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OPINION REVIEW

Sexual dysfunctions and their treatment in liver diseases

Rakesh Kumar Jagdish

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Abstract

Sexual dysfunction (SD) is a prevalent but very commonly ignored aspect in the treatment of liver diseases and cirrhosis. The etiology of SD is multifactorial and therefore treatment strategies are complex, especially in females. Phosphodiesterase inhibitors are useful and effective in erectile dysfunction in males but in females, no single drug is available for SD, therefore multimodal treatment is required depending upon the cause. The foremost and fundamental requirement in both genders is to be stress-free and have adequate control of liver diseases. Improved quality of life is helpful in improving SD and vice versa is also true. Therefore, patients suffering from liver diseases should come forward and ask for treatment for SD, and physicians should actively enquire about SD while history taking and evaluating these patients. SD results in deterioration of quality of life, and both are modifiable and treatable aspects of liver diseases, which are never addressed actively, due to social taboos and fears of SD treatment in the presence of liver diseases. The diagnosis of SD does not require costly investigations, as the diagnosis can be established based on validated questionnaires available for both genders, therefore detailed targeted history taking using questionnaires is essential. Data are emerging in this area but is still at an early stage. More studies should be dedicated to SD in liver diseases.

Key Words: Sexual dysfunction; Erectile dysfunction; Female sexual function index; International index of erectile function; Phosphodiesterase inhibitors; Hepatic venous pressure gradient

Core Tip: Liver diseases and cirrhosis related sexual dysfunction (SD) is present in a significantly high proportion of both genders but is often underestimated, ignored, or overlooked. Due to its multifactorial causations, detailed history taking, examination, and addressing potential causes are required for the diagnosis and management of SD. More randomized controlled trials should be planned for both genders regarding the newer treatment options.

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INTRODUCTION

Sexual dysfunction (SD) in liver diseases has a multifactorial origin, and this is often the most ignored aspect in both male and female patients. This review attempts to break the myths and social taboos on the sexual aspect, and the quality of life in patients with liver diseases, with simplified diagnostic criteria, common causes, treatment, and other management options. The aim is to provide support for the sexual life of liver disease patients as SD can have a negative effect on the quality of life and can cause psychological issues such as less emotional satisfaction and general unhappiness and depression [1]. It should be noted that, infertility issues related to cirrhosis are separate to SD, therefore are not discussed in this review.

SEXUAL DYSFUNCTIONS

In men, SD is categorized into disorders of desire (low libido), arousal (erectile dysfunction), or orgasm (premature or delayed ejaculation, or anorgasmia), according to their occurrence in the cycle of sexual response[2]. The most common presentations are premature ejaculation, which is arbitrarily defined as ejaculation within one minute (60 s) of vaginal penetration, or erectile dysfunction (ED), which is the inability to obtain and maintain a penile erection in order to achieve satisfactory sexual activity. ED is considered to be common in cirrhosis; predominantly due to endocrine dysfunction and sarcopenia. There is a paucity of data on the precise prevalence of and predictive factors for ED in cirrhosis. Phosphodiesterase type 5 (PDE5) inhibitors were found to be useful for ED in the non-cirrhotic and cirrhotic population in a recent randomized controlled trial (RCT) of tadalafil in cirrhotics[1].

In females, SD is more complex and predominantly manifests as low libido, vaginal dryness, dyspareunia, orgasm inability, or menstrual abnormalities[3].

INCIDENCE AND PREVALENCE OF SD

Patients with cirrhosis have a high prevalence of ED ranging from 25% to 92% in various studies[4-8] as shown in Table 1. In a recent RCT[1], it was found to be 70.3%, whereas in females, studies on prevalence are limited, but it has been shown that the prevalence of abnormal menstrual cycles was seen in 58%, decreased sexual interest in 42%, and no sexual activity in 56% of females[3,9]. In a study of women with non-alcoholic liver disease, reduced sexual desire was found in 33%, reduced arousal in 18%, not feeling orgasm in 25%, and dyspareunia in 21%, related to decreased vaginal lubrication. Prospective studies are needed especially in female patients.

RISK FACTORS CONTRIBUTING TO ED IN LIVER DISEASES IN BOTH GENDERS

The causation of SD in both genders is multifactorial, although there are only a few studies in females as compared to males. Female sexual function involves hormonal, neurological, vascular, psychological, and emotional aspects. Dysfunction may be triggered or maintained by any of these, or by the interplay between them. The hormonal interplay is grossly disturbed in chronic liver diseases (CLD) along with multiple other non-hormonal factors contributing to SD in CLD. Possible contributing factors to SD in both genders[10,12] in liver diseases are shown in Figure 1.

Table 1 Erectile dysfunction prevalence studies

Disease etiology and age	Prevalence	Study	Ref.	Journal, year	Method used
Cirrhosis of liver	70.3%. ED increases with increase in CTP score	Tadalafil improves erectile dysfunction and quality of life in men with cirrhosis: A randomized placebo controlled trial	Jagdish *et al*[1], India	*Hepatology International*, 2021	IIEF-5
Chronic viral liver diseases	60%, age < 50 years, 88% age > 50 years. ED	Erectile dysfunction in patients with chronic viral liver disease: Its relevance to protein malnutrition	Toda *et al* [4], Japan	*J Gastroenterology*, 2005	IIEF-5 and medical outcomes study short form 36 (SF-36)
Hepatitis B-aged 40-59 yr (SD 50.2 ± 5.7)	Total 24.6%, (CHB-8.6%, HBV-LC-41.2%)	Erectile dysfunction in patients with liver disease related to chronic hepatitis B	Kim *et al* [5], Korea	*Clinical and Molecular Hepatology*, 2015	Erectile function of patients was evaluated by the Korean version of IIEF-5
Hepatitis C, age - 20-80 yr (SD; 50 ± 17.19) yr	30%	Erectile dysfunction in patients with chronic hepatitis C virus infection	Hunter *et al*[6], Egypt	*Arab J Gastroenterol*, 2014	An Arabic validated version of the five-item IIEF-5
Alcoholic liver diseases (age < 56 yr)	61%	Sexual dysfunction in men with alcoholic liver cirrhosis. A comparative study	Jensen *et al*[7], UK	*Liver*, 1985	All groups had a significantly (*P* less than 0.025) raised prevalence of sexual dysfunction when compared to men without chronic disease
Chronic liver disease (mean age 54.8 ± 10.8 yr)	50.6%	Assessment of sexual function in patients with chronic liver disease	Simsek *et al*[8], Turkey	*Int J Impot Res*, 2005	International index of erectile function

CHB: Chronic hepatitis B; ED: erectile dysfunction; HBV: Hepatitis B virus; IIEF-5: International index of erectile function.

Non hormonal causes

Reduced quality of life due to depression (which can affect all phases of sexual function), anxiety, and stress are important factors. ED itself is associated with poor health-related quality of life[11,13] and depressive symptoms in both cirrhotic and non-cirrhotic patients.

Low serum albumin is a significant factor in ED and a low international index of erectile function (IIEF) score in liver diseases has been reported in multiple studies[4]. Hypoalbuminaemia results in water retention and loss of muscle volume which decreases sexual desire, and physical function. The ratio of albumin-bound to free testosterone may be influenced by decreased production of albumin which might then influence sexual desire and sleep-related erection. A study by Paternostro *et al*[14] showed that liver dysfunction, diabetes mellitus, hypertension, and high hepatic venous pressure gradient (HVPG), are key risk factors for ED in male cirrhotic patients.

Sarcopenia, as measured by the appendicular skeletal muscle index, was found to be associated with ED in a recent study[1,12]. This appears to be clinically significant as patients with low muscle mass are more likely to have low albumin, low power, frailty, and poor sexual performance. The concept of sexual frailty is similar to frailty in other organ systems. Musculoskeletal health needs to be highlighted in the evaluation of cirrhosis along with sexual health. More randomized trials should be conducted to understand the pathogenesis and underlying mechanisms and possible treatment options in this area.

Clinical features of hypogonadism, such as ED, infertility, decreased libido, and testicular atrophy, are often seen in patients with advanced liver disease, and it has been shown that the severity of liver cirrhosis correlates with the degree of ED[4].

Effects of drugs: Propranolol has a negative impact on erectile function, which is widely used to treat portal hypertension[15,16], but the doses which are required to cause ED are higher than the doses commonly used in CLD. Although Paternostro *et al*[14] found that beta-blockers were not associated with ED, beta-blockers generally cause vasodilation, and inhibit the release of renin, angiotensin II, and aldosterone production. These effects result in decreased adrenergic outflow from the sympathetic nervous system, resulting in SD[17]. However, a large meta-analysis[18] did not support the notion that beta-blocker (BB) therapy is associated with a relevant risk of SD, which was also supported by a recent RCT[1]. Spironolactone, an anti-mineralocorticoid and aldosterone antagonist, has anti-androgenic properties and can cause decreased libido and SD[16,17].

Hormonal causes (sex hormones)

Studies have shown that free and albumin-bound testosterone, rather than total testosterone concentration, correlated positively with sexual desire and sleep-related erection in healthy subjects[19]. Therefore, it is possible that the reduced production of albumin may affect the ratio of free testosterone

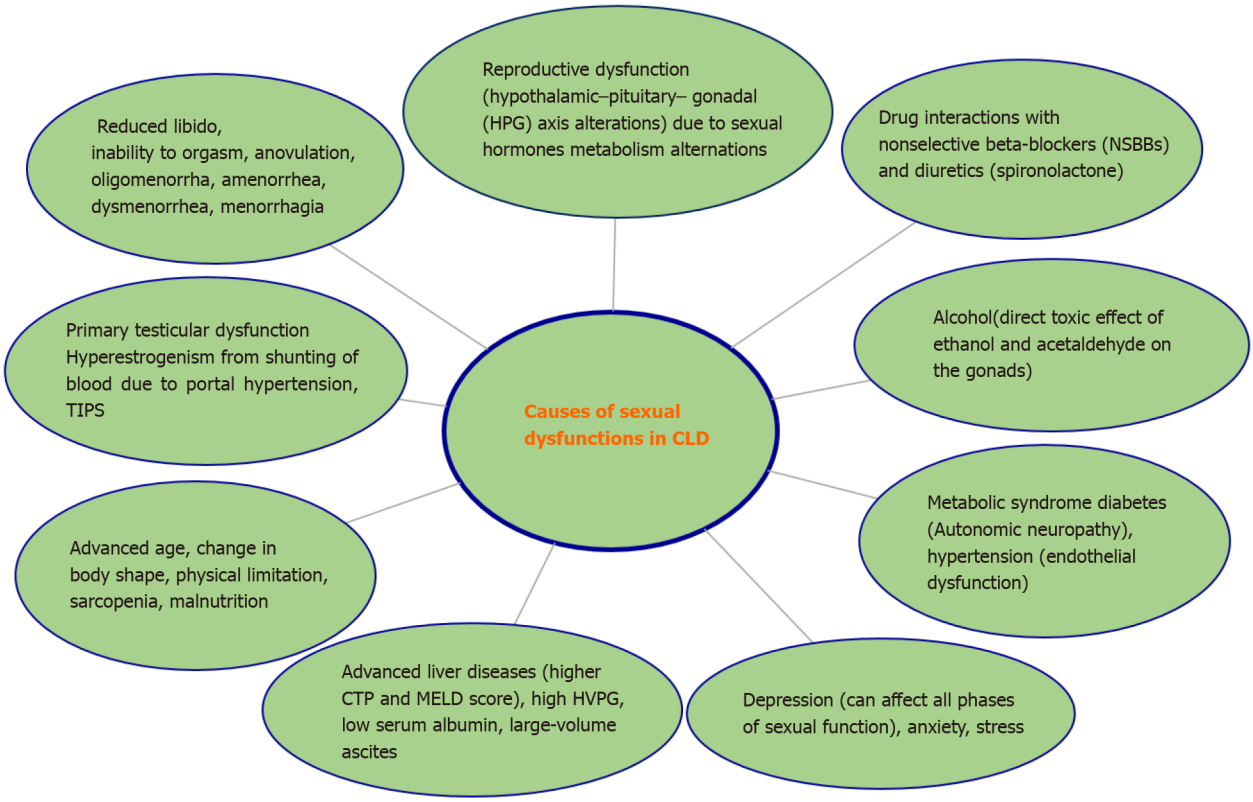

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Figure 1 Causes of sexual dysfunction in chronic liver diseases. CLD: Chronic liver diseases; HVPG: Hepatic venous pressure gradient.

to albumin-bound testosterone, as well as the total amount of testosterone, possibly modifying cell or tissue response to this sex hormone in cirrhotic patients. Sex hormone-binding globulin (SHBG) levels [20,21] are elevated in patients with cirrhosis due to increased hepatic production, but the pathogenesis of this remains unclear. Rising levels of SHBG have been shown to correlate with the severity of fibrosis in patients with chronic liver disease[22]. Due to the binding of testosterone to SHBG, the total serum testosterone value can remain normal or occasionally be raised in this patient group, despite reduced levels of free (presumed biologically active) testosterone[23].

