.2	150	850	216.0	337.0	411.5	

[1]The values are expressed as mean ± standard deviation (SD).

[a]*P*< 0.05.

von-Willebrand factor (vWF) was significantly higher in higher Child-Turcotte-Pugh (CTP) class, model for end-stage liver disease (MELD) group, serum ferritin (SF) group and in patients with elevated D-dimer level. SIRS: Systemic inflammatory response syndrome; PLT: Platelets.

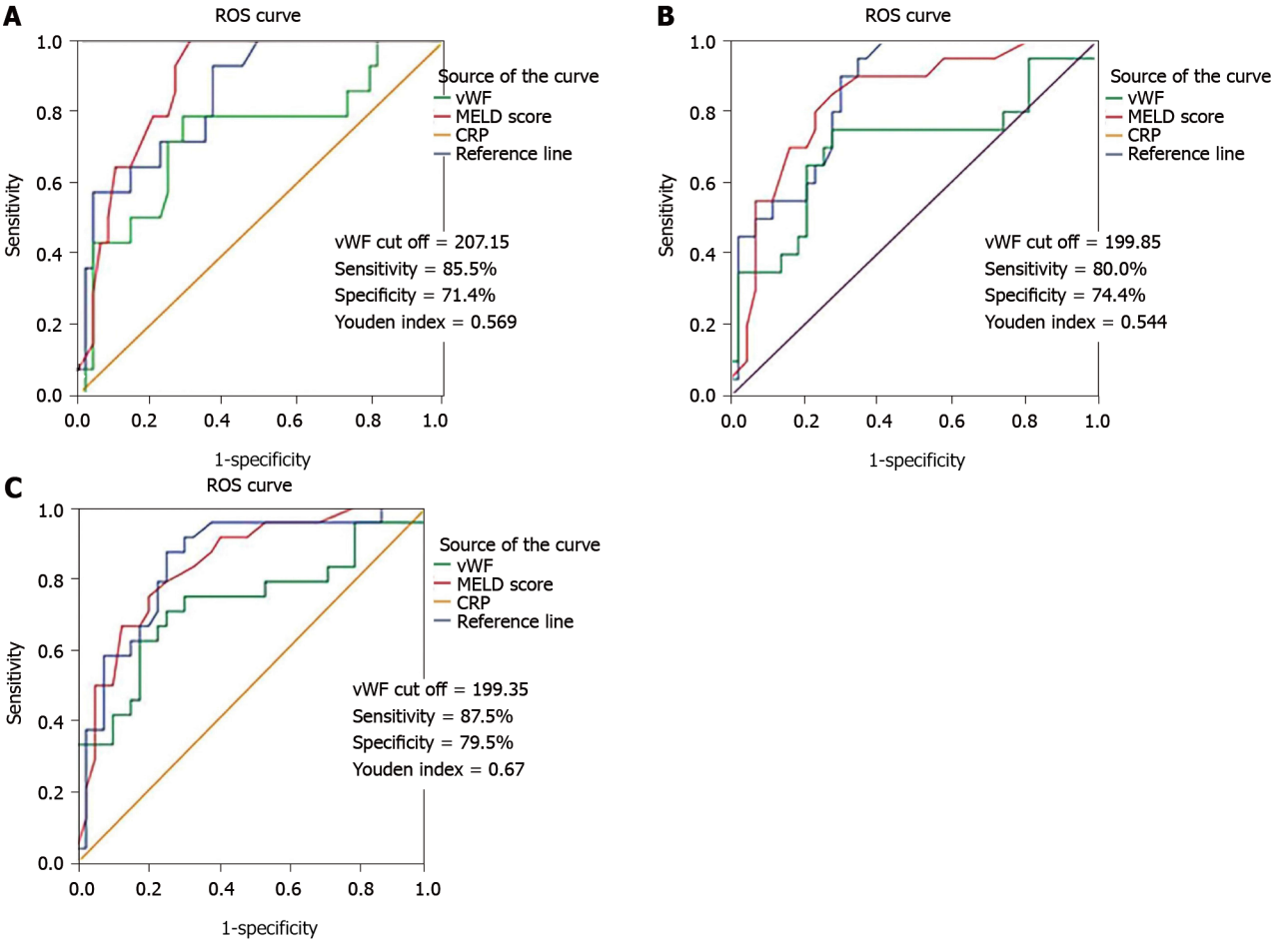

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Figure 3 Receiver operating characteristic analysis of the diagnostic performance of von-Willebrand factor, model for end-stage liver disease score, and C-reactive protein for mortality. A: 3-mo mortality; B: 6-mo mortality; C: 1-yr mortality. The difference between the area under the curve (AUC) values for von-Willebrand factor (vWF) and model for end-stage liver disease (MELD) score and between the AUC values for vWF and C-reactive protein (CRP) regarding all three follow-up periods was not statistically significant (Z = 1.459; P = 0.1444 and Z = 1.063; P = 0.2876 for 3-mo mortality, Z = 1.385; P = 0.1662 and Z = 1.601; P = 0.547 for 6-mo mortality, Z = 1.276; P = 0.20192 and Z = 1.366; P = 0.1718 for 1-yr mortality, respectively). ROC: Receiver operating characteristic.

Regarding the complex pathogenesis of PH and its influence on liver disease progression, we tried to make a deeper insight into the role of ED and SI, into their mutual interaction, and also into their interaction with the numerous and complex hemostatic abnormalities within coagulopathy associated with chronic liver disease. In this context, we analyzed the relation between vWF and some biological variables that reflect SI or that are considered to have some prognostic potential in patients with liver cirrhosis. Our study showed that vWF was not only associated with CTP and MELD score, but it was also significantly associated with some other variable and prognostic indicators in these patients.

Since our analysis confirmed a strong significant correlation only between vWF and D-dimer concentration, we wanted to analyze this relation more profoundly. D-dimer concentration is a specific indicator of fibrin turnover and the most widely used indicator of active coagulation and fibrinolysis. Hyperfibrinolysis is present in approximately one-third of cirrhotic patients[20], and in some of them, low-grade disseminated intravascular coagulation has also been registered[21]. It has been established that the abnormalities in the fibrinolytic system were more pronounced in patients with advanced, decompensated cirrhosis[20,22]. Still, the main dilemma regarding hyperfibrinolysis in these patients is whether it occurs mainly as a primary phenomenon or is induced secondarily as a response to activated coagulation, most commonly within disseminated intravascular coagulation. Previously reported data related to the prognostic relevance of D-dimer levels in cirrhotic patients have confirmed a significant association between elevated D-dimer concentration and liver dysfunction[22]. Although some authors suggest that the intense ascites reabsorption stimulates hyperfibrinolysis in patients with advanced disease[23,24] still, endotoxemia is probably the key factor that induces hyperfibrinolysis through endothelial activation and release of fibrinolytic substances[25]. It seems that the crucial role of ED in these developments may explain the relationship between elevated vWF and D-dimer concentration. In addition to the strong correlation, we also registered significantly higher vWF values in patients with elevated (> 500 μg/mL) D-dimer levels (355.9 ± 156.2 *vs* 205.2 ± 38.5, P = 0.0232). More importantly, our

Table 3 Univariate Cox proportional model and univariate logistic regression analysis[1]

Parameter	Univariate Cox proportional model						Univariate logistic regression analysis					
	3-mo		6-mo		1-yr		3-mo		6-mo		1-yr	
	Sig.	Exp (B)	Sig.	Exp (B)	Sig.	Exp (B)	Sig.	Exp (B)	Sig.	Exp (B)	Sig.	Exp (B)
vWF	0.004a	1.004	0.005a	1.006	0.000a	1.004	0.008a	1.005	0.005a	1.006	0.002a	1.007
MELD	0.000a	1.144	0.000a	1.157	0.000a	1.116	0.000a	1.191	0.000a	1.157	0.000a	1.176
CRP	0.000a	1.029	0.001a	1,044	0.000a	1.025	0.001a	1.044	0.001a	1.044	0.002a	1.046
Vitamin D	0.077	0.923	0.013a	0.877	0.040a	0.939	0.096	0.918	0.013a	0.877	0.061	0.931
Ferritin	0.119	1.001	0.333	1.001	0.016a	1.001	0.104	1.001	0.333	1.001	0.015a	1.003
aPTT	0.000a	1.05	0.068	1.052	0.000a	1.051	0.059	1.049	0.068	1.052	0.067	1.055
TT	0.258	1.034	0.588	1.02	0.426	1.021	0.292	1.041	0.588	1.02	0.529	1.023
D-dimer	0.061	1	0.014a	1	0.059	1	0.003a	1.001	0.014	1	0.008	1
	Dependent variable-survival in days; significant for $^aP < 0.05$						Dependent variable-mortality no/yes; significant for $^aP < 0.05$					

[1]von-Willebrand factor (vWF), model for end-stage liver disease (MELD) score, and C-reactive protein (CRP) were significantly associated with 3-mo, 6-mo, and 1-yr survival and significant predictors of 3-mo, 6-mo, and 1-yr mortality.
$^aP < 0.05$.
aPTT: activated partial thromboplastin time; TT: thrombin time.

study also confirmed that elevated D-dimer levels were a significant predictor of 3-mo mortality (P = 0.003). Some previous studies have proven that in patients with liver cirrhosis elevated D-dimer levels were related to poor outcomes and high short-term mortality[26,27]. Still, as far as we are aware, elevated D-dimer levels have not been specifically related to 3-mo mortality previously. These findings confirm the important role of ED underlying the hemostatic abnormalities as well as the relation between ED, procoagulant tendency, and short-term mortality in cirrhotic patients.

Taking into account the important prognostic role of SI, especially in advanced disease, we analyzed the SIRS occurrence, its relation to CRP as SIRS indicator, and its relation to vWF as an indicator of ED. Considering the fact that ED and SI coexist and support each other, we assumed that SIRS would be accompanied by higher vWF values. On the contrary, the analysis did not confirm a significant difference between vWF values in patients with and without SIRS (P = 0.7241). The positive linear correlation between CRP and vWF (r = 0.315) and the absent relation between vWF and SIRS mainly indicates that the applied ACCP / SCCM criteria for SIRS do not reflect the presence of SI adequately. Some previous studies have shown that ACCP/SCCM criteria are generally not suitable for use in cirrhotic patients[28,29], which has raised interest in CRP as an indicator of SIRS and also as a prognostic indicator in cirrhotic patients. According to some findings, elevated CRP in cirrhotic patients is not only a reliable indicator of active bacterial infection[30], but it may also reflect persistent low-grade SI even outside the context of active infection[6]. Moreover, one of the most significant limitations

of MELD score is that the formula does not include a variable that reflects inflammation, such as leukocyte count or CRP, suggesting that MELD score does not take into account the presence of SI, a condition that from a prognostic point of view has great importance in cirrhotic patients[31]. Regarding the predictive value of CRP for mortality, our study confirmed that along with vWF and MELD score, CRP has been significantly associated with death and that CRP has been a significant mortality predictor in all three follow-up periods, which was the most important finding regarding this issue. The ROC analysis comparing the corresponding AUC values for mortality did not show a significant difference between the diagnostic efficacy of vWF and CRP, indicating that in cirrhotic patients vWF and CRP were a significant mortality predictor with a similar predictive value, which, according to our knowledge on this topic, has not been reported previously.

Elevated SF is registered in about 30% of patients with advanced liver disease, and it is mainly due to the release of ferritin from the damaged hepatocytes[32,33]. Previous research has shown a significant association between SF and almost all known predictors of poor outcome in decompensated patients (MELD score, CTP score, leukocyte count, sodium level, ACLF stages, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatorenal syndrome)[34], but as far as we are aware, the relation between vWF and SF in cirrhotic patients has not been previously evaluated. Except for the significant positive correlation between vWF and ferritin (r = 0.360, P = 0.04), we also confirmed significantly higher vWF levels in patients with higher SF concentration (P = 0.0278). Despite the well-known relation between SF and liver cirrhosis, several studies have also confirmed the significant prognostic value of SF for mortality[32,33]. Walker *et al*[33] confirmed that SF was higher than 400 μg/L in all uncensored cirrhotic patients and that SF above 500 μg/L was an accurate predictor of 6-mo and 1-year mortality. The exact pathophysiological mechanism that explains this relation is not completely understood. It is presumed that an increased hepatic iron concentration promotes additional oxidative hepatocellular injury and also stellate cell activation, which can explain the ferritin involvement in the progression of liver disease and the relation between SF and mortality in cirrhotic patients[32]. Regarding mortality prediction, our study confirmed that in patients with liver cirrhosis SF was significantly associated with 1-year survival (P = 0.016) and that SF was a significant predictor of 1-year mortality (P = 0.015). Unlike some studies [32], our research did not confirm an association between SF and 3-mo and 6-mo mortality and a significant predictive value for short-term mortality in cirrhotic patients.

It is known that chronic liver disease is related to high prevalence of vitamin D deficiency that according to some data might reach up to 90%[35]. It is also considered that vitamin D deficiency additionally worsens liver dysfunction; it is related to decompensation and has a negative impact on the prognosis and survival especially in advanced liver disease[36,37]. Our study confirmed that vitamin D concentration was significantly associated with 6-mo and 1-year survival and that vitamin D was a significant predictor of 6-mo mortality (P = 0.013). However, according to some data, the cut-off value that defines vitamin D deficiency (20 ng/mL) does not appear to be a significant risk factor in cirrhotic patients[36]. In this context, one study showed that mortality was significantly affected only when a vitamin D cut-off value of 6 ng/mL was applied[36]. Despite the well-established predictive value of vitamin D in cirrhotic patients, our study did not confirm a relationship between vWF and vitamin D deficiency. We did not show a significant correlation between vWF and vitamin D (r = 0.064) or significantly higher vWF values in patients with vitamin D deficiency (P = 0.0907). The negative prognostic influence of vWF in cirrhotic patients is mainly due to its prothrombotic potential, which is a factor for progression of PH. On the other hand, the prognostic potential of vitamin D is mostly due to its effect on the immune system. Hence, it seems that in this case, these two parameters are involved differently in the pathogenesis of liver disease progression, which may explain the absence of a direct association between them.

CONCLUSION

In patients with liver cirrhosis, vWF is elevated and significantly related to the stage of the disease and other prognostic and inflammatory indicators. vWF is significantly associated with death and is a significant predictor of 3-mo, 6-mo, and 1-year mortality similar to MELD score and CRP. The significant prognostic value of CRP in cirrhotic patients confirms the important prognostic role of SI in these patients and highlights the importance of recognizing the condition for more accurate mortality prediction. Although generally reflecting an increased prothrombotic state, hyperfibrinolysis and elevated D-dimer levels in these patients should be analyzed in relation to clinical presentation, stage of disease, and other hemostatic parameters. The significant interaction between the variables analyzed in the study has reflected the complex and dynamic interaction between ED, SI, and cirrhosis-related coagulopathy that occurs in patients with liver cirrhosis.

ARTICLE HIGHLIGHTS

Research background

Endothelial dysfunction (ED) and systemic inflammation (SI) play an important role in the pathogenesis of portal hypertension (PH). Von-Willebrand factor (vWF) is an indicator of ED that favors a prothrombotic state, and hence it is directly involved in the progression of PH. Although previous research confirmed its prognostic value in cirrhotic patients, its relation to other prognostic indicators has not been properly evaluated. By analyzing the relation between vWF and other biological variables with certain prognostic potential, our research provides an insight into the complex relation between ED, SI, and liver-disease related coagulopathy in cirrhotic patients.

Research motivation

Although Model for End-stage Liver Disease (MELD) score is the most widely used prognostic score in cirrhotic patients, it does not take into account the presence of circulatory dysfunction or SI and it does not assess the coagulopathy properly. This raises the need for further research towards identifying new biological variables with certain prognostic potential in cirrhotic patients and evaluating their prognostic value for mortality. This could lead toward defining new prognostic scores or improve the predictive value of those currently in use. Recent researchers have suggested that some biological variables such as vWF, C-reactive protein (CRP), ferritin, and vitamin D possess certain prognostic potential in cirrhotic patients, but this area has not been widely investigated.

Research objectives

We tried to analyze the relation between vWF and liver cirrhosis and the relation between vWF and several inflammatory indicators and other variables that have certain prognostic potential in cirrhotic patients. We also tried to evaluate the prognostic value of vWF and several parameters in terms of 3-mo, 6-mo, and 1-year mortality.

Research methods

We conducted an analytic prospective study that enrolled 71 patients with liver cirrhosis and portal hypertension. At enrollment, we performed detailed examinations (abdominal ultrasound, complete blood count, biochemical blood analysis, basic hemostasis, D-dimer, vWF concentration) in order to assess the stage of the liver disease after which we followed the patients for 1 year. We analyzed the relation between vWF and chronic liver disease and between vWF and several prognostic and inflammatory indicators. We prospectively evaluated the prognostic value of vWF and several other variables (MELD score, CRP, ferritin, vitamin D, activated partial thromboplastin time, thrombin time, D-dimer concentration) in terms of 3-mo, 6-mo, and 1-year mortality, and we compared the diagnostic efficacy of vWF for morality to other significant mortality predictors.

Research results

Our study confirmed a significant relation between vWF and the stage of liver disease, CRP, ferritin, and D-dimer concentration. The study also confirmed that in patients with liver cirrhosis vWF, MELD score, and CRP were significantly related to 3-mo, 6-mo, and 1-year survival and significant predictors of 3-mo, 6-mo, and 1-year mortality. Our study did not confirm a significant difference between the diagnostic performance for mortality of vWF and MELD score and between the diagnostic performance of vWF and CRP.

Research conclusions

In patients with liver cirrhosis, vWF is a significant and relevant mortality predictor similar to MELD score and CRP, which highlights the important role of the ED in the pathogenesis of PH. Elevated CRP is a significant mortality predictor in patients with liver cirrhosis, which emphasizes the importance of recognizing the presence of SI for accurate mortality prediction. The relation between vWF and D-dimer concentration, ferritin, and CRP reflects the complex and dynamic interaction between ED, SI, and cirrhosis-related coagulopathy that occurs in patients with liver cirrhosis.

Research perspectives

Future research should be focused on identifying specific clinical settings in which vWF would have more accurate prognostic value.

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FOOTNOTES

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META-ANALYSIS

Effects and safety of natriuretic peptides as treatment of cirrhotic ascites: A systematic review and meta-analysis

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Abstract

BACKGROUND

Natriuretic peptides are involved in the cascade of pathophysiological events occurring in liver cirrhosis, counterbalancing vasoconstriction and anti-natriuretic factors. The effects of natriuretic peptides as treatment of cirrhotic ascites have been investigated only in small studies, and definitive results are lacking.

AIM

To examine the effects and safety of natriuretic peptides in cirrhosis patients with ascites.

METHODS

We searched MEDLINE, Web of Science, Scopus, Cochrane Library and Embase for all available studies applying intravenous administration of any natriuretic peptide to patients suffering from cirrhotic ascites. Inclusion was not limited by treatment duration or dose, or by follow-up duration. Both randomised controlled trials and non-randomised studies were eligible for inclusion. The primary outcome was change in renal sodium excretion. Secondary outcomes included safety measures and changes in renal water excretion, plasma aldosterone concentration, and plasma renin activity.

RESULTS

Twenty-two studies were included. Atrial natriuretic peptide (ANP) was the only intensively studied treatment. Sodium excretion increased in response to

continuous ANP infusion and was more pronounced when infusion rates of > 30 ng/kg/min were administered compared with ≤ 30 ng/kg/min ($P < 0.01$). Moreover, natriuresis was significantly higher in study subgroups with mild/moderate ascites compared with moderate/severe and refractory ascites ($P < 0.01$). ANP infusions increased renal water excretion, although without reaching a statistically significant dose-response gradient. Plasma aldosterone concentration and plasma renin activity were significantly lower at baseline in study subgroups achieving a negative sodium balance in response to an ANP administration compared with treatment non-responders ($P < 0.01$). Blood pressure decreases occurred less frequently when ANP doses ≤ 30 ng/kg/min were applied. The quality of evidence for a natriuretic response to ANP was low, mainly due to small sample sizes and considerable between-study heterogeneity. Data were sparse for the other natriuretic peptides; B-type natriuretic peptide and urodilatin.

CONCLUSION

Intravenous ANP infusions increase sodium excretion in patients with cirrhotic ascites. Continuous infusion rates > 30 ng/kg/min are the most effective. However, safety increases with infusion rates ≤ 30 ng/kg/min.

Key Words: Atrial natriuretic peptide; B-type natriuretic peptide; Urodilatin; Cirrhosis; Ascites; Refractory ascites

Core Tip: Pharmacotherapies for cirrhotic ascites have remained largely unchanged for the past four decades. 5%-10% of cirrhosis patients with ascites become refractory to available treatments, and the majority of these patients require frequent large-volume paracenteses. This justifies a continued search for new and improved treatments for ascites. This is the first systematic review and meta-analysis to investigate the effects and safety of natriuretic peptides as treatment of cirrhotic ascites. We demonstrate a significant natriuretic effect of intravenously administered atrial natriuretic peptide and summarise the safety findings.

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INTRODUCTION

The cirrhosis-derived health-care burden is growing globally[1]. Patients suffering from liver cirrhosis may develop portal hypertension with substantial neuro-hormonal impacts. Portal hypertension directly induces vasodilation of the splanchnic vascular system with blood pooling in the splanchnic bed. This reduces the central blood volume, leading to arterial hypotension. When these pathophysiological regulatory events become manifest, vasoconstrictor and anti-natriuretic factors are activated, ultimately resulting in retention of sodium and water[2,3]. Ascites formation is the most common manifestation of decompensation due to cirrhosis with portal hypertension. After the appearance of ascites, patients experience a poor prognosis, frequent hospital contacts, and an impaired quality of life [3,4]. In general, dietary sodium restriction, anti-aldosterone agents, and loop-diuretics are effective treatments to manage ascites[4,5]. However, 5%-10% progress to refractory ascites[6]. Invasive treatments, such as liver transplantation, transjugular intrahepatic portosystemic shunt, or implantation of an automated low-fluid ascites pump (Alfapump®), are restricted to a minor proportion of patients with refractory ascites, while the majority require frequent hospitalizations for large-volume paracentesis[3,4]. New pharmacotherapies to counteract the mechanisms involved in ascites development, as well as to treat established ascites and reduce the need for paracenteses in refractory ascites, are highly warranted.

Atrial natriuretic peptide (ANP) is the most extensively studied member of the natriuretic peptide family, which also comprises B-type natriuretic peptide (BNP) and two active forms of C-type natriuretic peptide (CNP), as well as the renal paracrine peptide urodilatin[7,8]. Urodilatin is identical to ANP except for an N-terminal extension of 4-amino acids, which makes it less vulnerable to degradation by the renal neutral endopeptidase[8,9]. Therefore, the renal effects of urodilatin are stronger than those

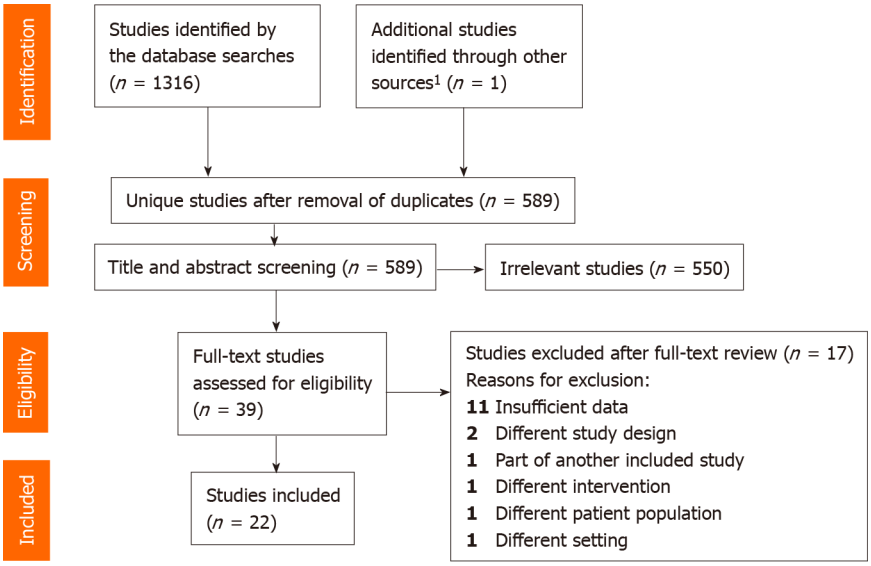

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Figure 1 Flow diagram of study identification. [1]Publication identified through screening of reference lists of all studies in the "Eligibility" assessment.

of ANP[10].

The majority of patients with cirrhotic ascites have marked activation of vasoconstrictor systems[11-14]. Moreover, plasma concentrations of ANP and BNP are elevated in patients with cirrhosis and ascites[15,16], consistent with a physiological attempt to counterbalance vasoconstriction and anti-natriuretic factors. However, in advanced cirrhosis, endogenous natriuretic peptides cannot override the pathophysiological events leading to ascites formation[11].

Earlier studies, that have investigated natriuretic peptides as treatment of cirrhotic ascites, have been too small to provide a definitive result. Thus, a review of the literature is justified. This systematic review and meta-analysis evaluates the benefits and harms of intravenous administration of the natriuretic peptides, ANP, BNP, or urodilatin in patients with liver cirrhosis and ascites. We aim to perform a meta-analysis of available data, especially regarding the effects on natriuresis, to determine the therapeutic potential of natriuretic peptides in cirrhotic ascites. Moreover, we intend to identify patient subpopulations for which treatment with natriuretic peptides may be particularly beneficial.

MATERIALS AND METHODS

This systematic review was performed in accordance with the PRISMA guidelines and the Cochrane Handbook[17,18]. The preregistered review protocol (CRD42020195619) is available in PROSPERO.

We included trials evaluating mono-therapy of ANP, BNP, urodilatin, or any synthetic equivalent of these in patients with cirrhosis and ascites. Randomised controlled trials (RCTs) and non-randomised studies, *e.g.*, trials investigating the pharmacodynamics of natriuretic peptides in cirrhosis patients with ascites and dose-finding trials, were considered for inclusion.

Trials were included regardless of dose and duration of the intervention or follow-up. Treatment administration had to be intravenous. As sodium retention is a main characteristic of decompensated cirrhosis, and existing pharmacological treatments aim to increase renal sodium excretion, the primary outcome in this systematic review was the maximum difference in natriuresis from baseline. To further evaluate renal and systemic treatment effects, secondary outcomes comprised the maximum difference in diuresis, plasma osmolality, urine osmolality, plasma creatinine concentration, plasma aldosterone concentration, and plasma renin activity from baseline. Change in bodyweight and waist circumference were included as objective surrogate measures of ascites burden. Non-serious and serious adverse events (AEs) were assessed focusing on the expected risk of blood pressure decreases and hypotension.

Trials were eligible for inclusion regardless of publication status, publication year, or language. The databases MEDLINE, Web of Science, Scopus, Cochrane Library and Embase were searched for eligible publications on the 13th of August 2020 (Supplementary material, page 1). References were managed in the reference sorting tool Covidence® (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia).

Data management

Study screening and data extraction to a spreadsheet with predefined variables were performed independently by two reviewers (RHG and MBK). Discrepancies were resolved through discussion

before analyses. To characterise study populations, we collected background details including age, gender, cirrhosis aetiology, ascites severity, dietary sodium restriction, and diuretic withdrawal prior to treatment. We extracted data concerning the intervention including the name of the experimental intervention (generic and international non-proprietary name), comparator intervention, duration and dose of treatment, and whether a bolus or continuous infusion was applied. Finally, we collected information on study design, size of the trial, and follow-up duration.

Outcomes measured at baseline, during the intervention and at the end of follow-up were extracted from the text, tables or figures using a measuring tool from Adobe Acrobat 2017 (Adobe Inc., California, United States). The baseline outcome value was selected at the time point closest to the intervention exposure. For data concerning primary and secondary outcomes, the peak response or concentration measured during the intervention was used for trials administering a continuous intervention, while the peak response or concentration measured after the intervention was used for bolus infusion trials. Analyses were made using means and standard deviations (assumptions regarding units and unit conversions are described in Supplementary material, page 2).

Risk of bias, quality assessments, and evidence

Risk of bias assessments were performed at study level using the Cochrane risk of bias tool version 2 (RoB2)[19] for RCTs. An overall risk of bias judgment; low, moderate, or high was independently conducted by RHG and MBK. Non-randomised studies were assessed using the Newcastle-Ottawa-Scale (NOS)[20] (Supplementary material, pages 2 and 3) with a score from 0 (lowest) to 9 (highest). The score was transformed to generate a RoB2-comparable entity; NOS-scores 8-9 were classified as "low", 5-7 as "moderate", and ≤ 4 as "high". The Grading of Recommendations Assessment and Evaluation was used to judge the quality of evidence for the study questions[21].

Data synthesis and statistical analyses

The effects of natriuretic peptides were summarised as overall peak mean and compared with overall baseline mean. This was performed for all trials and for study subgroups stratified by type of natriuretic peptide and continuous *vs* bolus infusions. When non-randomised studies outnumbered RCTs, baseline sodium excretion and peak sodium excretion were meta-analysed separately. We were unable to gather data allowing meta-analyses of safety data. Safety outcomes were therefore reviewed without statistical analyses.

The statistical software Stata, version 16.1, (StataCorp Lp, TX, United States) was used for data analyses. We applied a random-effects model with a DerSimonian-Laird tau^2 estimator for all meta-analyses. I^2 was used as a consistency measure. In situations with high levels of heterogeneity, meta-regressions were performed to elucidate study moderators. Moreover, the reasons for heterogeneity were addressed through subgroup analyses and leave-one-out analyses.

Small study effects were assessed using the Egger regression-based test. Funnel plots were included for graphical illustration of publication bias, while Trim-and-fill analysis were performed to estimate and account for this.

RESULTS

The literature search identified a total of 589 unique references. Ultimately, 22 publications describing 22 studies were included for data extraction (Figure 1)[17] covering 34 subgroups eligible for review and analyses (Table 1). ANP was studied in 19 studies (215 patients)[11-15,22-35], BNP in one study (seven patients)[16], and urodilatin in two studies (21 patients)[36,37].

The 22 included studies were conducted in eight countries and published between 1988 and 2007. Eighteen studies were non-randomised without a comparator, while four were cross-over RCTs using either placebo (3) or albumin (1) as a comparator. As the majority of included trials were non-randomised studies, we were unable to perform paired comparisons to compute effect sizes in the subsequent data analysis, except for urodilatin which was assessed in two RCTs. Fifteen studies investigated a continuous drug infusion, six studies applied a bolus injection, and one study assessed both a continuous infusion and a bolus injection (Table 1).

We classified ascites as mild, moderate, severe, or refractory. In a few situations, necessary assumptions were made regarding treatment dose and ascites severity to permit comparability (Table 1 and Supplementary material, pages 3 and 4).