ASSESSMENT OF SD

There are various well-validated questionnaires for SD and its risk factor assessment. The female sexual function index (FSFI) and the IIEF in males are the two most validated objective tools for SD severity assessment, with various domains and questions pertaining to each domain, which are then scored into numerical values[10] (Figure 2).

Females

The FSFI: A total of six domains are assessed, with a total set of 19 questions. All domains have the highest score of 6, and the total maximum score is 36, where a score of 0 represents no sexual activity during the preceding month. An overall score below 26.55 is indicative of female SD[10,24]. Female sexual dysfunction (FSD) is classified into three main clinical types. For each diagnosis, the disorder is experienced at least 75% of the time for at least six months (except for medication-induced FSD), resulting in significant distress. The three types, some or all of which may be present, are (1) Sexual interest/arousal disorder, which is defined as reduced or absent sexual interest, responsiveness, erotic thoughts and sexual pleasure; (2) Female orgasmic disorder (absence, infrequency, reduction, delay of orgasm); and (3) Genito-pelvic pain/penetration disorder (difficulty in vaginal penetration, marked vulvovaginal or pelvic pain during penetration, fear or anxiety about pain in anticipation of, during, or after penetration, and tightening or tensing of pelvic floor muscles during attempted penetration).

Males

The IIEF[25]: A 15-item questionnaire that assesses five domains of male sexual function using 5- to 6-point Likert scales, with 0 or 1 signifying a low frequency or ability and 5 signifying a high frequency or

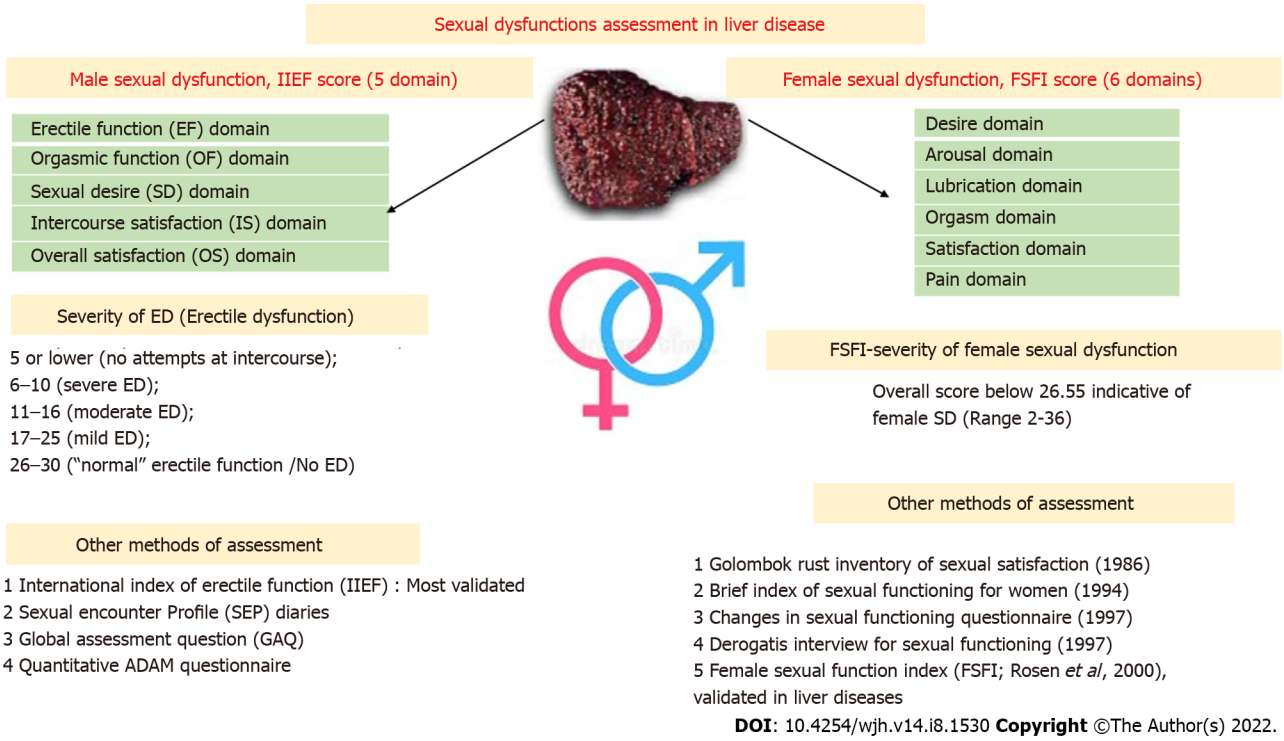

Figure 2 Assessment of sexual dysfunction in liver disease.

ability. These domains include erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction. The erectile function domain possible scores range from 1 to 30. The severity of ED is defined using the IIEF-EF domain's score: 5 or lower, (no attempts at intercourse at all); 6 to 10, severe ED; 11 to 16, moderate ED; 17 to 21, mild to moderate ED; 22 to 25, mild ED; and 26 to 30, is normal erectile function[1]. The IIEF has also been validated in the Indian population[26].

Other questionnaires: The quantitative ADAM (qADAM) questionnaire[27] is a new tool in quantifying the severity of hypogonadism, sexual encounter profile (SEP), and Global Assessment Question for assessing sexual performance[1]. The total qADAM score ranges between 10 and 50, with 10 being the most symptomatic and 50 being the least symptomatic.

The SEP diaries record yes/no responses after sexual encounters. There are five questions, but two questions are most frequently used to assess SD. SEP2 is "Were you able to insert your penis into your partner's vagina?" and SEP3 is "Did your erection last long enough for you to have successful intercourse?"

QUALITY OF LIFE IN PATIENTS WITH ED AND CIRRHOSIS

Approximately 92% of patients with cirrhosis and SD showed a significant impact on their quality of life as assessed by the SF-36 score[28]. Other scores related to the quality of life are the Generalized Anxiety Disorder 7 (GAD-7) questionnaire[29], where a GAD-7 score of ≥ 10 indicates a probable diagnosis of GAD, and the Patient Health Questionnaire (PHQ)[30] where a PHQ-9 score ≥ 10 is an indication of major depression. Both anxiety and depression are implicated in the causation of ED and SD. In a recent study, we have shown that treatment of ED with tadalafil results in a significant decline in the GAD-7 and PHQ-9 scores, with a significant improvement in scores of five of the eight domains of SF-36 when compared with placebo[1].

MANAGEMENT, CLINICAL APPROACH AND TREATMENT

Adequate management of the primary liver disease is of paramount importance, as most of the treatment targeting SD may not be feasible or even contraindicated in Child C cirrhosis[31]. Therefore, adequate control of liver diseases and co-morbidities is warranted. The clinical approach and treatment of SD in liver diseases are summarized in Figures 3 and 4.

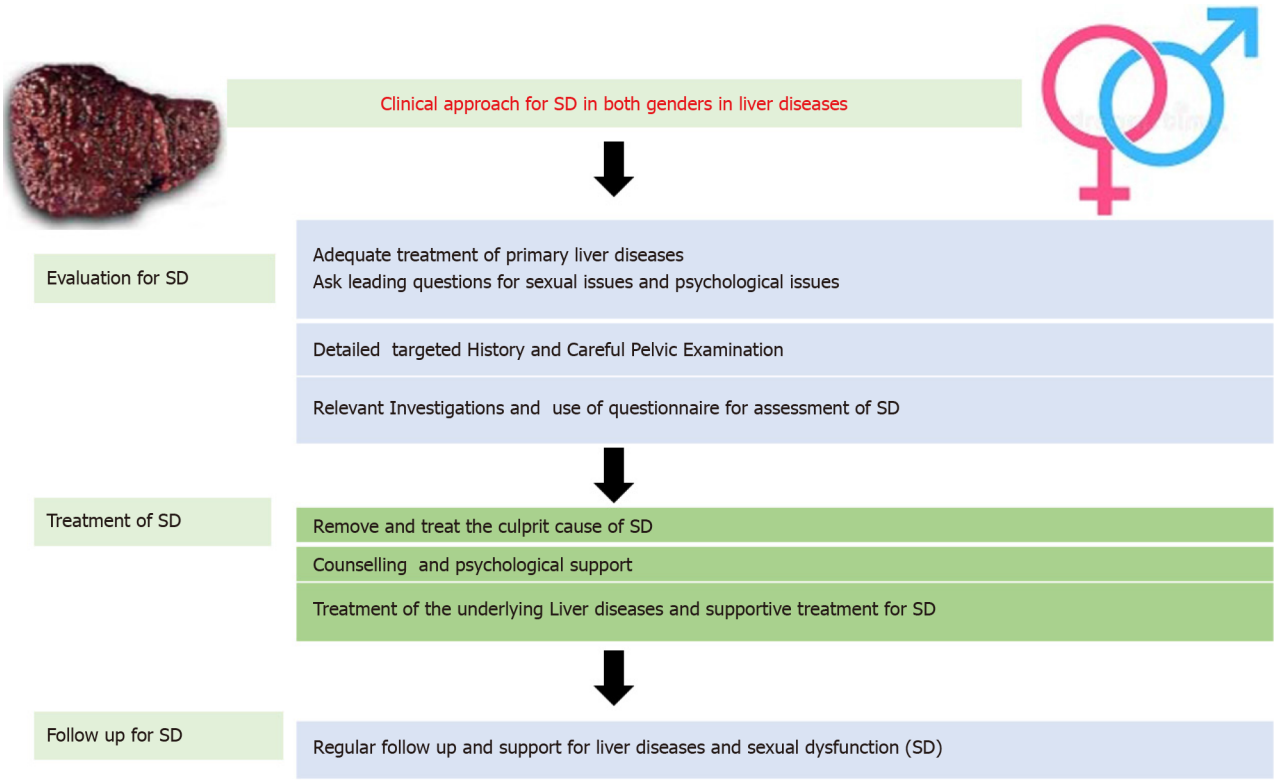

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Figure 3 Clinical approach to sexual dysfunction in both genders with liver diseases.

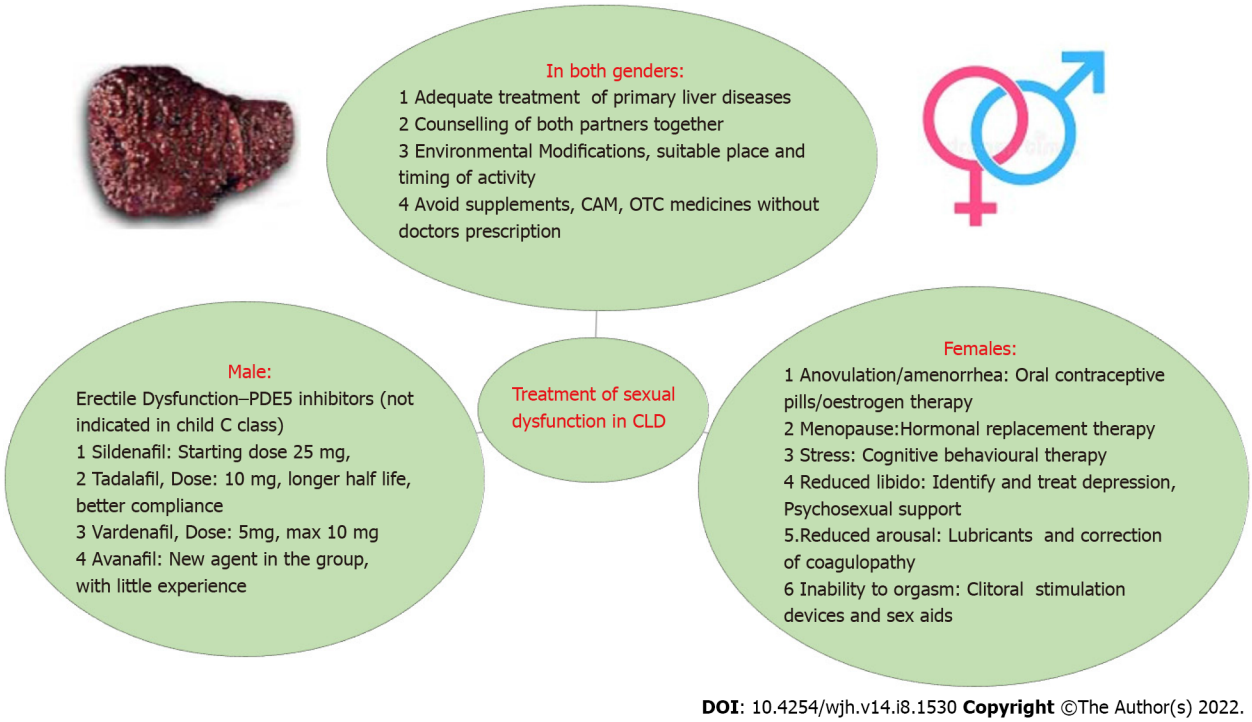

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Figure 4 Treatment of sexual dysfunction in chronic liver diseases. CLD: Chronic liver diseases; CAM: Crassulacean acid metabolism; OTC: Over the counter.