Background characteristics

Two hundred and forty-three patients with cirrhosis and ascites were treated with intravenous natriuretic peptide (Table 1). The overall ratio of male and female participants was 3:1. Mean age ranged between 44.5 and 64.3 years (median 56.2 years). Ascites severity was reported in 21 of the included studies, covering 239 participants. Eighty-four (35%) had mild ascites, 81 (34%) had moderate ascites, 22 (9%) had severe/tense ascites, and 52 (22%) had refractory ascites. Only one study did not withdraw loop-diuretics and anti-aldosterone agents prior to the intervention[23]. The time from discontinuation

Table 1 Main characteristics of the 22 included studies [34 subgroups; receiving bolus injection (*n* = 8) or continuous infusion (*n* = 26)]. Atrial natriuretic peptide was tested in 19 studies (215 patients), B-type natriuretic peptide in one study (7 patients), and urodilatin in two studies (21 patients)

Ref.	Country	Study type	Patient *n*	Drug	Comparator	Adm	Dose (ng/kg/min or ng/kg)	Duration (min)	Post-infusion follow-up (min)	Age (mean)	Male (%)	Ascites severity: Mild/moderate/severe/refractory	S- or P-sodium (mmol/L) mean ± SE	Quality assessments (risk of bias)	
														NOS	RoB2
Abraham *et al* [31], 1995	United States	Non-random	6	ANP	None	Cont	150	180	180	44.5	67	6/0/0/0	NR	Low	-
			6	ANP	None	Cont	150	180	180	49.5	100	1/3/2/0			
Ando[32], 1991	Japan	Non-random	10	ANP	None	Bolus	1000	NA	90	57.1	80	0/5/5/0	138.3 ± 0.7	Low	-
			6	ANP	None	Bolus	1000	NA	90	58.7	67	0/3/3/0	134.2 ± 0.2		
Badalamenti *et al*[33], 1992	Italy	Non-random	9	ANP	None	Bolus	1000	NA	90	57.8	78	0/5/4/0	134.7 ± 1.6	Low	-
			4	ANP	None	Cont	20	60	0	63.3	75	0/3/1/0		Moderate	
Carstens *et al* [36], 1998	Denmark	RCT	15	Urodilatin	Placebo	Cont	20	60	60	50.6	60	15/0/0/0	137.0 ± 0.5	-	Low
Carstens *et al* [37], 2007	Denmark	RCT	6	Urodilatin	Placebo	Cont	20	90	90	54.0	67	0/0/0/6	133.3 ± 2.0	-	Low
Ferrier *et al* [14], 1989	Switzerland	Non-random	7	ANP	None	Cont	36	60	60	54.3	57	0/7/0/0	NR	Moderate	-
Fried *et al*[34], 1990	United States/Switzerland	RCT	11	ANP	Placebo	Cont	15	120	90	NR	82	11/0/0/0	NR	-	Moderate
		RCT	8	ANP	Placebo	Cont	30	120	90	NR	88	8/0/0/0			
		RCT	9	ANP	Placebo	Cont	60	120	90	NR	89	9/0/0/0			
Gerbes *et al* [35], 1988	Germany	Non-random	4	ANP	None	Cont	50	30	90	NR	NR	NR	NR	Moderate	-
Ginès *et al*[22], 1992	Spain	Non-random	5	ANP	None	Cont	50	60	0	57.0	60	0/5/0/0	NR	Moderate	-
			11	ANP	None	Cont	50	60	0		73	0/11/0/0			
Heim *et al*[23], 1990	Germany	Non-random	8	ANP	None	Bolus	500	NA	60	54.5	75	0/8/0/0	NR	Low	-
Jespersen *et al* [12], 1995	Denmark	Non-random	9	ANP	None	Bolus	2000	NA	120	49.0	89	0/7/2/0	NR	Low	-
Laffi *et al*[15], 1989	Italy	Non-random	8	ANP	None	Bolus	1000	NA	900	56.4	75	0/0/0/8	131.8 ± 2.6	Low	-
Laffi *et al*[24],	Italy	Non-	5	ANP	None	Cont	100	45	75	58.0	60	0/5/0/0	NR	Low	-

1989		random	4	ANP	None	Cont	100	45	75	56.4	25	0/4/0/0			
			6	ANP	None	Cont	100	45	75	54.0	83	0/6/0/0			
La Villa *et al* [16], 1995	Italy	Non-random	7	BNP	None	Cont	13.86[1]	60	60	56.0	57	0/7/0/0	135.0 ± 1.0	Low	-
Legault *et al* [25], 1993	Canada	Non-random	5	ANP	None	Cont	15	120	120	NR	80	5/0/0/0	139.0 ± 2.0	Low	-
			7	ANP	None	Cont	15	120	120	NR	71	7/0/0/0	133.0 ± 1.0		
Miyase *et al* [26], 1990	Japan	Non-random	6	ANP	None	Bolus	500	NA	90	58.0	NR	6/0/0/0	NR	Low	-
Morali *et al* [13], 1991	Canada	Non-random	5	ANP	None	Cont	15	120	60	54.0	80	5/0/0/0	133.0 ± 1.0	Low	-
			12	ANP	None	Cont	15	120	60	53.0	67	0/0/0/12	138.0 ± 1.0		
Morali *et al* [11], 1992	Canada	Non-random	6	ANP	None	Cont	15	120	0	56.8	100	0/0/0/6	NR	Low	-
			4	ANP	None	Cont	15	120	0	64.3	100	0/0/0/4			
Salerno *et al* [27], 1988	Italy	Non-random	7	ANP	Placebo	Bolus	1000	NA	60	56.6	100	0/2/5/0	NR	Low	-
Tobe *et al*[28], 1993	Canada	Non-random	8	ANP	None	Cont	15	120	0	52.4	100	8/0/0/0	NR	Low	-
Tobe *et al*[29], 1993	Canada	Non-random	6	ANP	None	Cont	15	120	0	58.0	67	0/0/0/6	NR	Low	-
Wong *et al*[30], 1993	Canada	RCT	3	ANP	Albumin	Cont	15	120	60	58.0	67	3/0/0/0	135.0 ± 5.0	-	Low
			10	ANP	Albumin	Cont	15	120	60	54.0	70	0/0/0/10	134.0 ± 1.0		

[1]The continuous infusion of B-type natriuretic peptide (BNP) was administered with 4 pmol/kg/min. The dose was converted to ng/kg/min using the molar mass of BNP.

RCT: Randomised controlled trial; Non-random: Non-randomised trial; ANP: Atrial natriuretic peptide; BNP: B-type natriuretic peptide; NOS: Newcastle-Ottawa-Scale; ROB2: Risk of bias tool version 2; Adm: Mode of administration; Cont: Continuous infusion; S- or P-sodium: Serum or plasma sodium; NR: Not reported; NA: Not applicable.

of diuretics until intervention in the remaining 21 studies ranged between one day and 14 d (median 7 d). Finally, a sodium restrictive diet was applied before the intervention in 18 studies ranging from 15 to 150 mmol/d (median 20 mmol/d). The duration of continuous infusions ranged from 30 to 180 min (median 120 min) with post-infusion follow-up period ranging from 0 to 180 min (median 60 min). Post-bolus follow-up ranged from 60 to 900 min (median 90 min). For studies administering a continuous infusion, the time point for peak natriuresis in general coincided with the treatment duration. For studies using a bolus injection, a peak response after 30 min was most commonly observed.

We found a substantial inter-study variation in definitions of responder and non-responder subgroups. As the majority of included trials introduced dietary sodium restriction prior to intervention, we redefined treatment responsiveness as the achievement of a negative sodium balance during or after the intervention.

One study investigated the natriuretic effect of continuous BNP infusion. No significant change in natriuresis was observed, nor any effect on diuresis and blood pressure[16]. Therefore, due to the lack of effect on natriuresis, BNP did not receive further attention in our analyses.

Natriuresis

Continuous infusion of ANP increased sodium excretion from 5.6 μmol/min [95% confidence interval (CI): 3.7-7.4] pre-intervention (baseline) to 27.0 μmol/min (95%CI: 41.5-103.5) at peak response. Interestingly, the peak response was significantly higher in study subgroups receiving an infusion rate of > 30 ng/kg/min compared with ≤ 30 ng/kg/min ($P < 0.01$) (Figure 2). The between-study heterogeneity was considerable (peak $I^2 = 88\%$, baseline $I^2 = 89\%$). Leave-one-out analyses did not recognise a single study responsible for the large I^2. To search for interactions to explain the heterogeneity we performed subgroup analyses exploring the effects of infusion duration, ascites severity, and dietary sodium restriction. No statistically significant differences were observed between subgroups exposed to short-term or long-term infusions (threshold at 60 min) ($P = 0.07$), nor between subgroups with a marked or moderate restriction of dietary sodium intake (threshold at 20 mmol/d) ($P = 0.11$). The baseline and peak sodium excretion were higher in subgroups with mild/moderate ascites compared with moderate/severe and refractory ascites ($P < 0.01$). Meta-regression found that the effect size was moderated by treatment dose and ascites severity, although adjusting for these parameters had only limited influence on the overall I^2. Effect size was not affected by quality assessment score, mean age, dietary sodium intake, and treatment duration. Study subgroups exposed to a bolus ANP injection were heterogeneous (peak $I^2 = 94\%$, baseline $I^2 = 93\%$) affecting the interpretability of the results. However, overall sodium excretion increased marginally (Supplementary Figure 1). Leave-one-out analyses did not identify any single study responsible for the heterogeneity. As only seven studies (covering eight study subgroups) applied a bolus injection, subgroup analyses and meta-regression were not justified. However, we performed a sensitivity analysis excluding the single study that did not withdraw diuretics prior to ANP injection. This manoeuvre slightly lowered the baseline and peak natriuresis.

Two cross-over RCTs investigated the natriuretic effect of urodilatin in cirrhosis patients with ascites [36,37], originating from the same study site. The patients in the earliest and larger trial had mild/moderate ascites and received 60 min infusion with 20 ng/kg/min, while the patients in the latest trial had refractory ascites and received 90 min of infusion with 20 ng/kg/min. Both studies reported a significant increase in sodium excretion as a response to the treatment compared with placebo, although the response was most pronounced in the larger trial investigating cirrhosis patients with mild/moderate ascites (Figure 3). The results suffered from substantial heterogeneity ($I^2 = 64\%$).

A summary of the findings with quality of evidence are listed in Table 2. For natriuresis, the primary outcome of this systematic review, the quality of evidence was assessed as low for ANP and very low for urodilatin. These conclusions were primarily based on few RCTs being available, resulting in the dominance of non-randomised studies contributing to considerable heterogeneity, inconsistency, and imprecision due to small sample sizes.

Diuresis

Water excretion increased from baseline 1.3 mL/min (95%CI: 1.0-1.6) to 2.2 mL/min (95%CI: 1.7-2.7) in study subgroups receiving a continuous ANP infusion. The peak response tended to be higher in study subgroups receiving an infusion rate of > 30 ng/kg/min compared with ≤ 30 ng/kg/min ($P = 0.08$) (Figure 4). In our subgroup analysis, we found higher baseline and peak water excretion in study subgroups with non-refractory ascites compared with refractory ascites ($P < 0.01$). Dose, ascites severity, and quality assessment score explained between-study heterogeneity to some extent and especially in the low-dose group. When we adjusted for these factors overall heterogeneity remained substantial ($I^2 = 77\%$), but was reduced to moderate in the low-dose group ($I^2 = 54\%$). We were unable to identify parameters explaining heterogeneity in the high-dose group. Effect size was unaffected by mean age and treatment duration. Leave-one-out analyses also failed to identify any single study to explain the heterogeneity for this outcome.

Study subgroups exposed to bolus ANP injections had a tendency to increase diuresis with an estimated effect size similar to continuous infusion (Supplementary Figure 2). However, there was also considerable heterogeneity in this analysis (peak $I^2 = 94\%$, baseline $I^2 = 90\%$).

Urodilatin significantly increased urine production in the first and larger RCT[36] compared with placebo. However, in the most recent RCT with patients suffering from refractory ascites, water excretion was largely unaltered by urodilatin compared with placebo[37] (Supplementary Figure 3).

Bodyweight, waist circumference, osmolality and creatinine

Data were sparse on bodyweight and non-existent on waist circumference, thus these outcomes did not receive further evaluation. Two studies measured urine osmolality[14,24]. Laffi *et al*[24] reported a reduction in urine osmolality at the end of ANP-infusion and at the end of the recovery period compared with baseline for a subgroup of four participants. An increase was observed for five participants in another subgroup, while urine osmolality was unaffected in a third subgroup of six participants. Ferrier *et al*[14] reported no significant change in urine osmolality; however, a tendency

Table 2 Effect of natriuretic peptides as treatment of cirrhotic ascites grouped by drug

Outcomes	Anticipated absolute effects (95%CI)		No of participants (study subgroups)	Publication bias			Quality of evidence (GRADE)	Comments
	Baseline	Peak		Egger test	Trim-and-fill[1]			
					Baseline	Peak		
Atrial natriuretic peptide								
Natriuresis (μmol/min)							Low[2,3]	
Continuous infusion	5.6 (3.7-7.4)	27.0 (19.4-34.7)	152 (23)	$P < 0.0001$	4.2 (2.1-6.1)	22.9 (14.3-31.5)		
Low dose (≤ 30 ng/kg/min)	3.6 (2.0-5.3)	15.6 (8.7-22.4)	89 (13)					
High dose (> 30 ng/kg/min)	24.2 (13.8-34.5)	72.5 (41.5-103.5)	63 (10)					
Bolus injection	10.3 (4.3-16.2)	18.4 (8.8-28.0)	63 (8)	$P < 0.0001$	8.0 (1.2-14.8)	5.1 (-6.9-17.1)		
Diuresis (mL/min)							Very low[2,4]	
Continuous infusion	1.3 (1.0-1.6)	1.8 (1.2-2.3)	62 (8)	$P < 0.0001$	1.0 (0.7-1.3)	1.8 (1.3-2.4)		
Low dose (≤ 30 ng/kg/min)	1.0 (0.7-1.3)	15.6 (8.7-22.4)	89 (13)					
High dose (> 30 ng/kg/min)	1.8 (1.2-2.4)	2.8 (1.8-3.8)	63 (10)					
Bolus injection	1.1 (0.8-1.5)	2.3 (1.3-3.2)	63 (8)	$P < 0.001$	0.7 (0.2-1.1)	2.0 (1.1-2.9)		
P-Aldosterone (pmol/L)							Very low[2,4]	
Overall	1182 (879-1484)	739 (553-925)	148 (21)	$P < 0.0001$	1110 (802-1418)	497 (306-689)		
Continuous infusion								
Low dose (≤ 30 ng/kg/min)	1895 (929-2861)	1110 (670-1551)	56 (8)					
High dose (> 30 ng/kg/min)	753 (491-1015)	607 (412-801)	50 (8)					
Bolus injection	1095 (449-1741)	435 (58-812)	42 (5)					
P-Renin activity (ng/mL/h)							Very low[2,4]	
Overall	5.0 (3.7-6.2)	5.6 (4.2-6.9)	137 (20)	$P < 0.0001$	4.4 (3.2-5.6)	3.2 (1.8-4.6)		
Continuous infusion								
Low dose (≤ 30 ng/kg/min)	5.5 (2.8-8.2)	4.0 (2.2-5.8)	50 (7)					
High dose (> 30 ng/kg/min)	2.6 (1.8-3.4)	6.5 (3.7-9.2)	54 (9)					
Bolus injection	10.5 (3.8-17.2)	11.0 (2.9-19.1)	33 (4)					
Bodyweight	NA	NA	NA	NA	NA	NA	NA	Data too sparse for synthesis and quality assessment
Waist circum-	NA	NA	NA	NA	NA	NA	NA	No available data

ference							
P-Osmolality	NA	NA	NA	NA	NA	NA	NA	Data too sparse for synthesis and quality assessment
U-Osmolality	NA	NA	NA	NA	NA	NA	NA	No available data
P-Creatinine	NA	NA	NA	NA	NA	NA	NA	Data too sparse for synthesis and quality assessment
AEs	Risk of any AEs: 20 AEs/70 participants = 29%		70 (10)	NA	NA	NA	NA[5]	20 AEs were reported in two studies covering 6 study subgroups. 3 studies (4 subgroups) observed no AEs
BP reduction	Subgroups reporting BP drops			NA	NA	NA	NA[6]	Details outlined in Supplementary Table 2
Continuous infusion	53%		125 (19)					
Bolus injection	100%		63 (8)					
B-type natriuretic peptide								
NA							NA[7]	Data too sparse for synthesis and quality assessment
Urodilatin[8]								
Natriuresis (μmol/min)	Mean difference (Urodilatin *vs* Placebo): 75 (-33-183)		21 (2)	NA	Mean difference; NA		Very low[2,4]	
Diuresis (mL/min)	Mean difference (Urodilatin *vs* Placebo): 2.5 (-0.9-5.8)		21 (2)	NA	Mean difference; NA		Very low[2,4]	
AEs	Risk of any AEs: 6 AEs/21 participants = 29%		21 (2)	NA	NA		NA[5]	6 AEs were reported in the two studies
BP reduction	No BP drops observed for subgroups		21 (2)	NA	NA		NA[6]	Details outlined in Supplementary Table 2

[1]For natriuresis and diuresis trim-and-fill analyses were performed separately for continuous and bolus applications, for all other outcomes exclusively on overall data. Trim-and-fill analyses were restricted to outcomes of atrial natriuretic peptide studies.

[2]Since the vast majority of studies were non-randomised studies, rating started at low quality of evidence. For ularitide, two cross-over randomised controlled trials were available justifying rating initiation from high quality of evidence. No limitations due to risk of bias, since the majority of included studies obtained a low risk of bias score and the remaining studies a moderate risk of bias (Table 1). Overall results suffered from substantial or considerable heterogeneity with large *I2*. However, the importance of inconsistency for decision-making is uncertain, since the majority of studies were early phase non-randomised clinical trials. Nevertheless, quality of evidence was rated 1 down for the presence of serious inconsistency. Study populations were considered similar without indirectness in interventions.

[3]Rated 1 up because of a dose-response gradient. Rated 1 up because of large effect defined as a more than two-fold increase in the outcome measure.

[4]Outcomes for atrial natriuretic peptide: Rated 1 down for imprecision due to small sample sizes. Outcomes for urodilatin: Rated 2 down for imprecision due to small sample sizes and the possibility of no effect.

[5]We were unable to estimate quality of evidence for the occurrence of adverse events (AEs), although our results indicated that one atrial natriuretic peptide or urodilatin induced AE may be expected to occur in approximately every fourth patient exposed to the drugs.

[6]We were unable to estimate quality of evidence for the occurrence of blood pressure drops, although our results clearly indicated a frequent occurrence of the event when exposed to atrial natriuretic peptide. For the two urodilatin studies, blood pressure reductions were not observed.

[7]Only one trial with seven participants evaluated the effect of B-type natriuretic peptide and two studies with 21 participants in total evaluated the effect of urodilatin. A justified quality of evidence assessment requires more data.

[8]For urodilatin, data were too sparse for plasma aldosterone concentration and renin activity to justify quality of evidence grading. Furthermore, no data were available for the outcomes bodyweight, waist circumference, plasma and urine osmolality, and plasma creatinine.

Patients or population: Patients with liver cirrhosis and ascites.

Setting: Hospital

Intervention: Intravenous bolus injections or continuous infusions of natriuretic peptides (atrial natriuretic peptide, B-type natriuretic peptide, or urodilatin).

Duration of continuous infusions: 30 to 180 min; Follow-up period bolus injections: 60 to 900 min; Follow-up period continuous infusions: 0 to 180 min; Comparison: Baseline levels/concentrations.

ANP: Atrial natriuretic peptide; BNP: B-type natriuretic peptide; AEs: Adverse events; BP: Blood pressure; NA: Not applicable.

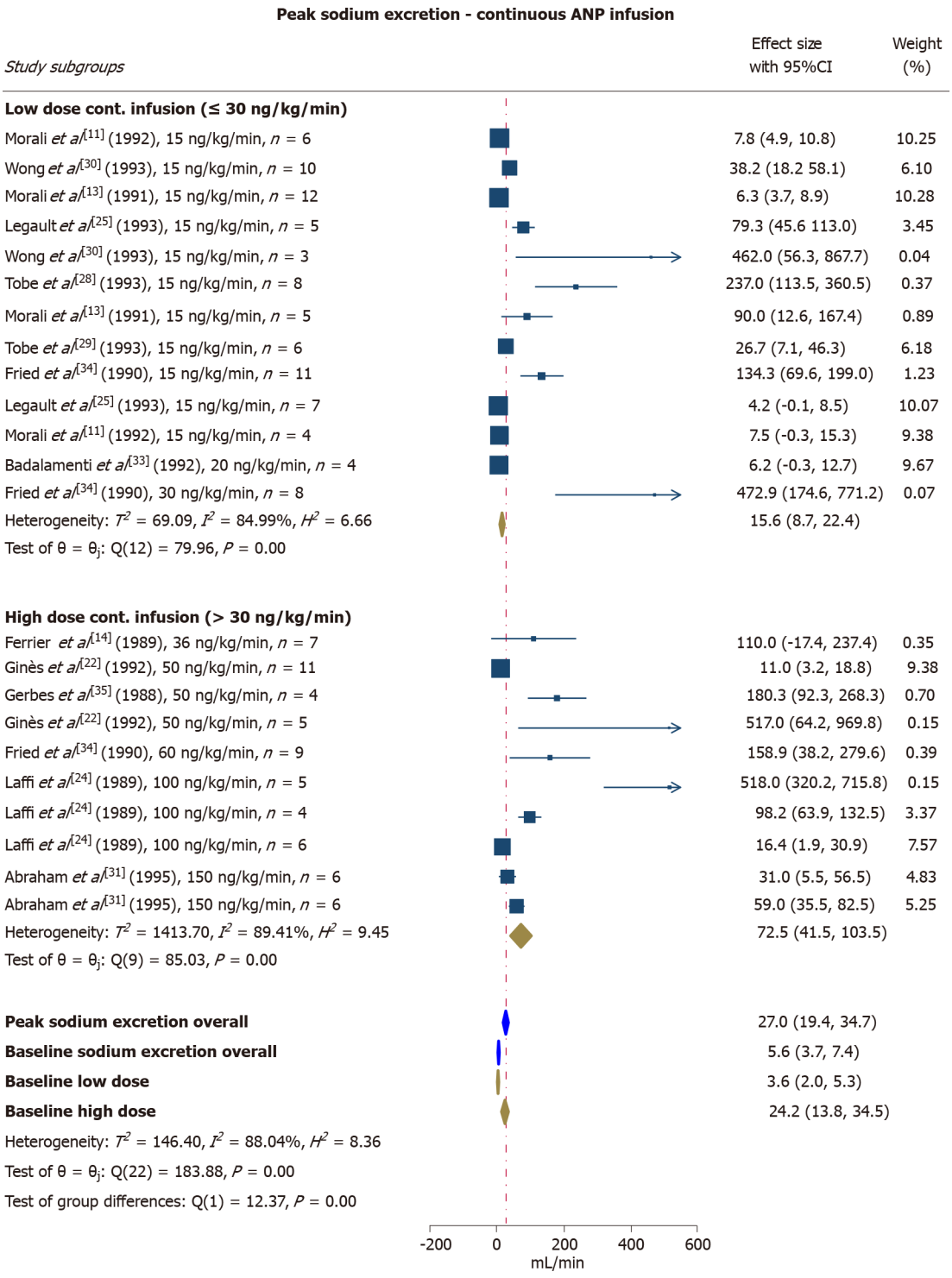

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Figure 2 Effect of intravenous continuous atrial natriuretic peptide infusion on sodium excretion (μmol/min). Analyses were performed on overall data and separately for studies applying low dose infusion (≤ 30 ng/kg/min) and high dose infusion (> 30 ng/kg/min). ANP: Atrial natriuretic peptide; CI: Confidence interval.

towards a reduction was observed in the recovery period compared with baseline. None of the included studies published data on changes in plasma osmolality. Plasma creatinine was unaffected in the seven subjects investigated by Ferrier *et al*[14].

Plasma aldosterone concentration and renin activity

Repeated measurements of plasma aldosterone concentration were performed in 13 studies covering 21 study subgroups exposed to intravenous ANP, while another two studies covering three study subgroups restricted aldosterone concentration measurements to baseline. Plasma renin activity was

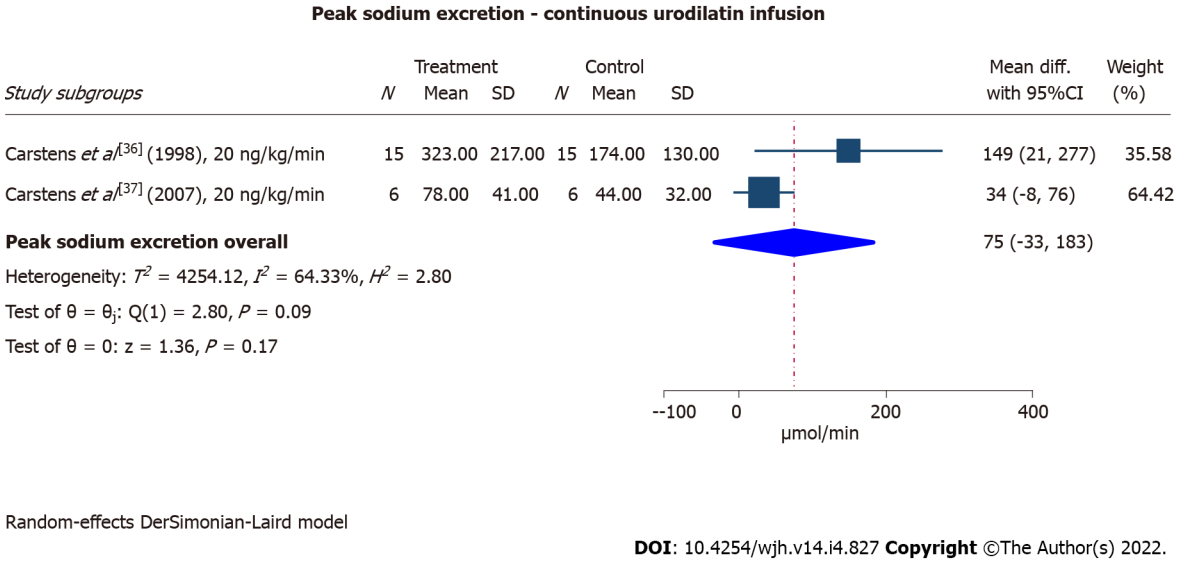

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Figure 3 Effect of intravenous urodilatin and placebo infusion on sodium excretion (μmol/min). As both studies were cross-over randomised controlled trials we used peak natriuretic response with urodilatin and placebo infusion to generate the forest plot. CI: Confidence interval.

measured before and after initiation of the intervention with ANP in 20 study subgroups originating from 12 studies. Two additional studies with three study subgroups measured plasma renin activity exclusively at baseline. Hence, for evaluation of ANP effects on these two parameters we defined peak response as the value that deviated the most from baseline.

We obtained an impression of decreased aldosterone concentration when exposed to ANP, which was similar for low- and high-dose continuous infusions as well as bolus injections (Supplementary Figure 4). For plasma renin activity, no overall significant change was observed, although study subgroups exposed to high-dose continuous ANP infusion tended to increase their plasma renin activity (Supplementary Figure 5). For both outcomes, heterogeneity was considerable and meta-regression revealed minor modulation by administration approach (bolus or continuous) and ascites severity. Of specific interest, subgroup analyses revealed that baseline plasma aldosterone concentrations and renin activity were significantly lower in study subgroups that achieved a negative sodium balance on intervention, responders, compared with non-responder study subgroups ($P < 0.01$) (Figures 5 and 6). Marked elevations of baseline plasma aldosterone concentration and renin activity were found in subgroups with refractory ascites compared with non-refractory ascites ($P < 0.01$) (Figures 5 and 6). For aldosterone analyses, four of six subgroups with refractory ascites were classified as treatment non-responders. Two of three subgroups with refractory ascites were non-responders in the renin activity analyses.

Only the largest study with urodilatin reported characteristics on plasma renin activity and aldosterone concentration. Plasma renin activity during the intervention decreased insignificantly from baseline levels, while plasma aldosterone concentration significantly decreased during the intervention compared with baseline.

Safety

Specific AEs (Supplementary Table 1) were reported in five of the included trials[16,24,34,36,37]. Three studies stated that AEs were absent[12,25,26], while no information on AEs was provided by 14 studies [11,13-15,22,23,27-33,35]. The most frequently reported AEs were facial flushing and hypotensive episodes. The latter were reported on an individual level in studies without any effect on blood pressure at study subgroup level.

In general, the included studies presented thorough illustrations or descriptions of the blood pressure course before, during and after the intervention, with only three studies[28-30] not mentioning this variable. Since the studies were of small size, we considered both statistical significance and trends as occurrence of blood pressure reductions. Blood pressure was unaffected in the three trials investigating urodilatin and BNP[16,36,37] (Supplementary Table 2).

Eighteen study subgroups exposed to ANP, including all eight subgroups that received a bolus injection, showed reductions in either mean arterial blood pressure or mean systolic blood pressure during the intervention while the blood pressure remained stable in nine study subgroups. Change in blood pressure was unreported in four study subgroups. Full recovery in the post-infusion follow-up period was reported in six study subgroups, all exposed to a bolus infusion. Partial recovery occurred in five study subgroups. No recovery was observed in one study subgroup subsequent to a continuous infusion. The remaining six study subgroups lacked follow-up data (Supplementary Table 2). Regarding

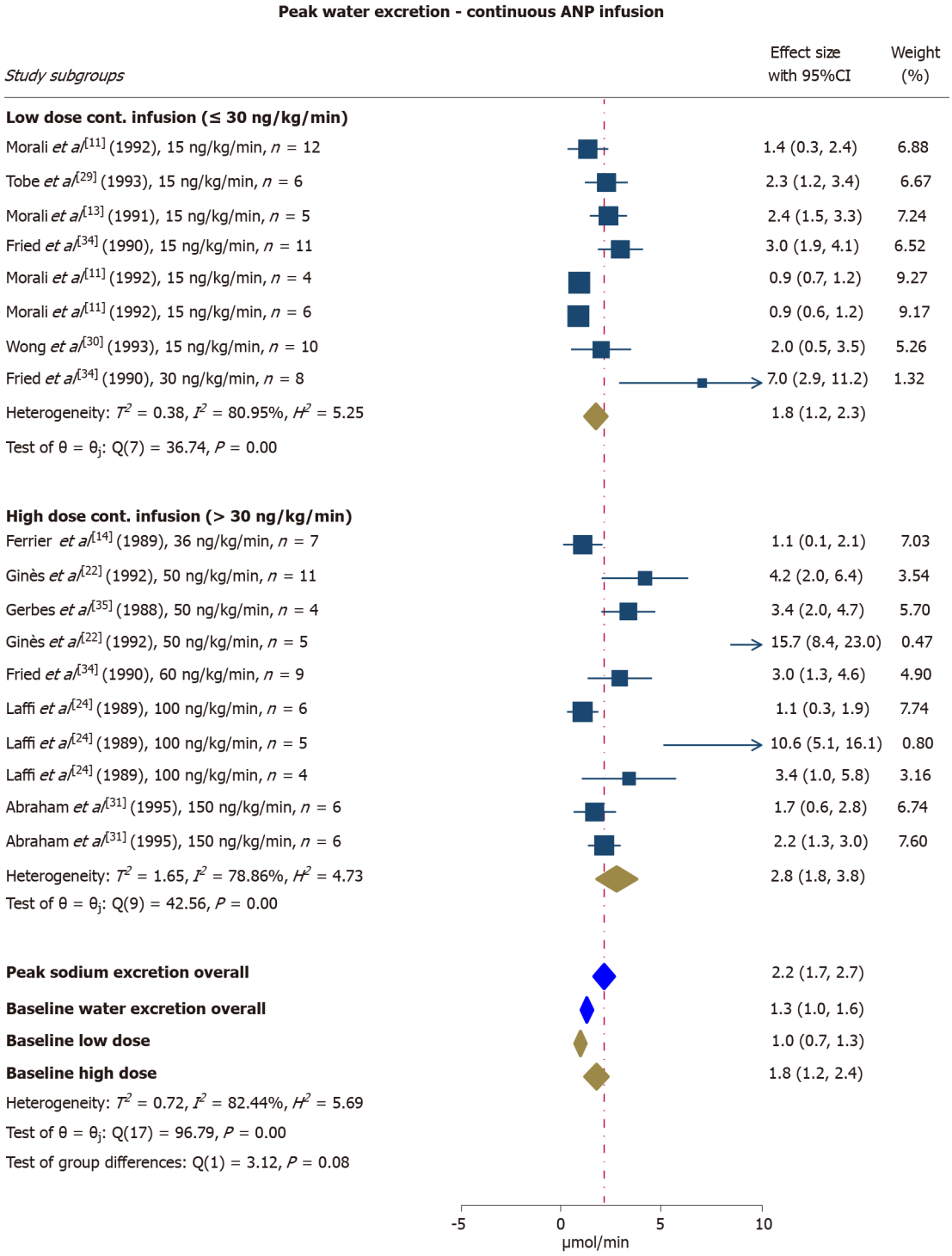

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Figure 4 Effect of intravenous continuous atrial natriuretic peptide infusion on water excretion (mL/min). Analyses were performed on overall data and separately for study subgroups receiving low dose infusion (≤ 30 ng/kg/min) and high dose infusion (> 30 ng/kg/min). ANP: Atrial natriuretic peptide; CI: Confidence interval.

the effect on blood pressure, our results clearly indicated continuous infusions of ≤ 30 ng/kg/min as well-tolerated, while higher doses almost guaranteed blood pressure reductions with a reduced likelihood of recovery in the follow-up period (Supplementary Table 2).