Both male and female patients

Counselling for partners: Counselling for both partners to understand the facts and myths, particularly regarding liver diseases and sexual activity can be advised.

Environmental modifications: Partners may accordingly be advised to change to a suitable environment, to change positions, or poses, as per the stage of the disease. Pelvic floor exercises and general exercises may be recommended to improve frailty, including sexual frailty.

Avoid stamina supplements: Avoid complementary alternative medications, herbs, and over the counter drugs available in many countries to boost stamina. Most of these drugs have not been studied and are strictly discouraged for use as they can be harmful in the presence of liver diseases.

Female patients

Female SD is subjective dissatisfaction, the management of which can vary among patients. A single drug is not the answer to female SD in contrast to her male counterpart's ED.

Management of female SD in cirrhotics is complex and depends on identifying and treating the predominant underlying causes, but often requires multimodality involvement for optimal results. Evaluation of the underlying causes includes a detailed history, hormonal analysis, pelvic ultrasound, gynaecological examination, and psychological evaluation, along with the status and stage of liver diseases. A new-onset SD can be a sign of advanced liver disease, coagulopathy, or other metabolic or cardiovascular disease.

Various treatment strategies[32,33] are shown in Figure 3. Modifications in lifestyle are recommended, such as possible pelvic floor exercises depending on cirrhotic stage, and cessation of smoking and alcohol consumption. If it is causing poor performance, even in a passive role in sexual activity, blood transfusion or intravenous iron therapy can be used to correct anaemia. Coagulation correction and management of bleeding should be kept in mind. Forced acts may risk bleeding. Arousal enhancement strategies include relationship couples counselling, sex education using videos and educational literature, sexual positions yoga, romantic songs, romantic dressing, making the environment suitable for sexual pleasure, and increasing foreplay time. Cognitive behavioural therapy is a type of psychotherapy that helps to remove sexual inhibitions and helps in enhancing interpersonal relationships and sexual involvement. Medical devices such as clitoral therapy devices for sexual arousal and orgasmic dysfunctions can be helpful in some cases. Oestrogen is effective in the treatment of dyspareunia related to menopause. Strict contraception should be used to avoid pregnancy, as pregnancy should be planned after discussion with the treating doctor for the best outcome. Transdermal testosterone was tried in postmenopausal women with hypoactive sexual desire disorder with variable results[34]. To date, there are no FDA-approved treatments available for FSD; thus, prospective studies and RCTs should be conducted in these patients[35].

SD in women after liver transplantation

Studies of liver transplant recipients have shown that 72% of females became sexually active after LT. 95% of females younger than 46 years had a regular menstrual cycle by the end of the first year of liver transplantation, but irregular bleeding and amenorrhea were present in 26% and 26%, respectively[36, 37]. An interval of at least 1-2 years after successful LT is recommended before considering pregnancy. Mycophenolate mofetil should be stopped before planning a pregnancy due to its teratogenicity.

Male patients

The basic mechanism of sexual arousal and further physiological changes involves nitric oxide (NO) and related mechanisms[38]. Phosphodiesterase (PDE) inhibitors competitively bind to PDE5 and inhibit c-GMP hydrolysis, thus enhancing the effects of NO. This increase in c-GMP in smooth muscle cells results in a prolonged erection. In view of the lack of direct effect of PDE5 inhibitors on corpus cavernosum and smooth-muscle relaxation, adequate sexual stimulation is necessary for an erection to occur. Therefore, foreplay and stress relief are required. The approved agents for the treatment of ED are sildenafil, tadalafil, vardenafil, and avanafil, but tadalafil[39] appears to be the best option due to its long half-life and flexibility in the timing of administration.

A recent RCT[1] demonstrated that therapy with tadalafil (10 mg/d) for 12-wk significantly improved erectile function, anxiety, depression, and quality of life, and was well tolerated by men with cirrhosis (CTP score 10) and ED, with no significant side effects. It also improves depression and quality of life related to ED[12,40,41].

Other treatment strategies for ED in CLD

Intramuscular testosterone supplementation[42]: Testosterone has been shown to be beneficial in increasing muscle mass and improving sarcopenia, which can indirectly benefit SD as sarcopenia has been shown to be associated with ED. This may help with sexual frailty. However, direct data on sexual function are not available.

Role of albumin: No direct evidence is available on the role of albumin, but it seems to be logical as it adequately controls the primary liver disease, which may help in sexual frailty. Our hypothesis suggests that reduced serum albumin may affect the ratio of free albumin to bound testosterone with a possible altered testosterone response. Therefore, improving albumin concentration might be helpful in ED and

Table 2 Studies of hepatic venous pressure gradient changes with phosphodiesterase-5 inhibitors

HVPG change	Intervention	Ref.
No significant change in HVPG	Tadalafil 10 mg	Jagdish *et al*[1], 2021, *Hept International*
N = 10, in seven patients, HVPG decreased and in three it increased but the decrease was not significant	Sildenafil 50 mg	Clemmesen *et al*[43], 2008, *World J Gastroenterology*
Lowers portal pressure in the acute setting by about 20%	75-100 mg of the phosphodiesterase-5-inhibitor udenafil	Kreisel *et al*[44], 2015, *Dig Liver Dis*
Decreased HVPG in 4/5 patients	Vardenafil 10 mg	Deibert *et al*[45], 2006, *Aliment Pharmacol Ther*
No effect on HVPG	Sildenafil	Tandon *et al*[46], 2010, *Clin Gastroenterol Hepatol*
HVPG decrease from 10 mmHg to 7 mmHg (case report)	Sildenafil 20 mg bd	Bremer *et al*[47], 2007, *J Med Case Rep*
HVPG decreased by 13%, and portal flow increased by 28% (case report)	Vardenafil	Deibert *et al*[48], 2018, *World J Gastroenterology*

HVPG: Hepatic venous pressure gradient.

testosterone is more likely to be beneficial in patients with high albumin levels. Therefore, regular albumin therapy and high protein intake may be indirectly helpful in improving SD. This hypothesis needs to be confirmed in prospective trials.

Liver transplantation and sexual function

Improvement in SD has not been consistently reported after liver transplantation. This requires more randomized trials for a better understanding. There are a few contradictory studies; therefore, further investigation in both genders is needed. Improvement in erectile function is associated with the absence of hypogonadism before living donor liver transplant[3,10]. However, after transplantation, up to 25% of patients report persistent SD, and approximately one-third of patients describe the appearance of de novo SD. The use of PDE-5 inhibitors has been reported in post-renal transplant recipients with ED and no drug interactions with immunosuppressants or side effects have been observed.

Side effects of PDE inhibitors

PDE5 inhibitors are generally well tolerated in the treatment of ED[1]. The most common reported adverse drug reactions include headaches, back pain, myalgia, flushing, nasal congestion, sore throat, and dyspepsia. In general, the pain is of mild to moderate severity, typically occurring 12-24 h post-administration and usually resolving within 48 h, with or without medical treatment, such as acetaminophen.

Studies of HVPG changes with PDE-5 inhibitors

In one study[14], higher MELD scores and higher HVPG values were found in patients with ED. PDE-5 inhibitors have been administered to lower portal pressure in cirrhosis but the results are conflicting[1,43-48] (Table 2).

CONCLUSION

Sexual dysfunction in liver diseases is one of the most ignored health issues and more prospective studies on this issue are needed which is potentially treatable. In particular, PDE inhibitors, especially tadalafil, are helpful for ED, and in females, treatment is multifactorial. Adequate control of primary liver diseases and causative agents of SD is warranted. All patients with liver diseases and cirrhosis should be evaluated, as SD is modifiable and treatable, and results in improved quality of life. More prospective and randomized controlled trials are needed on this topic, to understand the global epidemiology of the disease burden, possible underlying mechanisms, particularly related to non-hormonal aspects and sarcopenia, and possible newer treatment options.

FOOTNOTES

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MINIREVIEWS

Long-term liver allograft fibrosis: A review with emphasis on idiopathic post-transplant hepatitis and chronic antibody mediated rejection

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Abstract

Liver transplantation (LT) is a life-saving surgical procedure and the current standard of care for most patients with end stage liver disease. With improvements in organ preservation techniques, perioperative care, and immunosuppression, there is better patient and graft survival following LT, and assessment of the liver allograft in long-term survivors is becoming increasingly important. Recurrent or *de novo* viral or autoimmune injury remains the most common causes of chronic hepatitis and fibrosis following liver transplantation in adults. However, no obvious cause can be identified in many adults with controlled recurrent disease and the majority of pediatric LT recipients, as they have been transplanted for non-recurrent liver diseases. Serial surveillance liver biopsies post LT have been evaluated in several adult and pediatric centers to identify long-term pathological changes. Pathological findings are frequently present in liver biopsies obtained after a year post LT. The significance of these findings is uncertain as many of these are seen in protocol liver biopsies from patients with clinically good allograft function and normal liver chemistry parameters. This narrative review summaries the factors predisposing to long-term liver allograft fibrosis, highlighting the putative role of idiopathic post-LT hepatitis and chronic antibody mediated rejection in its pathogenesis.

Key Words: Liver allograft fibrosis; Long term; Idiopathic hepatitis; Chronic antibody mediated rejection

Core Tip: Pathological findings are frequently present in liver biopsies obtained after a year post LT. The significance of these findings is uncertain as many of these are seen in protocol liver biopsies from patients with clinically good allograft function and normal liver chemistry parameters. This narrative review summaries the factors predisposing to long-term liver allograft fibrosis, highlighting the putative role of idiopathic post-LT hepatitis and chronic antibody mediated rejection in its pathogenesis.

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INTRODUCTION

Advances in organ preservation techniques, perioperative care, and immunosuppression have resulted in greatly improved long-term survival in patients undergoing liver transplantation (LT). A continued assessment of the liver allograft to ensure optimal graft function is becoming increasingly important[1]. Recurrent or *de novo* injury is one of the most common causes of chronic hepatitis and fibrosis following LT[2,3]. However, no obvious cause can be identified in many adult recipients who have their original disease under control. The same is the case in the majority of paediatric LT recipients who have been transplanted for non-recurrent liver disease[4]. Centres which perform serial post-LT surveillance allograft biopsies have noted histological abnormalities without any clinical or biochemical dysfunction. Whether such abnormalities progress to long-term graft loss remains unknown and requires careful study[5-7].

This narrative review summarises the factors predisposing to long-term liver allograft fibrosis (LAF), highlighting the putative role of idiopathic post-LT hepatitis (IPLTH) and chronic antibody mediated rejection (CAMR) in its pathogenesis.

LONG-TERM LIVER ALLOGRAFT FIBROSIS

Allograft fibrosis is defined as the excessive accumulation of extracellular matrix proteins (including collagen) within the transplanted liver. The central event is the activation of hepatic stellate cells and portal fibroblasts in response to chronic injury[8]. When unchecked, progressive LAF inevitably leads to graft failure and loss.

Moreover, the prevalence and severity of LAF are reported to increase over time. It has been shown that 10 years after LT, normal histology is likely to be present in only 30% of patients. Data from six European transplant centres show an increasing incidence of LAF over time (54% at 5 years, 79% at 10 years)[7]. Interestingly, this phenomenon has been observed to occur more commonly in the paediatric population. Late post-transplant liver biopsies performed in this cohort of patients reveal LAF in 69% to 97% of all cases. Scheenstra *et al*[9] reported that the prevalence of LAF increased from 34% to 48%, 65%, and 69% among children at 1, 3, 5, and 10 years after LT, respectively.

Furthermore, apart from the incidence, the etiology and mechanism of LAF appear to be distinct between the adult and paediatric LT recipients. In adults, the original indication for LT is clearly important. Recurrent hepatitis C virus (HCV) infection is a common diagnosis and an important cause of LAF[10]. Venturi *et al*[11] noted a correlation between portal fibrosis and prolonged ischemic time, deceased graft, and post-transplant lymphoproliferative disease. In their study, they also highlighted biliary complications as a related factor for sinusoidal fibrosis, while vascular complications, positive autoantibodies, and high gamma-globulin level were related to centrilobular fibrosis. Immunosuppression with steroid therapy was not associated with decreased fibrosis. In a study by Rhu *et al*[12], liver scarring was common in patients with no clinical signs of graft dysfunction. Repeated transaminitis, positive autoantibodies, elevated gamma-glutamyl transferase, and experience of post-transplant lymphoproliferative disease were suspicious signs for fibrosis. HLA-DRB1 × 03/04 allele in LT recipients has also been shown to be significantly associated with portal fibrosis without influencing inflammation[13]. Other viral diseases including hepatitis E have been reported to cause LAF[14-19]. Furthermore, immune related indications of LT (auto-immune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, *etc*.) have been known to recur in the allograft, causing remarkable histological abnormalities. *De novo* autoimmune hepatitis has also been implicated as a causative factor in adult LAF.