Publication bias

For all outcomes, Egger tests indicated statistically significant publication bias (Table 2). For peak natriuresis induced by ANP, funnel plots showed large asymmetry (Supplementary Figure 6). This may

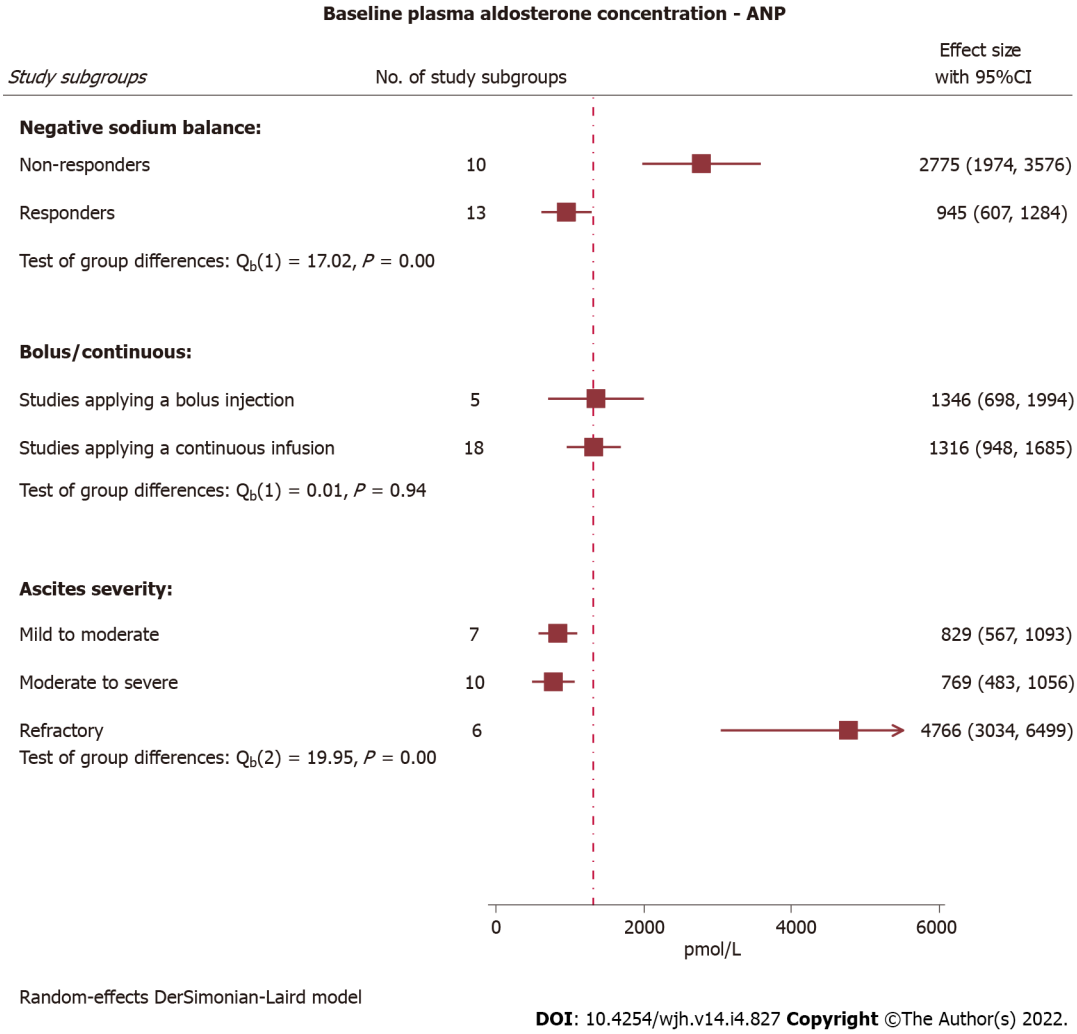

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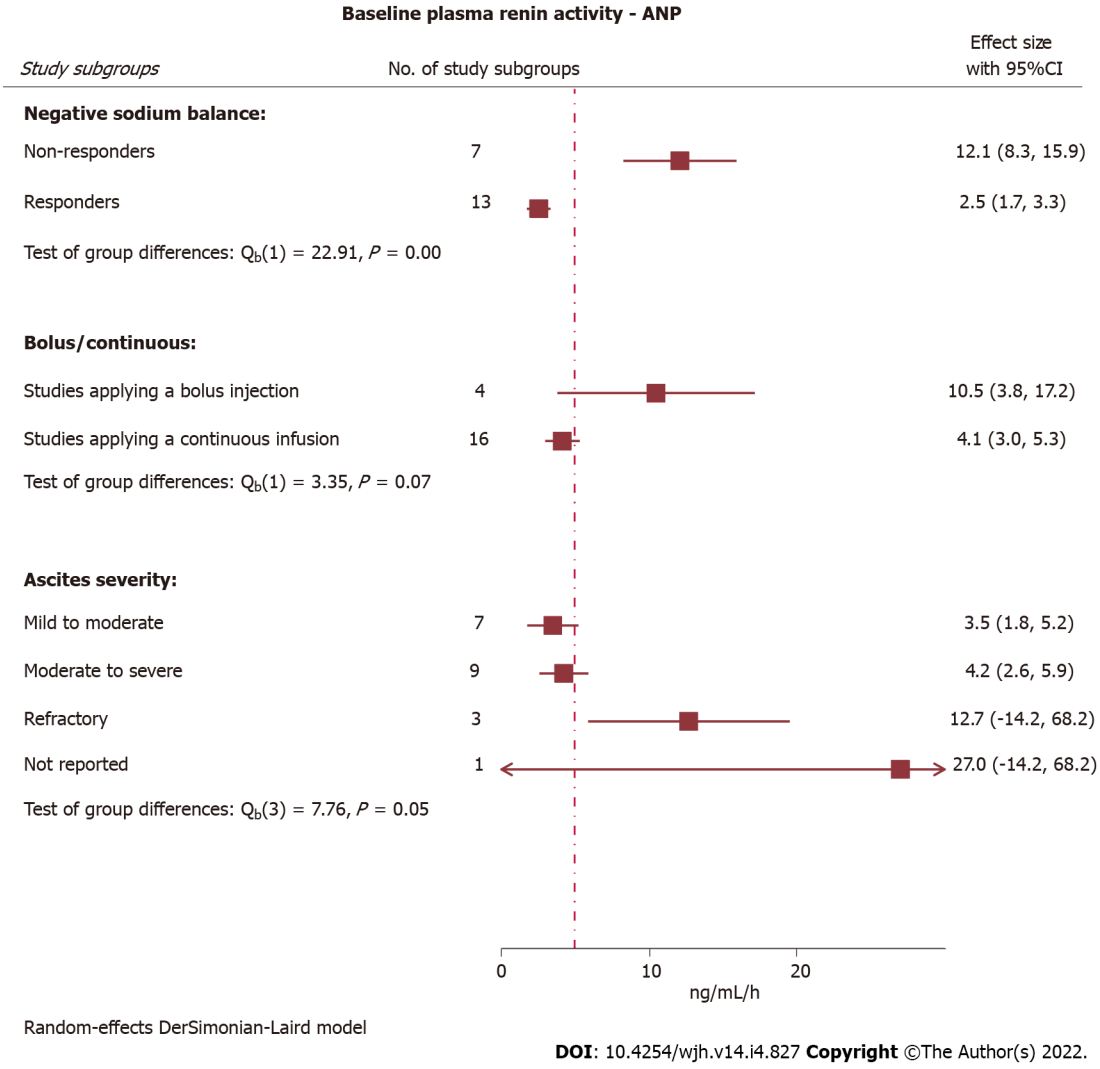
Figure 5 Baseline plasma aldosterone concentration in selected study subgroups. Non-responders failed to obtain negative sodium balance following intervention with atrial natriuretic peptide (ANP) and were characterised by significantly higher baseline plasma aldosterone concentration compared with ANP responders ($P < 0.01$). Baseline aldosterone levels were similar for subgroups exposed to bolus injections and continuous infusions, and extremely elevated in subgroups with refractory ascites compared with non-refractory ascites. Dotted line represents overall baseline mean plasma aldosterone concentration. ANP: Atrial natriuretic peptide; CI: Confidence interval.

be explained by only small sized studies being available. Nevertheless, for continuous effect parameters such as sodium and water excretion, large standard errors for small effects are unlikely. However, to overcome this disadvantage of funnel plots, we included trim-and-fill analyses to adjust for publication bias and the adjusted effect values are presented in Table 2. Baseline and peak values of the effect parameters decreased equally leaving our meta-findings unchanged.

DISCUSSION

We present here the first systematic review and meta-analysis of the benefits and harms of natriuretic peptides in patients with cirrhosis and ascites. In an attempt to investigate alternative treatments for cirrhotic ascites, this systematic review demonstrated a clinically relevant natriuretic effect in addition to diuretic and hormonal effects of natriuretic peptides. However, the levels of evidence are judged as low or very low, mainly due to considerable between-study heterogeneity, inconsistency, and imprecision due to small sample sizes. The most robust data exists for natriuresis induced by intravenous ANP administration, and especially when given by continuous infusion. Moreover, high-dose continuous infusions induce stronger sodium excretion than low-dose infusions, and subgroups with mild/moderate ascites have the most pronounced responses. Based on results from one single study, BNP is probably ineffective in this setting, although the patients included in the trial had marked sodium retention and activation of vasoconstrictor systems, and thus may have limited response to any natriuretic therapy[16]. Results from two studies investigating continuous urodilatin infusion suggest a clinically relevant natriuretic effect.



DOI: 10.4254/wjh.v14.i4.827

Figure 6 Baseline plasma renin activity in selected study subgroups. Non-responders failed to obtain negative sodium balance following intervention with atrial natriuretic peptide (ANP) and were characterised by significantly higher baseline plasma renin activity compared with ANP responders ($P < 0.01$). Baseline renin activity tended to be lower in subgroups exposed to continuous infusion than for subgroups exposed to bolus injection, while subgroups with refractory ascites had markedly elevated renin activity compared with non-refractory ascites. Dotted line represents overall baseline mean plasma renin activity. ANP: Atrial natriuretic peptide; CI: Confidence interval.

Since the effects of ANP were discovered in the early 1980's[38], investigations have been conducted to determine the therapeutic potential of natriuretic peptides in a broad range of diseases[39,40]. Through binding to specific natriuretic peptide receptors, ANP increases renal sodium and water excretion, increases glomerular filtration rate, inhibits aldosterone secretion, suppresses the release of arginine vasopressin, and reduces vascular resistance by smooth muscle relaxation[41-45]. These effects on fluid, electrolyte, and neuro-hormonal homeostasis reduce vasoconstriction and the intravascular volume, which may be beneficial for patients with fluid-accumulating disorders, including liver cirrhosis which is frequently complicated by ascites and peripheral oedema. Mild and moderate degrees of ascites are effectively treated with salt restriction and diuretics, but when ascites becomes refractory even to high doses of diuretics, treatment options are restricted to invasive procedures. Advances in diagnostic and therapeutic measures for refractory ascites remain unmet needs. Although a range of drugs, intended to alter essential events in the pathophysiological cascade, have undergone clinical investigations to relieve refractory ascites, none are currently approved.

Fyhrquist *et al*[40] were the first to report on the natriuretic and diuretic effects induced by intravenous ANP in refractory cirrhotic ascites and in the following decades further investigations with natriuretic peptides administered to cirrhotic patients were conducted. However, the trials sought primarily to elucidate the pharmacodynamics of natriuretic peptides in patients with cirrhosis and ascites, and some used ANP responsiveness to select eligible patients for other experiments *e.g.*, combined treatment of ANP and norepinephrine to stabilize blood pressure[33], compare ANP responsiveness with responsiveness to head-out water immersion[25], compare ANP responsiveness with sympathetic nerve activity[13], test the inhibitory potential of Angiotensin-II on ANP responsiveness [28], and compare ANP responsiveness before and after establishment of a peritoneovenous shunt[29].

Thus, the general limitations of the trials are the small number of participants, different study designs, short-term interventions, varying treatment doses, and differences in ascites severity of participants. These limitations manifest as considerable heterogeneity in the current meta-analysis and reduce the level of evidence obtained for all outcomes. Eight studies investigating a bolus ANP injection were also included in this work. Although natriuresis may be induced with this setup, due to a short half-life bolus injection as therapy has limited clinical potential. As continuous intravenous infusions are more effective and applicable, this may be the preferred method of administration for future trials.

We included the outcomes bodyweight and waist circumference in our protocol since they may function as surrogate markers of ascites burden. Unfortunately, only a few trials weighed their participants and none measured girth; thus, data on these parameters are too sparse for analysis. A maximum ascites reabsorption capacity of less than one litre per day[46] probably explains why these parameters were neglected for the majority of studies investigating short-term infusions of natriuretic peptides. However, due to the absence of these data the clinical relevance of natriuresis is still to be demonstrated. Serum sodium and potassium concentrations were not included as outcomes in the present study, although these electrolytes may be affected by natriuretic peptides. Baseline sodium concentration was measured in 12 study subgroups (Table 1). In only one study a follow-up measurement after ANP infusion, which was unchanged from baseline, was reported. The baseline serum sodium concentration generally reflects the disease severity, with the lowest concentrations in study subgroups characterised by refractory ascites, and thus, may be a potential predictor of ANP responsiveness. Exclusively at baseline, serum potassium concentration was measured in six study subgroups, which showed similar results. A RCT investigating longer-term urodilatin infusion is currently recruiting patients, and includes bodyweight, waist circumference, plasma sodium and plasma potassium as outcomes[47].

A notable finding of this meta-analysis is the observation that non-responders unable to achieve a negative sodium balance when treated with a natriuretic peptide were characterised by high baseline plasma aldosterone concentrations and renin activity. Plasma levels of these parameters indicate the severity of renin-angiotensin-aldosterone (RAAS)-activation, which directly parallels the degree of cirrhosis decompensation with portal hypertension. Therefore, we hypothesise that a threshold exists where RAAS-activation is too severe for ANP to counteract. Future trials may benefit from inclusion of these hormones to validate their ability to identify treatment responders and non-responders, and further investigate if a threshold predicting responsiveness can be estimated.

AEs and especially effects on blood pressure were assessed in this systematic review. The results indicated the incidence of any AE in every fourth patient receiving treatment with either ANP or urodilatin, and the AEs described were predominantly subjective measures, which may easily be explained by the well-known physiological effects of natriuretic peptides. Blood pressure is sensitive to intravenous administration of natriuretic peptides, which is well-documented in other diseases, *e.g.*, decompensated heart failure[48]. Our results clearly indicate that bolus ANP injections guarantee reductions in blood pressure, although with a high chance of rapid recovery. For continuous ANP infusions, doses ≤ 30 ng/kg/min are less likely to affect blood pressure compared with higher doses. Future trials are needed before a definite conclusion on the tolerability of natriuretic peptides in cirrhotic patients with ascites can be made.

The present study is limited to the inclusion of cirrhosis patients with ascites treated with either ANP, BNP, or urodilatin. Thus, we have not included results from studies investigating natriuretic peptide effects in cirrhosis patients without ascites. Furthermore, we have excluded studies focusing on interventions intended for blockade of natriuretic peptide degradation. One trial has tested the effects of sinorphan, an enkephalinase inhibitor, in patients with cirrhosis and ascites. Enkephalinase is a neutral membrane endopeptidase with a broad presence in plasma and tissues including the kidneys[49]. By blocking this enzyme, plasma levels of ANP and the second messenger cyclic guanosine monophosphate increase. Sixteen cirrhosis patients with persistent ascites received treatment with sinorphan in a double-blind, placebo-controlled, cross-over trial. Plasma concentrations of ANP increased significantly after drug administration and sodium excretion doubled (mean increase 17.2 μmol/min) in the 2-h post-administration period[49]. Interestingly, mean arterial blood pressure was unaffected. Therefore, blockage of ANP degradation may be of relevance for future trials, potentially with limited effects on blood pressure.

Another limitation of the present study is the age of the included studies. All but one of the studies were performed and published more than 20 years ago. The distribution of cirrhosis aetiologies has changed during the past two decades, particularly due to an increased frequency of cirrhosis due to metabolic disease. However, we focus here on cirrhosis decompensation with ascites, which occurs late in the disease course, allowing translation of the results to the present.

CONCLUSION

Ultimately, this systematic review and meta-analysis provides new perspectives into future medico-pharmaceutical research in patients with advanced cirrhosis and elucidates the course for future

research in the field. A clear natriuretic and diuretic effect of intravenous ANP is evident from our results, and we suggest that future trials would benefit from a RCT design with a comparator group, longer-term infusion, and clinical follow-up. The preferred dose has to be determined on the basis of larger natriuretic and diuretic effects with doses > 30 ng/kg/min, but better tolerability of doses ≤ 30 ng/kg/min. Although the natriuretic and diuretic effects are modest in patients with severe or refractory ascites, new treatments are most needed for this indication. This argues for a particular focus on these patients in future trials. To increase the treatment duration and maintain increased natriuresis, alternative methods of administration must be considered *e.g.*, continuous subcutaneous infusion. Finally, clinicians may find a prolongation of the interval between paracentesis as a sufficiently meaningful treatment effect and not necessarily a reversion from refractory ascites to treatment responsive ascites.

ARTICLE HIGHLIGHTS

Research background

Ascites formation is the most frequent event of cirrhosis decompensation with no approved non-invasive treatment for patients with refractory ascites. However, natriuretic peptides may induce beneficial effects in patients with cirrhosis and ascites, by counterbalancing vasoconstriction and anti-natriuretic factors.

Research motivation

Only small studies with a broad pallet of designs, have investigated the renal and systemic effects and safety of natriuretic peptides in patients with cirrhosis and ascites. We were motivated to compile results regarding natriuretic peptides as treatment for ascites to advance pharmaceutical research concerning this disease.

Research objectives

We aimed to systematically review the effects and safety of natriuretic peptides in patients with cirrhosis and ascites. We collected results regarding changes in renal sodium and water excretion, plasma aldosterone concentration, and plasma renin activity, and to perform meta-analyses providing overall estimates of the effects of natriuretic peptide infusions. Safety assessments, in particular blood pressure decreases, were included and descriptively summarised.

Research methods

We adhered to the PRISMA guidelines and the Cochrane Handbook. A review protocol was preregistered at PROSPERO. The databases MEDLINE, Web of Science, Embase, Scopus, and Cochrane Library were searched and references reporting the renal and systemic effects of mono-therapy with atrial natriuretic peptide (ANP), B-type natriuretic peptide, urodilatin, or any synthetic equivalent in patients with cirrhosis and ascites were considered for inclusion. Treatment administration had to be intravenous, but trials were included regardless of dose, follow-up duration, and whether a continuous infusion or bolus injection was administered. The study screening and data extraction were performed independently by two reviewers. A random-effects model was applied for the meta-analysis. Reasons for heterogeneity were explored through subgroup and leave-one-out analyses.