On the other hand, in the paediatric population, where the great majority of transplants are carried out for non-recurring disease, changes seen in late biopsies have no obvious attributable cause apart from a chronic immune-related damage (discussed below). Hence, while in adults a recurrence of

primary disease is the most common cause of late graft dysfunction, in children, unexplained idiopathic hepatitis and liver fibrosis are the main causes. Furthermore, compared to the adult population, wherein the histological abnormalities manifest as abnormal graft function, up to 90% of children who are otherwise clinically and biochemically normal will have some abnormality on protocol biopsy.

From a pathophysiological perspective, LAF is the result of sustained wound healing in response to repeated hepatocyte injury, leading to scar tissue formation and loss of hepatic architecture. Nonetheless, it is imperative to realise that the conventional concept of irreversible fibrosis has evolved and it is now considered to be a dynamic and reversible process. Hence, when the inciting injury stimulus is removed, LAF has shown to regress over time.

RISK FACTORS FOR LONG-TERM LIVER ALLOGRAFT FIBROSIS

Significant insights into the risk factors and natural history of LAF in clinically stable LT recipients have been obtained by correlating clinical and biochemical with histological findings on surveillance biopsy tissue obtained 5 and 10 years after paediatric LT[9]. LAF was strongly correlated with transplant-related factors such as prolonged cold ischemia time, young age at LT, high donor/recipient age ratio, and the use of a partial graft[9,10]. Venturi *et al*[11] noted a higher incidence of LAF in the presence of factors like prolonged ischemic time, deceased donor grafts, and post-transplant lymphoproliferative disease. The authors subdivided the risk factors based on the type of fibrosis. While biliary complications were more likely to result in sinusoidal fibrosis, vascular complications and high gamma-globulin levels were related to centrilobular fibrosis. Interestingly, episodes of rejection, chronic hepatitis, and the type of immunosuppression were not related to allograft scarring.

Other factors predicting a higher risk of LAF include the presence of autoantibodies with elevated immunoglobulin levels, repeated transaminitis, *de novo* hepatitis C infection, and hepatitis E (HEV) infection (genotype 3)[12,16]. The interplay of immunosuppressants and HEV is noteworthy. Post-LT HEV infection is usually acquired from the community. However, cases of HEV infection acquired from blood products or donor organs have also been reported[17,18]. Immunosuppression utilizing tacrolimus has been postulated as a risk factor for chronic liver disease, possibly by promoting viral replication[16]. Approximately 50%-80% of LT recipients infected with HEV develop chronic infection and 10-15% progress to diffuse scarring. Similarly, Torque Teno Virus (TTV), which is part of the normal human virome, may result in direct LAF without hepatitis[19]. HLA-DRB1 × 03/04 allele in LT recipients has also shown to be significantly associated with portal fibrosis without inflammation[12,13]. It is nonetheless sobering to realise that liver scarring is common in patients with no probable risk factors or clinical signs of graft dysfunction[12].

MECHANISMS OF LONG-TERM LIVER ALLOGRAFT FIBROSIS

It is noteworthy to consider that the paediatric immune system is quite distinctive from that of the adult population[20,21]. Depending upon the age at LT, their immune system is in various stages of development and maturation.

Multiple studies have proven that innate immunity plays a key role in the development of LAF[22]. At the cellular level, interferon (IFN)-λ stimulates LAF whereas both IFN-α/β and IFN-γ inhibit this event. Toll-like receptors (TLRs) participate in the development of fibrosis, while liver dendritic cells regulate inflammation and fibrosis in the liver microenvironment. Kupffer cells stimulate liver fibrosis whereas natural killer (NK) cells inhibit LAF by lysing activated hepatic stellate cells (HSCs) and inhibiting IFN-γ production. The imbalance between pro- and anti-fibrogenic agents is created by a common pathway which is incited by damaged hepatocytes. These cells in turn stimulate and activate HSCs by releasing damage-related reactive oxygen species and other fibrogenic substances. They also do it by recruitment of immune cells which promote cytokines and chemokines, causing further collagen deposition. This mutual stimulation between inflammation and profibrotic cells leads to a vicious circle of LAF. The two strongly associated precursor/ inciting events for 'idiopathic' LAF are IPLTH and CAMR which benefit from further elucidation, as below.

IDIOPATHIC POST-LIVER TRANSPLANT HEPATITIS

Identified in 5%-85% of adults and 32%-97% of children with normal liver biochemistry, IPLTH is an inclusive term for unexplained portal and/or lobular inflammatory lesions in the allograft[9,10,23-30]. These pathological features have been variedly labelled as nonspecific portal and/or lobular inflammation, unexplained hepatitis, interface hepatitis, portal lymphocytic inflammation, portal/parenchymal mononuclear inflammation, and allograft inflammation, leading to an underestimation of its true incidence[30-33]. Moreover, variations in centre-specific protocols of surveillance

liver biopsies make this conundrum even more byzantine[2]. Nevertheless, there are certain pathological features which are frequently observed in IPLTH. These include predominantly mononuclear (lymphocytes, histiocytes, and some plasma cells) portal inflammatory infiltrate associated with no significant bile duct damage or portal venulitis (Figure 1)[2,34]. Variable interface activity and/or centrilobular inflammation with spotty to confluent necrosis have also been reported. Further, periportal necroinflammatory activity is generally mild, and features of T-cell mediated rejection may occasionally be observed.

Although the term "idiopathic" implies an unexplained cause, there is increasing evidence to suggest that many cases of so-called IPLTH probably represent an immune phenomenon. The majority of patients have auto/allo-antibodies and other uncharacterized serum factors which react with donor hepatocytes and/or bile ducts. Over a fourth of patients with moderate to severe portal inflammation show positive antinuclear antibodies and/or anti-smooth muscle antibodies (titres 1:40-1:640)[5]. Their history is also significant for episodes of T-cell mediated rejection and histopathology shows features of acute or chronic rejection. Furthermore, patients on a long-term maintenance dose of corticosteroids have shown lesser degrees of inflammation and fibrosis, further suggesting that the whole process is immune mediated and may represent a hepatitic form of chronic rejection[10,34-39].

Recent genomic studies shed further light on this association. By using modular analysis, Londoño *et al*[5] explored the correlation between groups of co-expressed genes and semi-quantitative histological scores across liver samples. Of the 23 modules of genes identified, two were selected for further analyses on the basis of their significant correlation with portal inflammation and fibrosis. A significant correlation was noted between the modules and a 13-gene set specific for T-cell mediated rejection. The two modules were enriched in gene sets previously identified as being associated with allograft rejection across a variety of experimental and clinical settings. Significantly, the majority of their patients were on a very low dose of immunosuppressants, indicating insufficient immunosuppression as a cause of the chronic hepatitis.

Prevention and an early diagnosis of IPLTH are crucial as it is implicated in causing LAF and cirrhosis[33-36]. A study based on 158 asymptomatic paediatric LT recipients followed for over 10 years showed that a significant number of those who received cyclosporine-A as primary immunosuppression with withdrawal of corticosteroids at 3 mo post-LT developed unexplained chronic hepatitis[10]. The incidence and intensity of this inflammation increased with time; 22%, 43%, and 64% at 1, 5, and 10 years, respectively, developed chronic hepatitis. Of those with chronic hepatitis, 52%, 81%, 91% at 1, 5, and 10 years, respectively, progressed to graft fibrosis. Additionally, 15% progressed to cirrhosis at 10 years[10]. In another study based on 1287 LT recipients who were followed for over a decade, almost 40% of patients with allograft cirrhosis had no identifiable etiology apart from IPLTH[36].

Other long-term follow-up series have also shown similar data with regards to incidence and progression of inflammation[5,30]. Liver biopsies in clinically well LT recipients at a median interval of 13 years from LT showed histological abnormalities in 76% of liver biopsies (35% interface hepatitis, 12% moderate to severe hepatic steatosis, 9% cirrhosis, and 8% chronic rejection). Varma *et al*[13] showed that when the inciting factors were removed, serial allograft biopsies showed a reduction in inflammation and fibrosis, thus suggesting that IPLTH is not a terminal and progressive phenomenon.

CHRONIC ANTIBODY MEDIATED REJECTION

Conventionally, unlike cardiac or renal transplant recipients, LT recipients were believed to have an innate resistance to antibody mediated rejection (AMR) caused by donor specific antibodies (DSA)[40,41]. More recently, several series have reported an inferior survival in patients who were DSA positive, leading to a renewed interest in its effect on liver allograft structure and function and long-term outcome[42,43].

DSAs are antibodies formed by the recipient that bind to type I and type II human leukocyte antigens (HLAs) in the donor organ, potentially resulting in allograft injury[44]. Recipients exposed to a variety of non-self HLAs may have preformed DSAs prior to LT, whereas *de novo* DSAs form after LT in response to the new donor organ's HLAs[45]. Anti-HLA class I antibodies tend to appear in the early post-LT period, while anti-HLA class II antibodies (particularly anti-HLA-DQ antibodies) occur in the long-term. Non-self class II HLA molecules expressed by endothelial cells within the liver allograft are DSA targets. These get significantly upregulated by proinflammatory signals, resulting in antibody binding, crosslinking, and triggering of effector mechanisms like inflammation and fibrosis[46].

Nonetheless, the liver allograft has numerous inherent mechanisms which make it relatively resistant to AMR[6]. These include Kupffer cell-based scavenging and clearance of activated complements and immune complexes, relatively lower expression of class II DSA targets as compared to the kidney or the heart, and a large sinusoidal vascular bed which dilutes antibody-binding across a larger endothelial cell surface. Other liver based protective factors include a dual blood supply protecting the organ from ischemic damage and a high regenerative capacity which enables the liver to heal and recover from injury[45,46]. In view of these liver specific dynamics, the presence of preformed DSAs and a positive crossmatch test are not considered contraindications to LT. Moreover, many LT units do not routinely

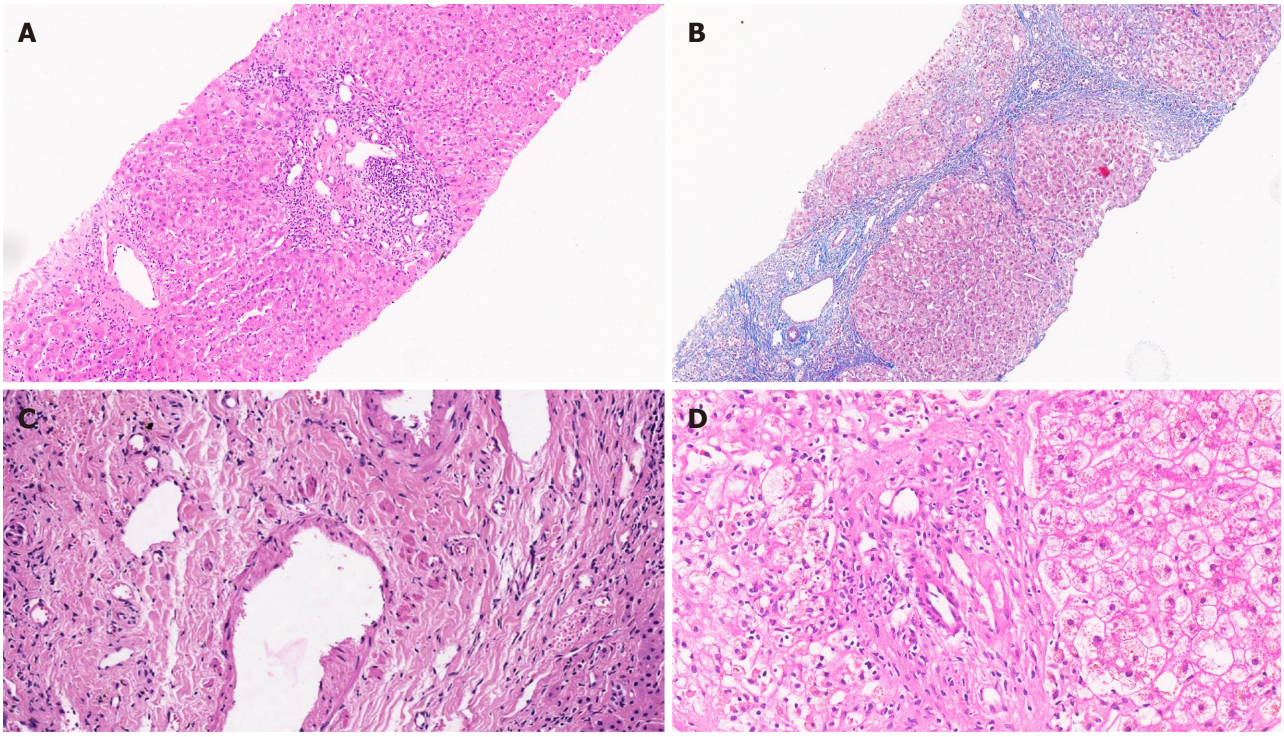

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Figure 1 Liver allograft biopsy. A: Liver allograft biopsy with portal fibrosis, portal inflammation, and mild centrilobular inflammation; (hematoxylin and eosin stain, ×10), B: Liver allograft biopsy with bridging fibrosis and portal/septal inflammation (Masson trichrome stain, ×10); C: Dense portal collagenization with portal venupathy in an allograft biopsy(hematoxylin and eosin stain, ×40); D: Bile duct loss in a portal tract. (hematoxylin and eosin stain, ×30).

perform a DSA test or cross-match prior to LT.

Descriptions of antibody subclasses and functional tests developed to separate complement binding and complement non-binding DSAs have provided better insights into the risks of DSAs in LT recipients. This understanding of the antibody mediated response in organ-transplant recipients is greatly facilitated by the introduction of solid-phase immunoassay technology for the detection and characterization of HLA antibodies[41]. The solid-phase immunoassay or Luminex assay which uses three types of antibody panels is more sensitive than complement-dependent lymphocytotoxicity assay and flow cytometry[44]. However, as described above, the clinical relevance of anti-HLA antibodies and DSAs detected on Luminex assay have not yet been conclusively elucidated. Moreover, there also remains the inability to sift through the panel and filter out the harmful antibodies from the more innocuous ones.

The lack of specific clinical, biochemical, or pathologic features makes CAMR a challenging diagnosis. The exact incidence of CAMR remains unknown but has been believed to occur in approximately 8%-15% of recipients who retain or form *de novo* DSAs against HLA class II molecules (especially DQ)[46]. Evidence in this regard has mostly come from long-term adult and paediatric LT recipients who had protocol liver biopsies or achieved operational tolerance. Histological findings strongly associated with persistent DSA include low-grade portal, periportal, and perivenular lymphoplasmacytic inflammation with low-grade interface and perivenular necro-inflammatory activity[47-50]. Dense portal collagenisation and obliterative portal venopathy have also been reported[48-50]. The above features, with or without positive complement component 4d (C4d) staining, is strongly associated with CAMR[48].

Several series have also indicated a direct correlation between DSAs against HLA class II molecules and LAF. A study from Japan showed that 88% of paediatric LT recipients with stable graft function and fibrosis or cirrhosis at 5 years post-LT exhibited DSA positivity[38]. Other series from Europe and Japan strongly associate the presence of *de novo* HLA antibodies to class II antigens (DSAs and non-DSAs) with CAMR, inflammation, and LAF[50-54]. Potential patho-mechanisms linking DSAs to LAF include destruction of microvasculature, non-microvascular antibody-dependent cell mediated cytotoxicity, activation of endothelial and stellate cells and portal myofibroblasts, and complement mediated chemotaxis[47,51,52].

Given the persistent ambiguity which hinders an unequivocal diagnosis of CAMR, a scoring system to better elucidate the features of CAMR has been proposed for "putative CAMR"[48]. The score is based on interface activity, lobular inflammation, portal tract collagenization, portal venopathy, presence of positive circulating DSAs, sinusoidal fibrosis, and HCV status. Nevertheless, it must be borne in mind that CAMR is a diagnosis of exclusion and other potentially confounding causes like recurrent disease and viral pathology need exclusion[55,56].

METRICS FOR LIVER ALLOGRAFT FIBROSIS

Irrespective of the etiology, there remains the need to qualify and quantify LAF. A scoring system for LAF allows for a reliable diagnosis, timely intervention, assessment of treatment efficacy, prognostication, and peer comparison. Currently available metrics for LAF have been adapted from those used for chronic hepatitis and, therefore, lack predictive power. A semiquantitative fibrosis scoring system specifically adapted to assess LAF has been proposed, which objectively defines portal, sinusoidal, and centrilobar fibrosis, providing a good representation of the whole hepatic acinus[57].Immunohistochemical assessment of alpha smooth muscle actin on graft biopsies have also been proposed as a modality, wherein a positive area percentage of over 1.05 predicted with a 90% specificity an increased risk of fibrosis on subsequent biospsies[8,58].

OPERATIONAL TOLERANCE AND LIVER ALLOGRAFT FIBROSIS: THE EQUIPOISE

An immunosuppression-free life remains the ultimate goal of transplantation. Allograft tolerance can be realised by immunological dampening and inhibition of the rejection response. True tolerance occurs when there is no demonstrable immunological response against the liver graft and is a rare event in transplantation[46]. Nonetheless, graft acceptance with minimal immunosuppression referred to as 'prope tolerance' can often be achieved in long-term post-LT survivors. Operational tolerance (OT) defined as the absence of rejection, and graft survival with normal function and histology in an immunosuppression-free, fully immunocompetent host on the other hand can be potentially achieved in up to 20% of well selected LT recipients[59]. It is important to realize that the current characterisation of CAMR resulted from such attempts at withdrawing immunosuppression[39]. The 2016 Banff update discussed pathological findings predictive of successful immunosuppression withdrawal and provided a guarded view on immunosuppression withdrawl[46].

Most evidence on immunosuppression withdrawal is anecdotal, retrospective, or lack a control-cohort. There also remains the undisputable fact that most protocol graft biopsies reveal sub-clinical histological damage. Furthermore, as detailed above, the risks of inadequate immunosuppression far outweigh the small potential for success. It is also crucial to note that OT is not a permanent stable state, but a dynamic one. Serial protocol liver biopsies are one way of evaluating OT, allowing for resumption of immunosuppression if there is injury to the graft. There always remains the need for immunological surveillance to ensure continued good graft histology and function. The lack of available, well-defined immune monitoring to detect immunoregulation or unresponsive states leads to an inability to objectively predict those who can successfully achieve OT. The key nonetheless, is the development of immune biomarkers which can reliably foretell the possibility of achieving OT, and at the same time predict the likelihood of its failure.

CONCLUSION

The incidence of allograft hepatitis and fibrosis continues to increase in long-term LT recipients and liver histology remains the only definite way to confirm these findings. There is emerging evidence that some of the graft fibrosis could be driven by inflammation, antibody mediated rejection, or even genetic predisposition. Protocol biopsies can identify cases of early allograft fibrosis, which then can potentially be reversed with optimised immunosuppression. Achieving OT remains the ultimate immunological goal of LT. However, in light of long-term sub-clinical immunological injury to liver grafts, this enthusiasm needs to be tempered. A prudent approach would be to base this decision on reliably predictive immune biomarkers.

FOOTNOTES

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MINIREVIEWS

Outcomes of patients with post-hepatectomy hypophosphatemia: A narrative review

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Abstract

Phosphate is an essential electrolyte for proper mineralisation of bone, buffering of urine, and diverse cellular actions. Hypophosphatemia (HP) is a clinical spectrum which range from asymptomatic to severe complications such as neuromuscular and pulmonary complications, or even death. Post-hepatectomy HP (PHH) has been reported to be 55.5%-100%. Post-hepatectomy, there is rapid uptake of phosphate and increased mitotic counts to aid in regeneration of residual liver. Concurrently, PHH may be due to increased urinary phosphorous from activation of matrix extracellular phosphoglycoprotein in the injured liver, which decreases phosphate influx into hepatocytes to sustain adenosine triphosphate synthesis. A literature review was performed on PubMed till January 2022. We included 8 studies which reported on impact of PHH on post-operative outcomes. In patients with diseased liver, PHH was reported to have either beneficial or deleterious effects on post-hepatectomy liver failure (PHLF), morbidity and/or mortality in various cohorts. In living donor hepatectomy, PHLF was higher in PHH. Benefits of correction of PHH with reduced post-operative complications have been shown. Correction of PHH should be done based on extent of PHH. Existing studies were however heterogenous; further studies should be conducted to assess PHH on post-operative outcomes with standardized phosphate replacement regimes.

Key Words: Hepatectomy; Hepatocellular Carcinoma; Hypophosphatemia; Phosphates; Liver neoplasms; Liver transplantation

Core Tip: Hypophosphatemia (HP) is a clinical spectrum which range from asymptomatic to severe complications such as neuromuscular and pulmonary complications, or even death. Post-hepatectomy HP (PHH) has been reported to be 55.5%-100%. Pathophysiologic mechanisms have been proposed. However, literature on the outcomes of patients following PHH is scarce. This is the first review to summarize existing literature on the pathophysiology of PHH in both healthy and diseased liver, and its impact on post-operative outcomes.

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INTRODUCTION

Phosphate is an essential electrolyte which is involved in several bodily functions. It is necessary for proper mineralisation of bone, buffering of urine, and diverse cellular actions such as energy metabolism, proliferation and specific functions of differentiated cells[1]. Given its essential roles, aberrancy in phosphate levels result in adverse impact on the body. Normal adult serum phosphate ranges from 0.81-1.45 mmol/L (2.5-4.5 mg/dL). Hypophosphatemia (HP) is defined as an adult serum phosphate level < 0.81 mmol/L (< 2.5 mg/dL)[2]. HP may also be subdivided according to its severity: mild (0.65-0.81 mmol/L, or 2.0-2.5 mg/dL), moderate (0.32-0.65 mmol/L, or 1.0-2.0 mg/dL) and severe (< 0.32 mmol/L, or < 1.0 mg/dL)[2]. The clinical presentation of HP is a spectrum; patients may be asymptomatic or present with mild symptoms such as fatigue, weakness or anorexia. However, HP may result in severe complications such as neuromuscular disturbances including encephalopathy, seizures, coma, pulmonary complications such as respiratory failure (in view of respiratory muscle weakness), cardiovascular complications such as impaired myocardial performance, hemolytic anemia, or even death[3]. Sequelae of patients with underlying malignancy such as nausea, vomiting and loss of appetite may result in HP from reduced dietary intake[4].

HP has been reported to be 0.2%-0.3% in all inpatients, 30% in intensive care unit (ICU) patients and 60-85% in sepsis[5-7]. Post-operative HP is also commonly reported following major abdominal surgery, including liver resection (hepatectomy)[8,9]. Post-hepatectomy HP (PHH) has been reported to be 55.5-100%[10-14]. Literature on the impact of PHH however, remains controversial. Immediately following hepatectomy, there is a drop in serum phosphate due to increased phosphate uptake in the regenerating injured liver, as well as increased urinary loss of phosphorous from activation of matrix extracellular phosphoglycoprotein in the injured liver[15,16]. Some studies have reported improved recovery of initial liver insufficiency in PHH, yet others reported increased major morbidity (cardiorespiratory, infections and haemorrhage)[10,11]. These studies were heterogenous in the extent of hepatectomy and PHH. In view of the lack of high quality evidence, this manuscript aims to review the pathophysiology, etiology, clinical significance and prognostic impact of PHH.

PATHOPHYSIOLOGY AND ETIOLOGY

The homeostasis of phosphate is a complex process. Phosphate regulation is maintained through intestinal phosphate absorption, renal phosphate excretion, and equilibrium of extracellular phosphate with that in bone or intracellular fluid[1]. Intracellular shift of phosphate is enhanced by respiratory alkalosis and insulin. Dietary sources of phosphate include eggs, milk, meat, soy-based products and foods with additives and preservatives[17]. Causes of HP include reduced dietary uptake, impaired intestinal absorption, increased phosphate excretion and intracellular shift of phosphate[2]. Metabolism of phosphate is closely linked to the calcium-parathyroid hormone (PTH)-vitamin D axis. Serum phosphate is mediated by PTH and 1,25 dihydroxyvitamin D (1,25$(OH)_2$D), which play critical roles in the regulation of phosphate homeostasis in the intestines, bone and kidneys; PTH is produced by the parathyroid glands and influence phosphate and calcium levels through the following: (1) Stimulation of bone resorption resulting in an increase in serum calcium and phosphate; (2) Inhibition of resorption of phosphate from tubular fluid in the kidneys resulting in decrease in serum phosphate; and (3) Stimulation of conversion of cholecalciferol to calcitriol in the kidney, whereby calcitriol is responsible for intestinal absorption of phosphate and calcium[18]. General causes of post-operative HP following major abdominal surgery has been postulated to be due to the result of hemodilution caused by bleeding or fluid administration during surgery[19]. Other contributory factors include diabetic ketoacidosis and refeeding syndrome especially in the context of malignancy and associated malnutrition[20]. Figure 1 summarizes the pathophysiology and etiology outlining HP.