Research results

Twenty-two studies were included and short-term ANP infusion was the only intensively studied treatment. Renal sodium and water excretion increased in response to continuous ANP infusion and was most pronounced when infusion rates > 30 ng/kg/min were applied. Furthermore, sodium excretion was higher in study subgroups with mild/moderate ascites compared with refractory ascites. The baseline plasma aldosterone concentration and renin activity were significantly lower in subgroups achieving a negative sodium balance compared with treatment non-responders, and may be relevant response predictors to include in future trials. Blood pressure decreases occurred less frequently with doses ≤ 30 ng/kg/min.

Research conclusions

Intravenous infusions of natriuretic peptides induce meaningful sodium and water excretion in patients with cirrhotic ascites. Continuous ANP infusions > 30 ng/kg/min induce the most pronounced renal effects, but with a higher risk of blood pressure decreases than doses ≤ 30 ng/kg/min.

Research perspectives

Future larger-scale clinical trials are justified to determine the therapeutic potential of natriuretic peptides in patients with cirrhotic ascites.

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FOOTNOTES

Author contributions: Gluud LL and Grønbæk H contributed equally to the work. All authors contributed to the design of the study. Gantzel RH and Kjær MB acquired the study data; Gantzel RH and Gluud LL performed the statistical analyses; all authors contributed to the interpretation of data; Gantzel RH drafted the manuscript; and all authors critically reviewed and approved the final manuscript.

Conflict-of-interest statement: Grønbæk H has received a research grant (No. ULA04-2019-1) from ADS AIPHIA Development Services AG (Switzerland) to investigate ularitide in patients with refractory ascites. Watson H owns stocks in Sanofi. Gantzel RH, Kjær MB, Jepsen P, and Aagaard NK have nothing to report. Financial support: Grønbæk H has received research funding from Intercept, Abbvie, NOVO Nordisk Foundation, Arla, and ADS AIPHIA Development Services AG. Grønbæk H is an advisory board member for Ipsen and speaker for Norgine. Gluud LL has received grants from Novo Nordisk, Alexion, and Gilead.

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CASE REPORT

Late polymicrobial transjugular intrahepatic portosystemic shunt infection in a liver transplant patient: A case report

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Abstract

BACKGROUND

Infection of a transjugular intrahepatic portosystemic shunt (TIPS) stent is a rare and serious complication that most commonly occurs during TIPS creation and revision. Patients typically present with recurrent bacteremia due to shunt occlusion or vegetation. To date there are approximately 58 cases reported. We present a patient diagnosed with late polymicrobial TIPS infection five years following TIPS creation.

CASE SUMMARY

A 63-year-old female status-post liver transplant with recurrent cirrhosis and portal hypertension presented with sepsis and recurrent extended-spectrum beta-lactamase *Escherichia coli* bacteremia. Computed tomography of the abdomen revealed an occluded TIPS with thrombus extension into the distal right portal vein, and focal thickening of the cecum and ascending colon. Colonoscopy revealed patchy ulcers in these areas with histopathology demonstrating ulcerated colonic mucosa with fibrinopurulent exudate. Shunt thrombectomy and revision revealed infected-appearing thrombus. Patient initially cleared her infection with antibacterial therapy and TIPS revision; however, soon after, she developed *Enterobacter cloacae* bacteremia and *Candida glabrata* and *C. albicans* fungemia with recurrent TIPS thrombosis. She remained on antifungal therapy

indefinitely and later developed vancomycin-resistant *Enterococcus faecium* with recurrent TIPS thrombosis. The option of liver re-transplant for removal of the infected TIPS was not offered given her critical illness and complex shunt anatomy. The patient became intolerant to linezolid and elected hospice care.

CONCLUSION
Clinicians should be aware that TIPS superinfection may occur as long as five years following TIPS creation in an immunocompromised patient.

Key Words: Transjugular intrahepatic portosystemic shunt; Endotipsitis; Colitis; Liver cirrhosis; Liver transplantation; Case report

Core Tip: Polymicrobial transjugular intrahepatic portosystemic shunt (TIPS) infection may occur in an immunocompromised patient many years following TIPS creation. Given the high morbidity and mortality associated with TIPS infection, it is important to consider this diagnosis early in a patient with recurrent septicemia, even without recent TIPS creation or revision. Early shunt thrombectomy is important for source control and optimization of antibiotic penetrance of the TIPS.

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INTRODUCTION

Transjugular intrahepatic portosystemic shunt (TIPS) creation is a routine therapy for patients with complications of portal hypertension such as refractory ascites or variceal hemorrhage[1]. Endotipsitis is a rare endovascular infection of a TIPS with a prevalence of approximately 1% and mortality rate of approximately 32%[2-4]. A rare but challenging complication of endotipsitis is persistent bacteremia following TIPS creation and revision. Early cases of TIPS infection, categorized as an infection within 120 days of TIPS creation, are commonly associated with gram-positive bacteria[4]. In the USA the use of prophylactic antibiotics prior to TIPS creation is routinely practiced, and according to the American Society of Interventional Radiology is considered a class IIB recommendation[2,5]. However, there are strict indications based on the guidelines by the British Society of Gastroenterology, which strongly recommend prophylactic antibiotics only for TIPS shunt for variceal bleeding, long/complex procedures, or in a patient with a previous biliary surgery or instrumentation[6].

TIPS stent colonization is believed to be secondary to lack of graft endothelization with the highest mortality rates associated with *Staphylococcus* and *Candida* species[2-4,7]. These microorganisms are difficult to treat given their ability to form biofilms, as can be seen in other endovascular infections such as prosthetic valve endocarditis[7,8]. Tacrolimus, a immunosuppressive medication prescribed to this patient, has been associated with decreased epithelization in animal and *in vivo* studies and therefore may further increase the risk of TIPS stent colonization in a patient[9,10]. This case report presents a rare event of polymicrobial TIPS infection occurring five years following TIPS creation in an immunosuppressed patient status post liver transplantation for hepatitis C cirrhosis. Additionally, polymicrobial endotipsitis cases were identified through PubMed to provide an updated overview of treatment courses and associated health outcomes.

CASE PRESENTATION

Chief complaints

A 63-year-old female presented with sepsis and recurrent extended-spectrum beta-lactamase *Escherichia coli* (*E. coli*) bacteremia.

History of present illness

Our patient initially presented to her local hospital with hypotension and sepsis in late August 2019 and completed a 10-d course of ertapenem while hospitalized for *E. coli* bacteremia. She presented locally

with similar symptoms a few days after discharge from the initial admission and was found to have recurrent *E. coli* bacteremia with the identical susceptibility pattern. Imaging demonstrated TIPS occlusion, and the patient was transferred to our institution with recurrent *E. coli* bacteremia from an unidentified source.

History of past illness

Our patient with a MELD-Na score of 23 underwent orthotopic liver transplantation in 2009 for hepatitis C cirrhosis. Her transplant course was complicated by recurrent hepatitis C infection resulting in bridging fibrosis/early cirrhosis despite viral eradication in 2011. She eventually developed portal hypertension related to a non-occlusive portal vein thrombosis and anastomotic stenosis of the hepatic vein and inferior vena cava. In 2014, she suffered acute gastric variceal bleeding and underwent semi-urgent treatment with balloon-occluded anterograde obliteration of a gastrorenal shunt and TIPS creation with a self-expanding poly-tetrafluoroethylene (ePTFE)–covered stent-graft.

Personal and family history

Medical history included hepatitis C, gastric varices, hypertension, well-controlled diabetes mellitus type 2, iron deficiency anemia and fibromyalgia. Family history included hypertension, lung cancer, and prostate cancer.

Physical examination

The patient was afebrile and displayed right upper abdominal tenderness.

Laboratory examinations

Laboratory findings included hemoglobin 8.6 (12-16 g/dL) (baseline of approximately 9-10), white blood cell (WBC) 5.39 (4-11 × 10^3/μL), platelets 89 (150-450 × 10^3/μL), alanine transferase 16 (< 55 U/L), aspartate transferase 19 (< 35 U/L), total bilirubin 0.5 (0.3-1.2 mg/dL), alkaline phosphatase 88 (40-150 U/L) and creatinine 1.2 (0.7-1.3 mg/dL). Gastrointestinal pathogens panel polymerase chain reaction was negative for enteroaggregative *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, shiga-like toxin-producing *E. coli*, *E. coli* O157, and shigella/enteroinvasive *E. coli*. Histopathology of cecum and ascending colon biopsies obtained a few days prior revealed extensive ulcerated colonic mucosa with fibrinopurulent exudates, consistent with active colitis. There was no evidence of cytomegalovirus associated inclusion bodies, microorganisms, granulomas, or malignancy. Bacterial culture of the extracted thrombus (Figure 1) during thrombectomy did not grow any organisms. Urine analysis showed no bacteria and rare white blood cells per high power field with no growth on culture.

Imaging examinations

Thickening of the cecum and ascending colon, and TIPS occlusion that extended into the distal right portal vein were noted on abdominal computed tomography (CT) (Figure 2A). Magnetic resonance imaging did not show significant hepatic biliary dilatation or biliary leak. Chest CT suggested multiple pulmonary septic emboli. Transthoracic echocardiography (TTE) obtained a few days prior showed mild reduced systolic ejection fraction and no obvious vegetations, though aortic, tricuspid, and pulmonic valves were not well visualized due to restricted patient mobility and excessive abdominal air.

FINAL DIAGNOSIS

The patient was diagnosed with a late TIPS infection.

TREATMENT

She underwent TIPS thrombectomy, venoplasty, and placement of a new Wallstent coaxial uncovered metallic stent at the portal end of the TIPS to extend the intraportal leading end of the shunt into a larger caliber portal vein, as the originally entered portal vein was small in caliber and formed an inflow narrowing into the TIPS at the leading edge of the PTFE-coated self-expanding stent. Anticoagulation after TIPS revision was not considered in this patient as she did not have a primary hypercoagulable disorder, and the flow in the shunt was brisk and was rendered clean by thrombectomy and clot extraction. She was discharged on a 6-wk course of IV ertapenem (1 g once daily). Patient successfully cleared her *E. coli* bacteremia.

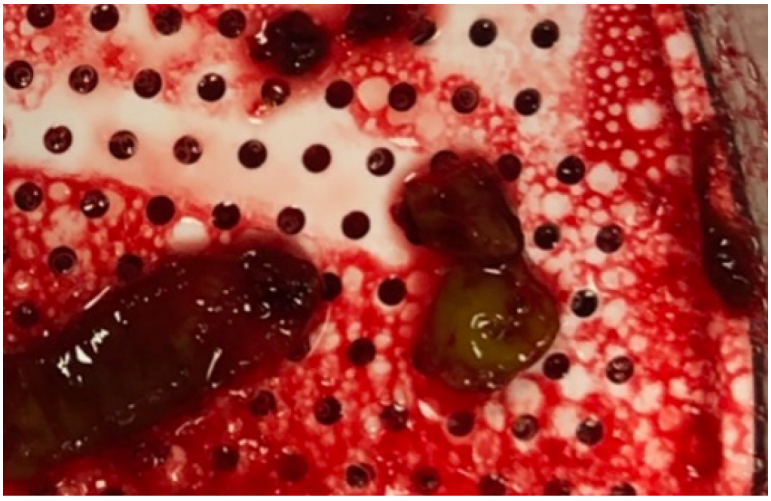

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Figure 1 Transjugular intrahepatic portosystemic shunt thrombectomy. Thrombus extracted during first transjugular intrahepatic portosystemic shunt thrombectomy was notable for size and infected appearance.

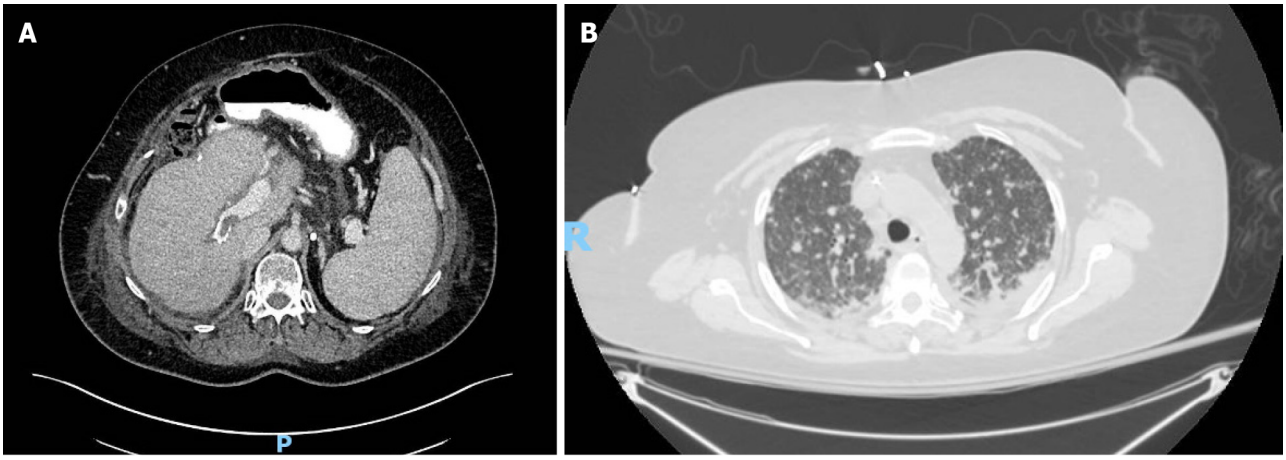

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Figure 2 Computed tomography images. A: Computed tomography of upper abdomen. An axial-contrast enhanced computed tomography of the upper abdomen showing an occluded (hypodense area) transjugular intrahepatic portosystemic shunt; B: Computed tomography of chest. An axial non-enhanced computed tomography of the chest showing diffuse bilateral ground-glass and interstitial opacities with innumerable bilateral pulmonary nodules and small bilateral pleural effusions.

OUTCOME AND FOLLOW-UP

Two and a half months later, our patient presented with a headache and was treated for *Enterobacter cloacae* and *Candida glabrata* septicemia. Vital signs were notable for fever (38.2°C) and tachycardia (114 bpm). Labs included hemoglobin 9.2 g/dL, WBC 4.34 × 10^3/μL, platelets 70 × 10^3/μL, alanine transferase < 6 U/L, aspartate transferase 33 U/L, and creatinine 0.9 mg/dL. Abdominal CT showed a new non-occlusive thrombus in the mid-TIPS and a right ovarian vein clot. Repeat shunt thrombectomy and revision was performed. Histopathology of the extracted thrombus confirmed *Candida* species, and *C. glabrata* and *C. albicans* grew in blood cultures. Additional infectious workup included a TTE that was non-revealing. A 6-wk course of IV ertapenem (1 g once daily) and IV micafungin (150 mg daily) was initiated at discharge.

Four weeks later she developed acute dyspnea and pleuritic chest pain and was readmitted with hypoxemic respiratory failure. TTE revealed grade 1 diastolic dysfunction without vegetation. Chest CT revealed numerous bilateral pulmonary nodules with diffuse ground-glass and interstitial opacities (Figure 2B). Blood cultures once again grew *C. glabrata* and *C. albicans*, and bronchoalveolar lavage grew *Candida* species. Given suspicion for refractory intravascular Candida infection, therapy was escalated to IV amphotericin (300 mg daily) and oral flucytosine (1000 mg twice a day). Balloon sweep of the TIPS was complicated by post-procedural shock and hypoxemia. In the setting of progressive acute kidney injury and pancytopenia, the patient was transitioned to IV micafungin (150 mg daily) and IV

voriconazole (400 mg twice daily for one day followed by 300 mg twice daily). Due to the combination of respiratory failure, active infection, and location of the TIPS that extended into the base of the right atrium, re-transplantation (and removal of the TIPS) was not offered due to exceedingly high surgical risk. She remained on antifungal therapy indefinitely and over the subsequent three months developed recurrent vancomycin-resistant *Enterococcus faecium* bacteremia. Newly identified thrombus along a peripherally inserted central catheter suggested the possibility of line associated septic thrombophlebitis as the source of her *Enterococcus* bacteremia. Bacteremia recrudesced despite line extraction, eventual resolution of her line associated clot, and an appropriate course of proactively dosed IV daptomycin (400-915 mg daily). Repeat TTE did not reveal signs of infective endocarditis and CT confirmed recurrent TIPS thrombosis as the likely source of her refractory *Enterococcus faecium* bacteremia that progressed to develop a daptomycin minimum inhibitory concentration > 256. Although the patient improved clinically and initially cleared her bacteremia with IV linezolid (600 mg daily), she developed severe thrombocytopenia and gastrointestinal bleeding that precluded further use of this agent. The patient elected to pursue hospice care and died shortly thereafter.