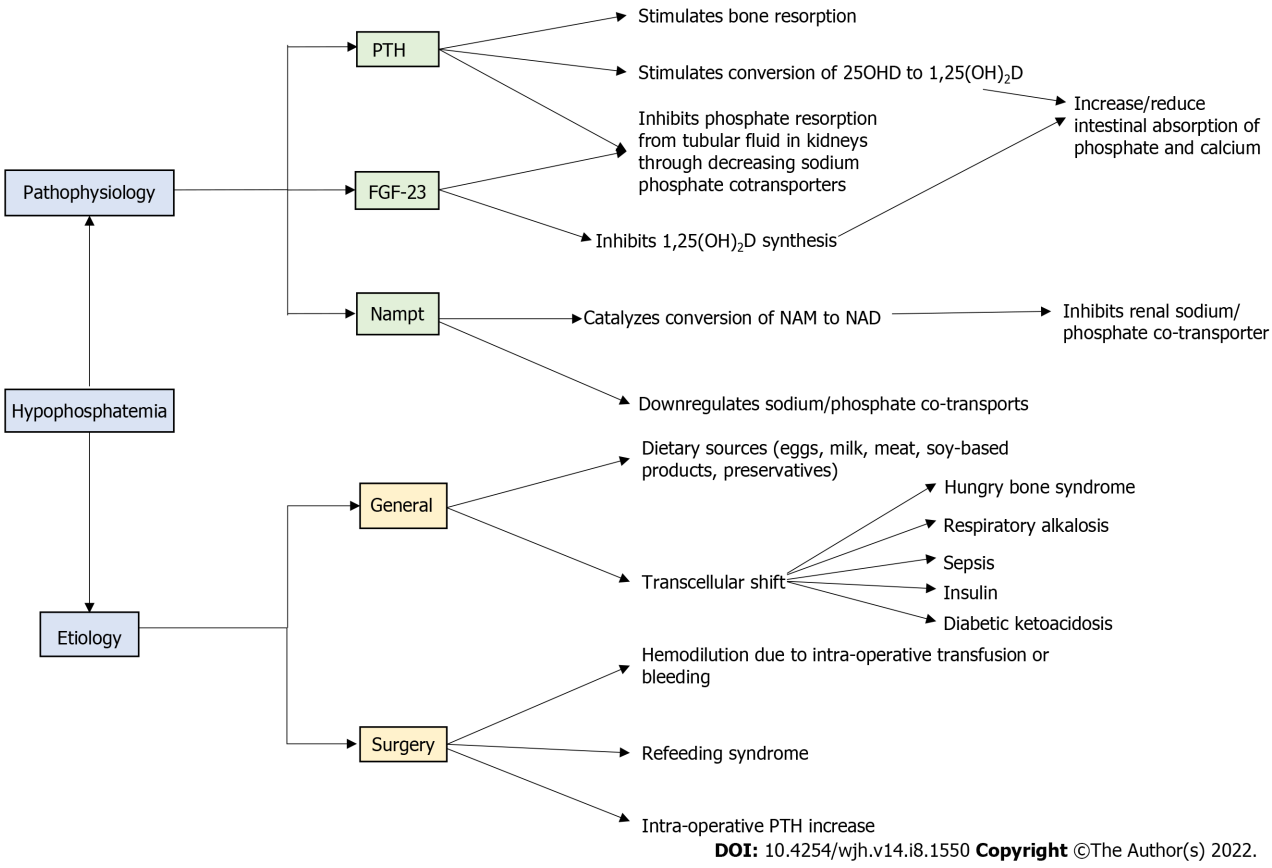

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Figure 1 Pathophysiology and etiology outlining hypophosphatemia. $1,25(OH)_2D$: 1,25 dihydroxyvitamin D; 25OHD: 25-hydroxyvitamin D; FGF-23: Fibroblast growth factor-23; NAD: Nicotinamide adenine dinucleotide; NAM: Nicotinamide; Nampt: Nicotinamide phosphoribosyltransferase; PTH: Parathyroid hormone.

Liver tissue contains 0.3% phosphate by weight[21]. PHH has been traditionally thought to be due to the increased metabolic demands by the regenerating liver[22]. Post-hepatectomy, there is rapid uptake of phosphate and increased mitotic counts in the regenerating residual liver, resulting in PHH[15]. However, it has been postulated that there are several pathophysiologic mechanisms behind HP following hepatectomy. Surgery has been shown to result in elevated PTH of up to 9 times intra-operatively[23]. PTH reduces renal proximal tubular phosphate uptake by decreasing the abundance of renal sodium phosphate cotransporters (Npt2a, Npt2c, and PiT-2) in the renal proximal tubule, resulting in increased fraction of excretion of phosphate (Fe-P) with resulting HP[24]. A study by Nafidi *et al* on 18 patients who underwent hepatectomy showed that intact-PTH (I-PTH) had significant increase on post-operative day (POD) 1 (from 4.5 ± 0.3 to 8.8 ± 0.9 pmol/L, $P < 0.01$)[25]. Phosphate levels was negatively correlated with I-PTH (r = -0.56: $P = 0.024$) on POD1, and Fe-P was positively correlated with I-PTH ($r = 0.52$: $P = 0.047$)[25]. An alternative explanation for PHH is secondary to increased urinary phosphorous loss due to the release of cathepsin B from activation of matrix extracellular phosphoglycoprotein in the injured liver[16,26]. This activation of matrix extracellular phosphoglycoprotein results in decreased concentration of phosphate influx into hepatocytes to sustain adenosine triphosphate (ATP) synthesis[16]. This is in contrary to the hypothesis that PHH is a result of influx of phosphate into liver for ATP synthesis which aids liver regeneration[22].

Phosphatonins, which are phosphaturic peptides that decrease renal sodium-dependent cotransport of phosphate, may also be responsible for PHH[27]. Fibroblast Growth Factor-23 (FGF-23) inhibits $1,25(OH)_2D$ synthesis and reduces the expression and activity of the sodium phosphate cotransporters in the renal proximal tubule, resulting in reduced intestinal and renal phosphate absorption[28]. FGF-23 is elevated in chronic kidney disease in view of higher phosphate and calcium concentrations[29]. Elevation in FGF-23 (which results in HP) has been demonstrated to be a strong predictor of mortality independent of renal function in patients with end-stage liver disease on transplant waiting list; this has been postulated to be due to the toxic effects of FGF-23 and increased risk of infections at above-physiological levels[30,31]. However, the effect of hepatectomy on FGF-23 levels has not been demonstrated[23].

Recently, translational studies have shown the role of nicotinamide (NAM) and nicotinamide phosphoribosyltransferase (Nampt) in the pathophysiology of PHH[32]. Nampt catalyzes rate-limiting step in conversion of NAM to nicotinamide adenine dinucleotide (NAD) which is essential for cellular

metabolism, energy production and deoxyribonucleic acid repair[33]. NAM inhibits intestinal and renal sodium-dependent inorganic phosphate (Na/Pi) transport system in rats[34]. Following hepatectomy, there is increase in Nampt and NAM. Excess Nampt and NAM influx in proximal tubular cells of the kidney results in downregulation of NaPi-IIa and NaPi-IIc protein levels[32]. In addition, Nampt catalyzes conversion of NAM to NAD, which inhibits renal Na/Pi transport in response to metabolic stimuli, resulting in PHH with hyperphosphaturia[35].

ADVANTAGES OF HYPOPHOSPHATEMIA

Search strategy

A literature review was performed on PubMed from inception till 11 January 2022 using a combination of search terms "hypophosphatemia" AND ("liver resection" OR "post-hepatectomy liver failure" OR "post-hepatectomy insufficiency"). The detailed search strategy is appended in Supplementary material (Supplementary Table 1). We obtained a total of 65 studies, of which 10 studies reported impact of HP on outcomes following hepatectomy; 2 studies did not have full-text available and had insufficient data in the abstract and hence were not included in our review[36,37]. We included 8 studies which reported on the impact of PHH on post-operative outcomes[10-14,38-40]. Table 1 summarizes the study characteristics of all included studies. Table 2 summarizes the median phosphate levels and difference in post-operative outcomes following liver resection for both healthy liver donors and diseased patients with PHH *vs* normophosphatemia (NP). Where applicable, overall mean and standard deviation values were combined from individual subgroups using methods described by Altman *et al*[41]. Figure 2 is a schematic representation of the advantages and disadvantages of PHH on post-operative outcomes with their respective proposed pathophysiology.

Summary of evidence on PHH

Literature has shown benefits of PHH with improvement in recovery from post-hepatectomy liver failure (PHLF). A retrospective study by Hallet *et al*[10] in 2016 investigated on the impact of PHH on post-operative liver function and recovery in 402 patients who underwent hepatectomy. They investigated on initial liver insufficiency (ILI), which was defined as serum bilirubin > 50 μmol/L and INR > 1.7 within 5 d post-operatively; patients who had PHH were also more likely to have ILI compared to NP (n = 44/223 (19.7%) *vs* n = 20/179 (11.2%), P = 0.02). However, they showed that of all patients with ILI, more patients with HP recovered from ILI compared to those with NP (90.9% *vs* 65.0%, P = 0.03).

Incidence of PHLF is reported to be 0.7%-35%, varying based on pre-operative liver function, underlying pathology and co-morbidities[42]. Definition of PHLF is controversial with lack of standardized definitions; the "50-50" criteria (serum bilirubin > 50μL/L and prothrombin time < 50% of normal on POD 5) was proposed by Balzan *et al*[43]. Consensus by the International Study Group of Liver Surgeries (ISGLS) in 2011 defined PHLF as post-operatively acquired deterioration in the ability of the liver (in patients with normal and abnormal liver function) to maintain its synthetic, excretory and detoxifying function, characterised by increase in the INR and hyperbilirubinemia on or after POD 5: Grade A (defined as abnormal laboratory values without change in clinical management), B (defined as requiring deviation from regular clinical management but without need for invasive treatment) and C (defined as requiring need for invasive treatment) has mortality of 0%, 12% and 54% respectively[44,45]. Intravenous phosphate replacement was given based on the serum phosphate levels in the study by Hallet *et al*[10] (Table 1). It is possible that more aggressive phosphate replacement in patients with PHH may have resulted in better improvement in ILI by creating influx of phosphate into hepatocytes to assist in liver regeneration[22]. Apart from liver (dys) function however, there was no difference in post-operative outcomes between PHH and NP in their study; there was no association between PHH and length of stay (PHH: median 7 (interquartile range (IQR) 6-10) d *vs* NP: median 7 (IQR 5-11) d, P = 0.55), morbidity (PHH: n = 13/223 (5.8%) *vs* NP: n = 12/179 (6.7%), P = 0.56) and mortality (PHH: n = 3/223 (1.3%) *vs* NP: n = 4/179 (2.2%), P = 0.50). It is important to note the definition of PHH defined in their study (PHH was defined as ≤ 0.65 mmol/L) correlates to moderate HP instead. Benefits of improved recovery from ILI in patients with PHH may only be seen in moderate or severe PHH, or due to more aggressive phosphate replacement in those subgroups.

Similar to the study by Hallet *et al*[10], Squires *et al*[13] demonstrated improved liver function with PHH. A retrospective study by Squires *et al* on 719 patients who underwent major hepatectomy showed that NP (defined as > 2.5 mg/dL or > 0.81 mmol/L) was associated with highest incidence of PHLF (12.3%), major complications (30.3%), 30 d mortality (6.5%) and 90 d mortality (7.1%), compared to moderate, severe or profound PHH[13]. Profound PHH had the lowest incidence of post-operative complications (PHLF: 3.4%, P = 0.008; major complications: 16.7%, P = 0.037; 30 d mortality: 0, P = 0.010; 90 d mortality: 3.4%, P = 0.166). Phosphate replacement was given based on surgeon discretion (n = 469/719, 69%). Multivariate analysis also showed that phosphate > 0.78 mmol/L on POD 2 is independently associated with significant risk of PHLF (Hazards ratio (HR) 1.78, 95% confidence interval (CI): 1.02-3.17, P = 0.048), major complications (HR 1.57, 95%CI: 1.02-2.47, P = 0.049), 30 d mortality (HR 2.70, 95%CI: 1.08-6.76, P = 0.031) and 90 d mortality (HR 2.51, 95%CI: 1.03-6.15, P = 0.044).