DISCUSSION

This case is a unique account of polymicrobial TIPS stent infection occurring five years after TIPS creation, the longest interval reported[4]. Shunt infection is most commonly reported during TIPS creation or revision, and infrequently with biliary-shunt fistulae that may form following TIPS creation [11]. Our patient presented with recurrent *E. coli* bacteremia of unclear origin and ongoing active colitis. While it is possible that bacterial translocation into the portal vein blood in the setting of active colitis could lead to *E. coli* bacteremia and seeding of the TIPS, it seems more likely that the colitis and occlusion of the TIPS occurred as a result of a low-flow state with seeding of the occlusive TIPS thrombus during ongoing *E. coli* bacteremia. Evidence that supports colitis likely being ischemic during the second episode of *E. coli* bacteremia include the colonic biopsy results obtained during lower endoscopy that corresponded to areas of active colitis noted on CT imaging which did not show any microorganisms based on limited histopathological analysis (no gram staining performed). Alternative common sources of *E. coli* bacteremia were investigated during her second episode of bacteremia, including urinary infection, biliary leak, cholangitis, and bacterial gastroenteritis, and work-up was non-revealing. Moreover, there was a very low suspicion for infective endocarditis given the respective organism involved and no history of IV drug use. This was confirmed on multiple TTEs which did not show any vegetation. In summary, the initial source of infection that may have seeded the TIPS was not clearly identified -- bacterial translocation due to confirmed, active colitis is a plausible explanation but an alternate source such as genitourinary or biliary are also possible given that records do not indicate a source of the initial *E. coli* bacteremia. It is important to note that during this patient's first *E. coli* bacteremia episode diagnostic imaging was delayed. Imaging was pursued during her second episode of *E. coli* bacteremia as the patient complained of right upper quadrant pain and this revealed an occluded TIPS that raised suspicion for endotipsitis. This patient continued to suffer from recurrent polymicrobial bacteremia and fungemia after multiple TIPS revisions likely from a chronically infected TIPS. The patient likely had incomplete stent endothelization given the use of tacrolimus, which increased her risk of stent colonization[9,10].

Determining treatment duration and whether liver transplantation should be considered in the clinical scenario of endotipsitis are challenging decisions. Available clinical care guidance derives from case reports and case series, hence underscoring the importance of reporting new cases. To date there are 59 cases[2,4,12-14] of TIPS stent infection with nine being polymicrobial (Tables 1 and 2)[15-19]. Initial treatment courses range from 2 to 6 wk of antimicrobial therapy, followed by long-term oral therapy and orthotopic liver transplantation when medical therapy fails (Table 2). Analysis of polymicrobial cases to date showed that infections resolved with liver transplantation (Table 2). If patients did not undergo transplantation they remained indefinitely on antifungal therapy. While TIPS thrombectomy and revision were not commonly undertaken, there should be consideration of early thrombectomy, as drug penetration and source control may be insufficient with only antimicrobial therapy. For instance, inadequate drug penetration has been noted with amphotericin for *Candida* infective endocarditis[7]. Hence, surgical intervention is recommended for left sided infective endocarditis involving fungal organisms, as well as for *S. aureus*, or other highly resistant organisms [15]. Considering the high mortality rate of fungal TIPS infections[14,20-24], approximately 60% (Table 1), it is unclear whether antifungal prophylaxis should be considered prior to a TIPS revision. Justification for this step is lacking due to paucity of prospective studies (not surprising given the rarity of this infection) and the absence of guidelines to identify high-risk patients.

In summary, we present a case of recurrent *E. coli* bacteremia due to a late TIPS infection and occlusion that later evolved to a polymicrobial, multidrug-resistant TIPS infection. The patient initially cleared her infection with antimicrobial therapy and TIPS thrombectomy. Unfortunately, the patient later developed recurrent polymicrobial bacteremia and fungemia from a chronically infected TIPS.

Table 1 Mortality rates associated with monomicrobial and polymicrobial endotipsitis

Microbial agent	Reported cases	Mortality rate, %
Enterococcus (faecalis, faecium)	14	21
Staphylococcus (aureus, epidermidis)	9	44
Escherichia coli	7	43
Candida (glabrata, albicans)	7	57
Lactobacillus (rhamnosus, acidophilus)	3	67
Streptococcus (sanguis, bovis)	2	0
Gemella morbillorum	2	0
Klebsiella (pneumonia, oxytoca)	3	33
Serratia marcescens	1	0
Enterobacter cloacae	1	0
Salmonella typhi	1	0
polymicrobial infection	9	50
total	59	39

Adapted from Alberto Garcia-Zamalloa.

Table 2 Polymicrobial cases of endotipsitis

Case and microbial agent	Treatment course	Outcome	Ref.
Case 1: Gram-positive and negative bacteria, and fungus	Antibiotic × 2 wk->antifungal × 2 ws->antibiotics->liver transplant	Resolved	[2]
Case 2: Gram-negative bacteria	antibiotics × 6 wk->antibiotics -> liver transplant	Resolved	[4]
Case 3: Gram-negative bacteria	antibiotic × 4 wk	Resolved	[20]
Case 4: Gram-negative bacteria and fungus	antibiotics and antifungal × 6 wk-> antifungal indefinitely	Resolved	[20]
Case 5: Gram-positive and negative bacteria	antibiotics->TIPS revision	Death	[21]
Case 6: Gram-positive and negative bacteria, and fungi	antibiotics->antibiotics, antifungal and TIPS revision-> antibiotic->antibiotics and TIPS revision->oral antibiotics indefinitely	Unknown	[22]
Case 7: Gram-positive and negative bacteria, and fungus	antibiotics and antifungals->liver transplant	Resolved	[23]
Case 8: Gram-positive and negative bacteria	antimicrobials × 4 wk ->liver transplantation	Resolved	[24]
Case 9: Gram-positive and negative bacteria, and fungi	antibiotics × 10 d-> TIPS thrombectomy & revision, and antibiotics × 6 wk-> TIPS thrombectomy and revision, and antibiotics and antifungals for 6 wk->balloon sweep of TIPS, antifungals indefinitely with antibiotics as tolerated	Death	Current case

TIPS: Transjugular intrahepatic portosystemic shunt.

CONCLUSION

TIPS infection is a rare event that most commonly occurs following TIPS creation and revision, though as illustrated in this case report it may occur many years following TIPS creation. Clinicians should be aware of this clinical complication early in the course so a TIPS thrombectomy can be performed for source control and to improve antibiotic penetration of the TIPS. Moreover, literature review shows that the highest mortality rates with endotipsitis are with *Candida* and polymicrobial infections. Given the refractory nature of these infections, liver transplantation should be considered to provide definitive

treatment when feasible. Lastly, the rarity of a TIPS infection limits the development of research studies, and current understanding of this entity relies mainly on case reports and case series, hence highlighting the need to continue to report new cases.

FOOTNOTES

Author contributions: Perez IDLC was the internal medicine resident physician providing care during initial hospitalization; Perez IDLC also drafted and participated in the revision process and final submission of this manuscript; Haskal ZJ was the interventional radiologist that performed the procedures described in this case and participated in the whole revision process; Hogan JI was the infectious disease specialist overseeing her care and participated in the whole revision process; Argo CK was the outpatient and inpatient hepatologist who provided specialized care, participated in the revision process and final submission of this manuscript.

Informed consent statement: Consent was obtained verbally over the phone from the husband as the patient passed away.

Conflict-of-interest statement: Dr. Haskal disclosures include working with Varian, Boston Scientific, WL Gore and Associates, and Becton Dickinson. All other authors have no actual or potential conflicting interest in the submission of this manuscript.

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CASE REPORT

Angiotensin converting enzyme inhibitor associated spontaneous herniation of liver mimicking a pleural mass: A case report

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Abstract

BACKGROUND

Spontaneous diaphragmatic herniation of the liver is a rare entity. It may mimic pulmonary mass especially in the absence of trauma. Cough is a common side effect of angiotensin converting enzyme (ACE) inhibitors that may cause diaphragmatic rupture due to a sudden increase in trans-diaphragmatic pressure. We present a case of ACE-inhibitor associated spontaneous herniation of the liver mimicking pleural mass.

CASE SUMMARY

An 80-year-old woman presented with dry cough for 1 mo and sudden onset of cramping abdominal pain for 1 d. She denied history of trauma, prior surgeries, smoking, alcohol or illicit drug use. She has a history of diabetes and was started on an ACE inhibitor 6 mo ago for the management of hypertension. Examination was remarkable for right upper quadrant tenderness. Lab work-up was unremarkable. Chest X-ray showed a right lower lung opacity suspecting right pleural mass. Chest computed tomography scan ruled out pleural mass, however, revealed herniated right lobe of the liver (3.9 cm × 3.6 cm × 3.4 cm) into the

thoracic cavity through the posterolateral diaphragmatic defect. Laparoscopic repair of the diaphragmatic defect was performed and the ACE inhibitor was stopped. Patients' symptoms had completely resolved on follow-up.

CONCLUSION

ACE inhibitor-associated cough may cause diaphragmatic liver herniation mimicking pleural mass. Early diagnosis, surgical repair and addressing the triggering factors improve patients' outcomes.

Key Words: Diaphragmatic hernia; Liver herniation; ACE-inhibitors; Cough; Non-traumatic diaphragmatic hernia; Case report

Core Tip: Diaphragmatic herniation of the liver secondary to angiotensin converting enzyme inhibitors induced cough is uncommon. Cough is a rare cause of diaphragmatic liver herniation and it may be overlooked. This case illustrates the importance of combining clinical presentation with cross-sectional radiological imaging for early diagnosis and surgical repair of diaphragmatic liver herniation and for better patient outcomes.

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INTRODUCTION

Spontaneous diaphragmatic herniation of abdominal organs into the thoracic cavity is an uncommon entity. A congenital defect in the diaphragm is the most common cause of diaphragmatic hernia with a reported incidence of 0.8-5 per 10000 births[1]. Acquired rupture of the diaphragm is most commonly caused by high-velocity blunt or penetration abdomino-thoracic trauma and postsurgical diaphragmatic defect that may result in herniation of abdominal contents into the thoracic cavity[2,3]. Spontaneous diaphragmatic herniation is an uncommon subtype of acquired hernia without history of trauma. Commonly herniated abdominal organs are the stomach, small or large intestines, mesentery and spleen [2,4,5]. Spontaneous herniation of the liver into the thoracic cavity due to a non-traumatic rupture of the diaphragm is unusual with only a few cases reported[4,6,7].

Clinical presentation of diaphragmatic hernias are variable depending upon the acuity of diaphragmatic rupture, size of the defect and underlying etiology. Majority of patients present with abdominal pain, chest pain, tachycardia, shortness of breath and cough, however, a subset of patients remain asymptomatic in cases of a small defect in the diaphragm[8]. Diaphragmatic liver herniation may mimic pleural malignancy. A high index of clinical suspicion is required for early identification of diaphragmatic hernias and differentiating them from pleural malignancy with a careful review of cross-sectional radiological imaging of chest and abdomen. We present a case of cough induced spontaneous diaphragmatic herniation of the liver due to the use of angiotensin converting enzyme (ACE) inhibitor.

CASE PRESENTATION

Chief complaints

An 80-year-old female presented for evaluation of dry cough for 4 wk.

History of present illness

Patient's cough was severe, persistent, without associated hemoptysis or sputum production. She also reported the sudden onset of upper abdominal pain and mild shortness of breath for 1 d prior to visiting the hospital.

History of past illness

She had past medical history of diabetes mellitus and hypertension and was started on an ACE inhibitor 6 mo ago for the management of hypertension. She denied history of previous surgery or recent trauma.

Personal and family history

Family history was unremarkable.

Physical examination

On examination, the patient was afebrile (98.6 F), tachycardiac (112/min) with an elevated blood pressure (140/80 mmHg) and respiratory rate of 20 breaths/minute. Abdominal examination was remarkable for mild right upper quadrant tenderness without evidence of Murphy's sign or skin bruising. The lower border of the liver was non-palpable; however, a percussion dullness was noted at the right fourth intercostal space of the chest in the midclavicular line. The patient was admitted for further evaluation.

Laboratory examinations

Her baseline blood work including complete blood count, liver function tests and basic metabolic panel were unremarkable except for low hemoglobin and hematocrit (Table 1).

Imaging examinations

Ultrasound of the abdomen showed normal echotexture of the liver without evidence of liver lesions, cholelithiasis, acute cholecystitis or hepatobiliary ductal dilation. Chest radiograph demonstrated a well-defined soft tissue mass noted just above the right hemidiaphragm making an obtuse angle suggesting pleural or extra-pleural mass (Figure 1). Given a suspicion of pleural malignancy, a high-resolution computed tomography (CT)-scan of the chest was performed which revealed a defect in the posterolateral aspect of the right diaphragm with a herniated right lobe of the liver into the thoracic cavity representing a mass measuring 3.9 cm × 3.6 cm × 3.4 cm (Figure 2).

FINAL DIAGNOSIS

Spontaneous liver herniation through the right diaphragm due to an ACE inhibitor associated cough.

TREATMENT

Laparoscopic surgical repair of the diaphragmatic defect was performed after the retraction of herniated liver into the abdominal cavity. The post-surgical hospital course was uneventful. Patient was discharged on day 3 of hospitalization. Her ACE inhibitor was switched to a calcium channel blocker (verapamil) for the management of hypertension.

OUTCOME AND FOLLOW-UP

At the 8-wk follow-up, the patients' symptoms were completely resolved and blood pressure was well controlled on Verapamil.

DISCUSSION

This case illustrates an unusual presentation of spontaneous diaphragmatic herniation of the liver secondary to ACE inhibitor associated cough. ACE inhibitors are common medications used for the management of hypertension and congestive heart failure. Approximately 5%-35% of patients develop ACE inhibitor associated dry cough with a reported onset within hours to months after initiation of therapy[9-11]. Coughing causes an opposing force on the diaphragm due to respiratory muscle discoordination. Abdominal muscle contraction causes an upward pushing force on the diaphragm against the downward and inward movement of the ribs[12]. Sustained cough increases the trans-diaphragmatic pressure gradient that may cause trivial injury to the diaphragm. This phenomenon may result in spontaneous herniation of abdominal organs into the thoracic cavity through diaphragmatic defects.

Our patient had an ACE inhibitor associated cough that caused a sudden increase in trans-diaphragmatic pressure and induced liver herniation through a diaphragmatic defect. The herniated

Table 1 Baseline lab investigations

Lab investigation	Value	Normal range for female
Hemoglobin	9.7 g/dL	11.1-14.5 g/dL
Hematocrit, %	29	35.4-42.0
WBC count	10.1 × 10^9/L	4.0-11.0 × 10^9/L
Platelets	209 × 10^9/L	150-450 × 10^9/L
Urea	17 mg/dL	10-50 mg/dL
Creatinine	0.76 mg/dL	0.6-1.1 mg/dL
Hepatitis B surface antigen	0.357 (non-reactive)	1.0
Hepatitis C virus antibody	0.090 (non-reactive)	1.0
Total bilirubin	0.50	Up to 1.2 mg/dL
Direct bilirubin	0.20	< 0.2 mg/dL
Alanine transaminase	08	< 34 U/L
Alkaline phosphatase	96	44-147 U/L
GGTP	22	< 38 U/L
Aspartate aminotransferase	13	< 31 U/L

WBC: White blood cell; GGTP: Gamma-glutamyl transferase.

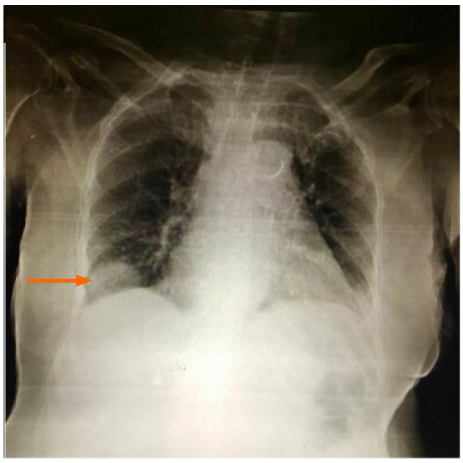

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Figure 1 Chest radiograph demonstrates a well-defined soft tissue mass noted just above the right hemi-diaphragm making an obtuse costophrenic angle suggesting pleural or extra-pleural mass.

liver closely mimicked a pleural mass leading to a diagnosis of suspected malignancy particularly in the setting of new onset of cough and shortness of breath. Our case was initially misdiagnosed as a pleural malignancy due to the rarity of the finding and confusing it with other causes of pulmonary origin. Investigation with chest CT scan ruled out pleural malignancy and revealed diaphragmatic defect with liver herniation. Pataka *et al*[13] presented a similar case of liver herniation which mimicked lung malignancy due to the gastrointestinal reflux associated with sustained cough.