Table 1 Summary of study characteristics of all included studies in the literature review

No	Ref.	Definition of HP	PHH, *n* (%)	NP, *n* (%)	Type of liver resection (%)	Histopathology (%)	Post-operative phosphate replacement regime	Phosphate replacement, *n* (%)
1	Buell *et al* [14], 1998	< 2.5 mg/dL	21/35 (60)	14/35 (40)	Major hepatectomy (NR); Cryosurgery (NR)	CRLM: 8 (23) HCC: 4 (11) Others: 23 (66)	For phosphate < 3.0 mg/dL: sodium phosphate or potassium phosphate HP: mean of 15 mmol/d on POD1, to 25 mmol/d on POD3 NP: mean of 5 mmol/d	NR
2	George *et al* [11], 1992	NR	44/44 (100)	0	Right hepatectomy and extended right hepatectomy	NR	NR	NR
3	Giovannini *et al*[12], 2002	Normal: > 2.5 mg/dL; Mild-moderate: 1.6-2.5 mg/dL; Severe: < 1.5 mg/dL	38/59 (64.4)	21/59 (35.6)	Major hepatectomy (58); Minor hepatectomy (42)	CRLM: 10 (17) ICC: 7 (12) HCC: 16 (27) GBC: 2 (3) Others: 24 (41)	If > POD3 and oral feeding cannot be resumed: parenteral phosphate (fructose 1-6 diphosphate or potassium phosphate) at 20-50 mmol/d	NR
4	Hallet *et al* [10], 2016	≤ 0.65 mmol/L	223/402 (55.5)	179/402 (44.5)	Major hepatectomy (52) Minor (48) Hepatectomy	CRLM: 260 (65) ICC: 53 (13) HCC: 27 (7)Others: 62 (15)	Based on serum phosphate: Intravenous potassium phosphate or sodium phosphate	NR
5	Serrano *et al*[38], 2019	Normal: > 2.5 mg/dL; Mild: 1.6-2.5 mg/dL; Moderate: 1.0-1.5 mg/dL; Severe: < 1.0 mg/dL	161		Living donor hepatectomy	NA	Elemental phosphate based on phosphate levels: < 1.1 mg/dL: 25 mmol 1.1-1.9 mg/dL: 20 mmol 2.0-2.3 mg/dL: 15 mmol 2.4-2.7 mg/ dL: 10 mmol	NR
6	Squires *et al* [13], 2014	Normal: > 2.5 mg/dL; Mild: 1.6-2.5 mg/dL; Moderate: 1.0-1.5 mg/dL; Severe: < 1.0 mg/dL	488/719 (67.9)	231/719 (32.1)	Extended left hepatectomy (6) Extended right hepatectomy (20) Left hemihepatectomy (23) Right hemihep-atectomy (39) Central hepatectomy (2) Non-anatomical (10)	CRML: 229 (32) HCC: 69 (9) ICC: 88 (12) Metastatic NET: 34 (5) Other: 299 (42)	Discretion of surgeon Median replacement: 55 mmol (range 10-170 mmol)	469 (69)
7	Tan *et al* [39], 2003	Normal: > 2.5 mg/dL; Moderate: 1.5-2.5 mg/dL; Severe: 1.0-1.5 mg/dL; Profound: < 1.0 mg/dL	89/95 (93.7)	6/95 (6.3)	Right-lobe living donor hepatectomy: Right hepatectomy (94); Left lateral segmentectomy (5); Left lobectomy (11)	NA	Based on phosphate deficit: intravenous or oral phosphate	NR
8	Yuan *et al* [40], 2010	Normal: > 2.5 mg/dL; Mild: 1.5-2.5 mg/dL; Moderate: 1.0-1.5 mg/dL; Severe: < 1.0 mg/dL	Overall: 100/102 (98) Mild: 56/102 Moderate: 25/102 Severe: 19/102	2/102 (2)	Living donor hemi-hepatectomy	NA	Severe HP: Intravenous phosphate	7/19 (36.8)

All categorical variables are expressed as *n* (%). CRLM: Colorectal liver metastasis; GBC: Gallbladder carcinoma; HCC: Hepatocellular carcinoma; ICC: Intrahepatic cholangiocarcinoma; ILI: Initial liver insufficiency; INR: International normalized ratio; LOS: Length of stay; NA: NET: Neuroendocrine tumor; Not applicable; NP: Normophosphatemia; NR: Not reported; PHH: Post-hepatectomy hypophosphatemia; PHLF: Post-hepatectomy liver failure.

Following hepatectomy, liver regeneration with hepatocyte proliferation and deoxyribonucleic acid synthesis begins immediately and is mostly completed after 72 h[46,47]. Failure to reach PHH suggest the lack of phosphate uptake for ATP synthesis in the liver, resulting in higher incidence of PHLF. This was supported by increased PHLF and mortality in patients who had phosphate nadir after POD3[13].

Table 2 Summary of laboratory values and post-operative outcomes comparing patients with post-hepatectomy hypophosphatemia and normophosphatemia

		Mean nadir phosphate, mg/dL[a]			Mean INR[a]			Length of stay, d			Post-hepatectomy liver failure			Any morbidity			30 d mortality		
No	Ref.	PHH	NP	*P* value	PHH	NP	*P* value	PHH	NP	*P* value	PHH	NP	*P* value	PHH	NP	*P* value	PHH	NP	*P* value
1	Buell *et al* [14], 1998	2.1 ± 0.1[b]	3.0 ± 0.2[b]	< 0.05	NR			16.22 ± 12.09	11.22 ± 7.03	NR	NR			17/21 (81)	4/14 (29)	< 0.05	1/21 (5)	0/14 (0)	NR
2	George *et al* [11], 1992	NR			NR			NR			1/44 (2)	0/0	NA	11/44 (25)	0/0 (0)	NA	NR		
3	Giovannini *et al*[12], 2002	1.7 ± 0.8 (POD3)		NR	NR			NR			NR			Mild-moderate: 4/23 (17) Severe: 9/15 (60)	3/21 (14)	< 0.001[c]	Mild-moderate: 1/23 (4) Severe: 3/12 (20)	1/21 (5)	NR
4	Hallet *et al* [10], 2016	1.52 ± 0.31[d]	2.72 ± 0.74[d]	< 0.01	1.51 ± 0.37	1.53 ± 0.91	0.83	7 (6-10)	7 (5-11)	0.55	44/223 (19.7)	20/179 (11.2)	0.02	Major morbidity: 13/223 (5.8)	Major morbidity: 12/179 (6.7)	0.56	9/223 (4.0)	4/179 (2.2)	0.31
5	Serrano *et al* [38], 2019	2.00[e] (recorded at median 1.6 d post-operatively)			NR			7.2 ± 3.4[e]		NR	10/161 (6.2)		NR	Any morbidity > 30 d: 19/161 (11.8)		NR	NR		
6	Squires *et al* [13], 2014	2.2 [1.7-2.8]			NR			NR			Moderate: 8.0% Severe: 8.5% Profound: 3.4%[f]	12.3%[f]	0.008	Major morbidity: Moderate: 20.1% Severe: 19.5% Profound: 16.7%[f]	30.3%[f]	0.037	Moderate: 3.8% Severe: 2.8% Profound: 0%	6.5%	0.010
7	Tan *et al*[39], 2003	2.6 (range 1.3-5.0)		NR	NR			NR			NR			Any morbidity: 8/95 (8.4)		NR	NR		
8	Yuan *et al* [40], 2010	1.89 ± 0.72 (POD3)		NR	Mild: 1.51 ± 0.26 Moderate: 1.43 ± 0.19 Severe: 1.95 ± 0.40	NR	< 0.001[g]	NR			14/100 (14)	0/2 (0)	NR	NR			NR		

[a]Values are reported on post-operative day 2 unless otherwise specified.
[b]Values described here excluded patients who received cryosurgery; the study included cohort of patients who received both major hepatectomy and cryosurgery.
[c]Comparing severe PHH (< 1.5 mg/dL) with other range of phosphate values.
[d]Values were reported in mmol/L in the original study and subsequently converted to mmol/L for standardization.
[e]Overall mean and SD was calculated through the combination of mean and SD from patients who had liver insufficiency and those without using methods described by Michael *et al*[37].
[f]Exact values of patients with normal, moderate, severe and profound post-hepatectomy hypophosphatemia (PHH) were not provided in the study.
[g]Comparing between each subgroup of PHH. All categorical variables are expressed as *n* (%), and all continuous variables are expressed in median (range), median [IQR], or mean ± SD unless otherwise specified. NA: Not applicable; NP: Normophosphatemia; NR: Not reported; PHH: Post-hepatectomy hypophosphatemia; POD: Post-operative day; SD: Standard deviation.

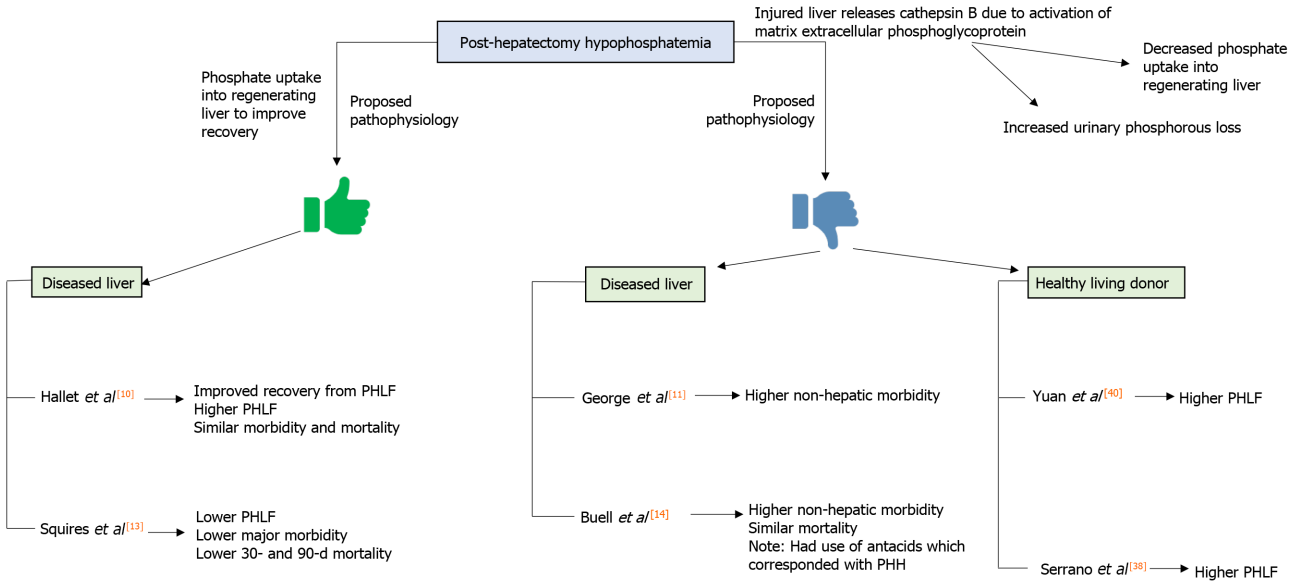

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Figure 2 Schematic diagram summarizing the postulated pathophysiology of the impact of post-hepatectomy hypophosphatemia on post-operative outcomes, as well as summary of the advantages (green) and disadvantages (blue) of post-hepatectomy hypophosphatemia in existing literature on post-operative outcomes. PHLF: Post-hepatectomy liver failure.

Unlike the study by Hallet *et al*[10] which showed increased PHLF, Squires *et al*[13] showed reduced PHLF with increased severity of PHH. This may be attributed to the patient selection in studies, where Squires *et al*[13] included patients with normal to profound PHH, whereas Hallet *et al*[10] only included patients with normal to moderate PHH. Severe and profound PHH may be more frequently seen in major hepatectomy in view of the extent of liver resection and burden on the regenerating liver. Failure to reach severe or profound PHH may indicate the liver's inability for adequate regeneration, and hence, worse outcome with higher PHLF during the initial post-operative day[10].

DISADVANTAGES OF HYPOPHOSPHATEMIA

Hepatectomy for liver pathology

The adverse effect of HP following hepatectomy was first shown by George and Shiu in 1992, where a retrospective study was conducted on 44 patients who underwent right or extended right hepatectomy [11]. They showed that severe HP (< 1.0 mg/dL, or < 0.32 mmol/L) was associated with increased major post-operative complications (cardiorespiratory $n = 5$, infections $n = 4$, haemorrhage $n = 1$, liver failure $n = 1$, $P < 0.001$). Protective effect of early phosphate replacement ($P < 0.05$) with fewer complications was also described, highlighting the importance of normalization of phosphate post-operatively. However, there is a lack of information on the extent of replacement and other outcome measures such as mortality.

Similarly, a retrospective study by Buell *et al*[14] in 1998 on 35 patients who underwent major hepatectomy and/or cryosurgery showed significantly higher post-operative complications (pancreatitis, pulmonary infections, gastrointestinal bleed, wound infection and ileus) in PHH compared to NP (HP: $n = 17/21$ (80%) *vs* NP: 4/14 (28%), $P < 0.05$)[14]. Length of hospital stay was 5 d longer (clinically but not statistically significant) in patients who had PHH compared to NP. Mortality was comparable between both PHH and NP. The authors defined PHH as phosphate < 0.81 mmol/L. Phosphate replacement was also initiated from POD 1 when phosphate < 0.97 mmol/L, with higher replacement in the PHH group compared to NP group. The authors noted a potential confounding factor responsible for HP in patients who underwent hepatectomy; use of antacid corresponded to PHH ($P < 0.05$). However, liver function (represented using aspartate aminotransferase as surrogate marker, HP: 462 U/L and NP: 440 U/L) was comparable. Cause of PHH may be due to the phosphate binding by antacids resulting in reduced phosphate absorption[48], rather than increased metabolic demands by regenerating liver, suggesting an improvement in liver function. While there is a correlation between PHH and increased morbidity and possibly length of stay, correlation does not equate to causation. Use of antacids may have resulted in PHH, and antacid use have been reported to result in post-operative ileus and predispose patients to pneumonia through airway colonisation[49,50]. Majority of patients who undergo hepatectomy are prescribed acid-suppressive therapy (H2 receptor antagonists or proton-

pump inhibitors) for stress ulcer prophylaxis[51]. The use of antacids following hepatectomy is however not routine. Hence the results by Buell *et al* should not be generalized to all patients who undergo hepatectomy[14].