The sensitivity of chest radiography to differentiate diaphragmatic liver herniation from the pulmonary mass is only 17% in right sided and 46% on left sided diaphragmatic defects[14]. Helical CT scan of the chest and abdomen is the radiological imaging of choice with a 73% sensitivity and a 90% specificity in the identification of diaphragmatic defects, herniated abdominal organs and differentiating them from pulmonary mass[15]. Small diaphragmatic defects may be difficult to locate on CT scan. In these cases, magnetic resonance imaging, diagnostic thoracoscopy or laparoscopy may assist in the identification of diaphragmatic defects and in the planning of surgical repair[8]. Surgical reduction of herniated abdominal contents and repair of the diaphragmatic defect is the treatment of choice. Laparoscopic and/or thoracoscopic repair is preferred over open laparotomy or thoracostomy because of less

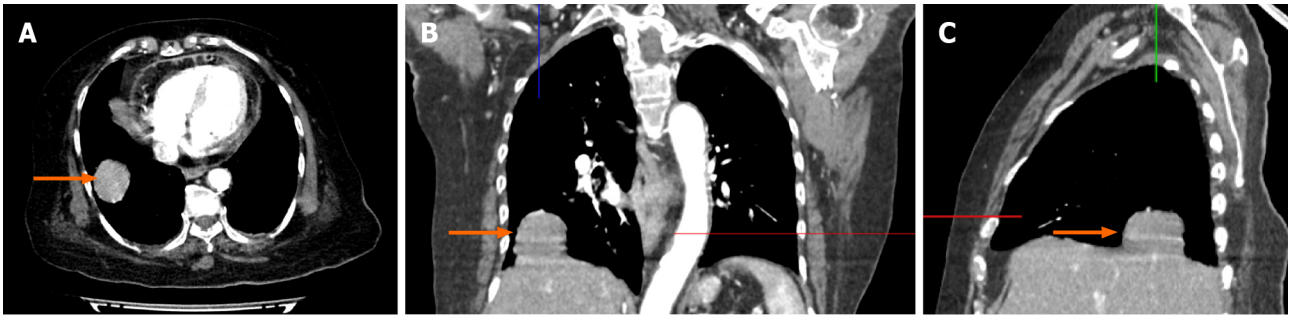

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Figure 2 Chest computed tomography images. A: Axial view showing herniated part of the liver through focal defect in the right hemi-diaphragm (arrow) mimicking a pleural/pulmonary mass; B: Coronal view shows extension of liver parenchyma into the thoracic cavity with hepatic artery within herniated liver (arrow); C: Sagittal view shows nubbin of liver parenchyma herniated through diaphragmatic defect posteriorly (arrow).

risk of morbidity and mortality with these minimally invasive modalities[8].

CONCLUSION

Spontaneous diaphragmatic herniation of the liver may mimic a pleural/pulmonary mass. A high index of clinical suspicion is required for early identification of non-traumatic diaphragmatic liver herniation particularly in individuals at risk of the increased transabdominal pressure gradient. ACE inhibitor associated cough is a known adverse reaction that rarely results in liver herniation. Early diagnosis with cross-sectional radiological imaging, surgical repair and addressing triggering factors improves patient outcome.

FOOTNOTES

Author contributions: Sethar S identified the abnormality and diagnosed the patient; Tebha SS and Zaidi ZA reviewed the literature, found relevant information, and wrote the manuscript; Virk MAA and Yousaf MN proofread, revisions and edits of the manuscript, and overall supervision in finalizing of the manuscript.

Informed consent statement: Informed consent was acquired from the patient before writing and publishing this case report and all accompanying images.

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CASE REPORT

Not all liver tumors are alike — an accidentally discovered primary hepatic leiomyosarcoma: A case report

Isabel Garrido, Patrícia Andrade, João Pacheco, Elisabete Rios, Guilherme Macedo

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Abstract

BACKGROUND

Primary hepatic leiomyosarcoma is a very rare entity that originates from smooth muscle. Preoperative diagnosis requires a high degree of suspicion due to atypical clinical presentation and non-specific imaging features.

CASE SUMMARY

We report the case of a 42-year-old man, with no relevant past medical history, accidentally diagnosed with a nodular liver lesion on a routine abdominal ultrasound. Liver function tests and hematology parameters as well as tumor markers were normal. A contrast-enhanced abdominal computed tomography scan revealed a heterogenous hepatic lesion measuring 40 mm 30 mm, adjacent to the left branch of the portal vein and the round ligament. Due to the unclear diagnosis, the patient underwent surgical resection. Histopathological and immunohistochemical examinations confirmed complete (R0) resection of a hepatic leiomyosarcoma. The patient remains without any signs of tumor recurrence for more than 2 years.

CONCLUSION

We report a rare case of accidentally diagnosed primary hepatic leiomyosarcoma originating from the portal vein or the round ligament. Although this tumor has aggressive metastatic potential, a tumor-free resection margin is essential to improve survival.

Key Words: Primary hepatic leiomyosarcoma; Portal vein; Round ligament; Surgical

resection; Case report

Core Tip: Sarcomas comprise only 1%-2% of all primary liver malignancies, and leiomyosarcoma is even rarer. We report a rare case of primary hepatic leiomyosarcoma accidentally diagnosed and surgically treated. The diagnosis requires a high level of suspicion because the clinical scenario and cross-imaging are not specific. Thus, histological examination is the only way to reach the diagnosis. In our case, the tumor probably originated from the left branch of the portal vein or the round ligament. Radical hepatectomy is the cornerstone of treatment. However, this tumor has aggressive metastatic potential and is usually diagnosed in locally advanced or metastatic disease. Two years after surgical resection, our patient is alive and with no evidence of tumor recurrence, likely because the diagnosis was established at an early stage and the surgery achieved a tumor-free margin.

Citation: Garrido I, Andrade P, Pacheco J, Rios E, Macedo G. Not all liver tumors are alike — an accidentally discovered primary hepatic leiomyosarcoma: A case report. *World J Hepatol* 2022; 14(4): 860-865
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INTRODUCTION

Sarcomas comprise only 1%-2% of all primary liver malignancies, and leiomyosarcoma is even rarer[1]. In the vast majority of cases of primary hepatic leiomyosarcoma, the tumor originates in the inferior vena cava. Clinical presentation and imaging features are non-specific and can mimic the most frequent primary liver tumors, namely hepatocellular carcinoma and intrahepatic cholangiocarcinoma. This tumor has aggressive metastatic potential and is usually diagnosed in a locally advanced or metastatic disease[2]. We report a rare case of primary hepatic leiomyosarcoma originating from the portal vein or the round ligament, accidentally diagnosed and surgically treated.

CASE PRESENTATION

Chief complaints

The patient was asymptomatic.

History of present illness

A 42-year-old man was referred for gastroenterology consultation due to a nodular hepatic lesion identified in a routine abdominal ultrasound.

History of past illness

Healthy, with no specific diseases.

Personal and family history

There was no family history of liver disease.

Physical examination

There were no relevant changes on physical examination.

Laboratory examinations

Liver tests were normal. The metabolic, virological and autoimmune study was negative. Alpha-fetoprotein and cancer antigen 19-9 were also normal.

Imaging examinations

A contrast-enhanced abdominal computed tomography scan was performed, revealing a nodular hepatic lesion measuring 40 mm × 30 mm, adjacent to the left branch of the portal vein, in the transition from the right to the left lobe (Figure 1). The tumor was contrast-enhancing, with heterogeneous areas inside.

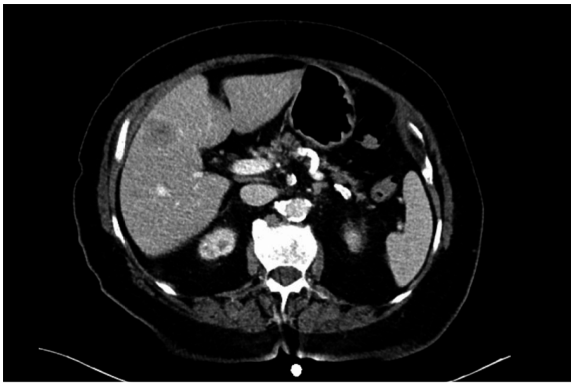

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Figure 1 Preoperative contrast-enhanced computed tomography scan demonstrated a heterogeneous mass in the liver adjacent to the left branch of the portal vein.

MULTIDISCIPLINARY EXPERT CONSULTATION

The imaging characteristics did not allow for a categorical distinction between hydatid cyst or other etiology. Therefore, it was decided at the multidisciplinary team meeting to perform surgical resection.

TREATMENT

In the explored laparoscopy, an exophytic lesion in segment IV was identified, involving the left branch of the portal vein and the round ligament; non-anatomic resection was performed without complications.

FINAL DIAGNOSIS

Histological examination showed complete resection of malignant mesenchymal neoplasia consisting of spindle cells with nuclei elongated to ovoid, with atypia and marked pleomorphism (Figure 2). In the immunohistochemical study, diffuse expression of actin and desmin was observed in neoplastic cells, in the absence of S100 expression. A chest-abdomen-pelvis computed tomography scan was performed in the postoperative period, with no evidence of residual or metastatic lesions. Moreover, this examination made it clear that it was a primary lesion. Thus, primary hepatic leiomyosarcoma was diagnosed with origin in the portal vein wall or the round ligament.

OUTCOME AND FOLLOW-UP

The patient remained under clinical surveillance. Two years after surgery, he is asymptomatic, with no evidence of tumor recurrence.

DISCUSSION

Primary hepatic leiomyosarcoma is a rare type of liver sarcoma; so far, less than 100 cases have been reported in the literature. Its diagnosis requires a high level of suspicion because the clinical scenario and cross-imaging are not specific[3]. In fact, patients are often asymptomatic or their symptoms are non-specific. Furthermore, alpha-fetoprotein and other serological markers are usually normal. Thus, histological examination is the only way to achieve the diagnosis, as in our case.

Primary hepatic leiomyosarcoma can arise from intrahepatic vascular structures, bile ducts or the round ligament. In our case, the tumor probably originated from the left branch of the portal vein or the round ligament. The first description of leiomyosarcoma by Perl in 1871 involved a tumor of the inferior vena cava[4]. Indeed, leiomyosarcoma of vascular origin often occurs in the inferior vena cava. On the other hand, those arising from portal vein are extremely rare and only 6 cases have been reported in the literature (Table 1)[5-10].

Table 1 Reported cases of leiomyosarcoma of the portal vein

Ref.	Wilson *et al* [5]	Sundaresan *et al*[6]	Boudjema *et al*[7]	Gohrbandt *et al*[8]	Gaignard *et al*[9]	Esposito *et al*[10]
Patient characteristics	Female, 28 yr	Female, 67 yr	Female, 44 yr	Female, 71 yr	Male, 53 yr	Male, 78 yr
Treatment	Operated - not resected	Left hepatectomy (R2)	Whipple with portal and biliary confluence reconstruction (R0)	Right hepatectomy with portal and biliary reconstruction (R0)	Right hepatectomy with portal and biliary reconstruction (R0)	Left hepatectomy (R0)
Outcome	Not available	Not available	Recurrence 27 mo after surgery. Died, 47 mo after surgery	Recurrence 36 mo after surgery. Alive	No recurrence. Alive	No recurrence. Alive

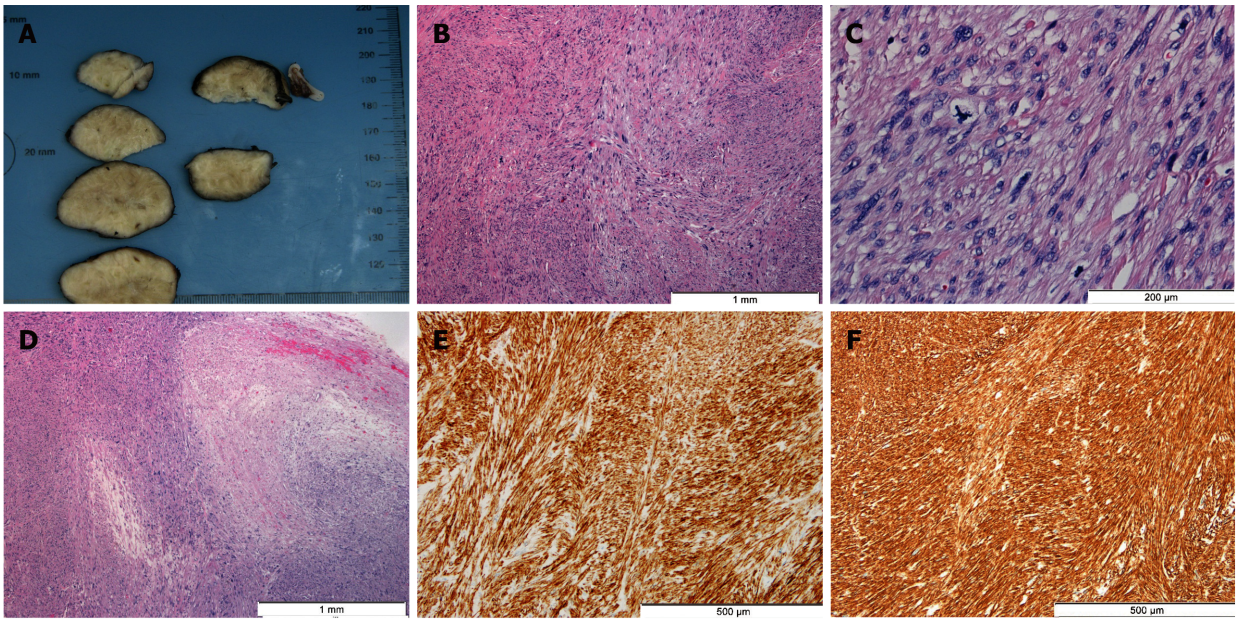

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Figure 2 Hepatic leiomyosarcoma. A: A well-circumscribed, white, solid mass of 4.3 cm exhibiting whorled features was seen on gross examination. B: Histologically, intersecting fascicles of spindle cells with elongate to ovoid nuclei displaying marked pleomorphism were observed [hematoxylin & eosin (HE), 40 ×]. C: Mitotic figures, including atypical ones, were frequent (7 mitosis/10 high power field) (HE, 200 ×). D: Foci of coagulative necrosis were observed (HE, 40 ×). E and F: Tumor cells were diffusely positive for desmin (100 ×) (E) and smooth muscle actin (100 ×) (F), in the absence of S100, c-Kit and discovered on GIST-1 (not shown).

No risk factors have been identified yet, although the increasing incidence of the tumor has been noted among immunosuppressed patients[11]. Nevertheless, more studies are needed to investigate the underlying pathogenetic mechanisms of this uncommon entity.

Due to the rarity of primary hepatic sarcomas in general and primary hepatic leiomyosarcoma in particular, the standard of care has not been defined. Radical R0 hepatectomy, in the form of wedge resection, segmentectomy, lobectomy or extended hepatectomy, is the cornerstone of successful management of primary hepatic leiomyosarcoma[12]. In our case, as the tumor was located in the liver, we were able to perform a surgical resection, obtaining a tumor-free margin. However, this tumor has aggressive metastatic potential and is usually diagnosed in locally advanced or metastatic disease. The role of adjuvant or neoadjuvant chemotherapy and radiotherapy regimens in these circumstances has not yet been established. Similarly, orthotopic liver transplantation remains controversial.

Little is known about the natural history of the disease. However, survival rates are relatively low, as the tumor is usually diagnosed in advanced stages. Chi *et al*[1] reported a median overall survival of 19 mo (range 0-181 mo) with 1-, 2- and 5-year survival rates of 61.2%, 41.1% and 14.5%, respectively. The smaller size of the lesion and tumor-free resection margin were identified as independent predictors of improved survival. Two years after surgical resection, our patient is alive and with no evidence of tumor recurrence, probably because the diagnosis was established at an early stage and the surgery achieved a tumor-free margin.

CONCLUSION

Primary hepatic leiomyosarcoma is a rare malignant disease with a poor prognosis. The authors report a rare case of resected primary hepatic leiomyosarcoma originating from the portal vein or the round ligament. Although there is no standard treatment due to the rarity of this disease, we showed that liver resection with a tumor-free resection margin could be an effective treatment in the early stages. Gastroenterologists should be alert to this unusual entity as early diagnosis requires a high degree of suspicion due to atypical clinical presentation and non-specific imaging features. We emphasize the need for a global database for these rare tumors to promote a better understanding and standardized treatment of these patients.

FOOTNOTES

Author contributions: Garrido I drafted the manuscript; Garrido I, Andrade P, Pacheco J, Rios E and Macedo G have revised and finalized the manuscript; All authors have approved the final version of the manuscript.

Informed consent statement: Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

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