Living donor hepatectomy

It is important to analyse this subgroup of healthy patients who underwent liver donor hepatectomy. The physiology of healthy patients with normal liver function differs from diseased patients with malignancy and/or liver dysfunction. A study by Yuan *et al* in 102 living donors who underwent hemi-hepatectomy showed a negative correlation between nadir phosphate level and peak total bilirubin (r=-0.337, P = 0.001) and international normalized ratio (INR) (r=-0.293; P = 0.004)[40]. Positive correlation was observed between severity of PHH and PHLF (r = 0.549, P = 0.023). The deleterious effects of PHH on liver function may be due to the activation of matrix extracellular phosphoglycoprotein in injured liver, resulting in decreased phosphate influx into hepatocytes to sustain ATP synthesis[16]. Hence, PHH may be associated with worse liver function and increased incidence of PHLF following hepatectomy. The authors additionally showed that in patients with severe HP (≤ 1.0 mg/dL, or ≤ 0.32 mmol/L), use of intravenous phosphate replacement resulted in better hepatic function (incidence of PHLF in severe PHH with replacement n = 0/7 (0%), without replacement n = 6/12 (50%)). However, it is important to note that while correlation was obtained for phosphate severity with PHLF, the R^2 value was 0.301 (not calculated in the study); only 30.1% of the variance may be explained by severity of HP on PHLF. In addition, unlike studies which examine the impact of PHH on hepatectomy in patients with underlying pathology (*e.g.*, HCC, cholangiocarcinoma), the study population by Yuan *et al*[40] was on healthy living donors. Healthy living donors have NP; in contrary, patients who undergo hepatectomy may have underlying chronic liver disease which commonly presents with HP due to malnutrition and vitamin D deficiency[52]. Pre-operatively, however, phosphate levels were reported to be normal[13,14]. This may be due to unreported pre-operative nutrition optimisation and phosphate replacement, and may have resulted in improvement in post-operative liver regeneration, compared to healthy living donors. Hence, results by Yuan *et al*[40] may not be applicable in majority of patients who undergo hepatectomy for underlying pathology. Similarly, Serrano *et al*[38] who investigated 161 patients who underwent living donor hepatectomy showed that intraoperative time and low postoperative phosphate levels through the first 38 h were good predictors of liver insufficiency (defined as serum bilirubin > 3 mg/dL and/or INR > 1.7 on POD 5 or more) (area under curve 0.731, sensitivity 60%, specificity 75.5%, positive predictive value 14%, negative predictive value 96.6%)[38].

In contrary, Tan *et al*[39] in 2003 retrospectively reviewed 95 living donors who underwent right hepatectomy showed NP with mean phosphate of 2.6 mg/dL (0.84 mmol/L), 2.7 mg/dL (0.87 mmol/L) and 2.9 mg/dL (0.94 mmol/L) on POD 1 to 2, POD 3 and POD 4 respectively[39]. Intravenous or oral phosphate replacement was given based on their existing deficits. The authors failed to demonstrate that PHH was more frequent in the subgroup of patients with morbidity. Of patients who had morbidity (n = 8/95 (8.4%)), incidence of PHH was however, not more frequent. A possible explanation behind this lack of statistical significance is that none of the patients included had profound PHH unlike the study by Yuan *et al*[40]. To add on, it is worth noting that the morbidity reported by Tan *et al*[39] were surgical complications such as pneumothorax, incisional hernia, intravenous line complications requiring occupational therapy and right pleural effusion and atelectasis. These complications are general surgical complications which should not be attributed to PHH. We caution to draw any conclusion from their study on the impact of PHH on post-operative outcomes.

MANAGEMENT OF HYPOPHOSPHATEMIA FOLLOWING HEPATECTOMY

Phosphate replacement regimes have been suggested by various studies and reviews but no international consensus statements have been put in place for recommended phosphate replacement[3,53,54]; Table 3 summarizes the list of example of phosphate replacement formulations, recommended doses and special considerations to note. While phosphate replacement is required for HP, it is also prudent to avoid over-aggressive replacement of phosphate. Phosphate replacement may result in hypocalcemia, metastatic calcification from HP, hypotension, hyperkalemia (in the event where potassium-containing phosphate replacement is used), dehydration and acute kidney injury[55]. These deleterious effects are more often seen in intravenous replacement; intravenous replacement may result in precipitation of calcium resulting in hypocalcemia and renal failure due to calcium phosphate precipitation in kidneys, resulting in cardiac arrhythmias. Hence, oral route is the preferred route of administration for mild-moderate HP and for patients who are able to tolerate orally. Should intravenous phosphate be used, its rate should be limited to maximum of 20mmol/hour[56]. The extent of increase in serum phosphate and potassium have been demonstrated using calculated sodium potassium phosphate ($Na_2K_5PO^4_6$) replacement, where infusion of $Na_2K_5PO^4_6$ with calculated phosphate dose (in mmol) of 0.5 x body weight x (1.25 -[serum phosphate]) resulted in mean rise in phosphate of 0.38 ± 0.04 mmol/L and mean rise in potassium of 0.3 mmol/L[57]. Repeat serum phosphate should be rechecked at 2-12 h following completion of phosphate replacement.

Table 3 Summary of phosphate replacement regimes for hypophosphatemia

Indications		Formulation	Route of administration	Composition	Recommended dosage	Special considerations
Mild hypophosphatemia (0.65-0.81 mmol/L)		Phospho-soda (C.B. Fleet Company, Virginia)	Oral	180mg $Na_2HPO^4 \cdot 7H_2O$ + 480 mg $NaH_2PO^4 \cdot H_2O$/mL Phosphate: 4.150 mmol/mL Sodium: 4.822 mEq/mL Potassium: 0	1000mg/d	Chronic renal failure / reduced glomerular filtration rate: to use half of recommended initial dose Causes diarrhoea
		Phospha 250 Neutral (Rising Pharmaceuticals, Inc., United States)	Oral	Elemental phosphorus 250 mg (8 mmol), Sodium 298 mg (13 mEq), and Potassium 45 mg (1.1 mEq)		
Moderate hypophosphatemia (0.32-0.65 mmol/L)	Not on ventilator	Phospho-soda (C.B. Fleet Company, Virginia)	(same as above)	(same as above)	If ≥1.5 mg/dL: 1 mmol/kg of elemental phosphorus (minimum of 40 mmol and a maximum of 80 mmol) in 3-4 doses over 24 h If < 1.5 mg/dL: 1.3 mmol/kg of elemental phosphorus (maximum of 100 mmol) in 3-4 doses over 24 h	(same as above)
	On ventilator	Sodium phosphate (Abbott Laboratories, North Chicago, Illinois)	Intravenous	142 mg Na_2HPO^4 + 276 mg $NaH_2PO^4 \cdot H_2O$/mL Phosphate: 3.0 mmol/mL Sodium: 4.0 mEq/mL	0.08 mg/kg over 2-6 h if recent and uncomplicated HP 0.16 mg/kg over 2-6 h if prolonged and has multiple causes Maximum of 20 mmol/h	Chronic renal failure / reduced glomerular filtration rate: to use half of recommended initial dose
		Potassium phosphate (Invenex Pharmaceuticals, Grand Island, New York)		236 mg K_2HPO^4 + 224 mg KH_2PO^4 /mL Phosphate: 3.003 mmol/mL 4.360 mEq/mL		Chronic renal failure / reduced glomerular filtration rate: to use half of recommended initial dose To avoid if potassium > 4mmol/L
Severe hypophosphatemia (< 0.32 mmol/L) / Critically ill patients, or with severe complications of hypophosphatemia		Sodium phosphate or potassium phospahte	(same as above)	(same as above)	0.08-0.16 mg/kg over 2-6 h	(same as above)

Similarly, for post-hepatectomy, there is no standardized regime for phosphate replacement. Table 1 summarizes the various phosphate replacement regimes and indications for replacement in existing studies for patients with PHH. Indications for phosphate replacement differed largely across the studies, with studies replacing phosphate only for severe PHH (< 1.0 mg/dL), *vs* studies which replace phosphate for < 3.0 mg/dL[14,40]. Nevertheless, the benefits of phosphate replacement has been described with reduced post-operative complications and improvement in liver function[11,40]. In view of the lack of standardized protocol for phosphate replacement in PHH, we suggest the use of the same regimen for phosphate replacement in HP (Table 3), with the use of oral replacement for mild-moderate PHH, and intravenous replacement for severe PHH or in critically ill patients.

PROGNOSTICATION OF POST-OPERATIVE COURSE FOLLOWING HEPATECTOMY

This summarized study and reviewed literature have shown equivocal evidence (Tables 1 and 2), with both benefits and disadvantages of PHH on incidence of PHLF and/or morbidity. However, exclusively for healthy patients with living donor hepatectomy, our literature review showed that these group of patients who had HP were more likely to have PHLF[13,40]. In contrary, patients with diseased liver (underlying malignancy and/or cirrhosis) who undergo hepatectomy may have improved liver regeneration and/or lower PHLF following hepatectomy, or have increase in post-operative morbidity [10,13,14]. This difference in outcome may be attributed to pre-operative nutritional optimisation and phosphate replacement in patients with diseased liver.

PHLF is a dreaded complication following hepatectomy with mortality risk; this is especially so in the context of patients with underlying cirrhosis and/or deranged liver function. Thus far, several studies have devised prognostic factors and prognostic scoring systems for the prediction of PHLF and

mortality following hepatectomy[58-61]. Established predictive factors of PHLF include Albumin-Bilirubin score, prothrombin time and Child-Pugh score[58,60,61].

NP has been shown to increase incidence of PHLF; multivariate analysis by Squires *et al*[13] on 719 patients who underwent major hepatectomy showed that POD 2 phosphate > 2.4 mg/dL (0.78 mmol/L) was associated with higher PHLF (HR 1.78, 95%CI: 1.02-3.17, P = 0.048), major complications (HR 1.57, 95%CI: 1.02-2.47, P = 0.049), 30 d mortality (HR 2.70, 95%CI: 1.08-6.76, P = 0.031) and 90 d mortality (HR 2.51, 95%CI: 1.03-6.15, P = 0.044)[13]. Nevertheless, the evidence on the use of phosphate as a prognostic marker of PHLF is scarce, and more studies are required to demonstrate any correlation between phosphate and PHLF.

CONCLUSION

The pathophysiology behind PHH remains poorly understood. This review summarized existing literature investigating the impact of phosphate on post-operative outcomes following hepatectomy. However, definition of PHH is variable and majority of studies are retrospective with small sample size. Phosphate replacement regimes were not standardized across the studies. The heterogeneity of the reviewed studies limits our understanding of PHH on post-operative outcomes following hepatectomy. Nevertheless, PHH is a common phenomenon and it is important for clinicians to ensure adequate replacement in view of deleterious effects of PHH. Well-designed randomized controlled trials should be conducted to fill in the knowledge gap on the impact of phosphate levels and phosphate replacement in patients undergoing hepatectomy.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Assessment of circulating levels of microRNA-326, microRNA-424, and microRNA-511 as biomarkers for hepatocellular carcinoma in Egyptians

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Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) is the fifth most common cancer. Differential expression of microRNAs (miRNAs)-326, miRNA-424, and miRNA-511 has been associated with the diagnosis and prognosis of HCC in different populations. However, limited information is available regarding their expression in Egyptian HCC patients.

AIM

To assess the role of circulating miRNAs-326, miRNA-424, and miRNA-511 in Egyptian HCC patients.

METHODS

This prospective observational study included 70 HCC patients and 25 healthy controls. The circulating levels of these three miRNAs were evaluated by real-time

PCR. Receiver operating characteristic curve analysis was used to test the diagnostic accuracy of microRNA expression levels.

RESULTS

All miRNAs were differentially expressed in HCC patients; miRNAs326 and miRNA-424 were upregulated, while miRNA-511 was downregulated. Both miRNA-326 and miRNA-424 showed sensitivity and specificity of 97%, 71.4%, and 52%, 60%, respectively, to differentiate HCC from controls. Moreover, miRNA-326 was associated with survival and could differentiate between Child grades (A *vs* B); miRNA-424 significantly differentiated early *vs* intermediate stages of HCC; while miRNA-511 was significantly correlated with response to modified Response Evaluation Criteria in Solid Tumors (mRECIST).

CONCLUSION

We conclude that miRNA-326, miRNA-424, and miRNA-511 have diagnostic and prognostic roles in Egyptian patients with hepatitis C virus-related HCC and should be considered for better disease management.

Key Words: Hepatocellular carcinoma; miRNAs-326; miRNA-424; miRNA-511; Modified response evaluation criteria in solid tumors

Core Tip: Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide. Recent cancer incidence data confirmed that the global age normalization rate of primary liver cancer is 10.1/100000, with a male/female ratio of 3:1. The diagnosis of HCC usually occurs in the late stages, resulting in an elevated death rate; this makes HCC the third deadliest malignancy. We examined whether circulating miRNAs326, miRNA-424, and miRNA-511 could serve as promising candidate non-invasive biomarkers for HCC. Such biomarkers could be used for targeted gene therapy research and may assist in monitoring the severity of HCC.

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INTRODUCTION

Liver cancer is the fifth most common cancer in men and is the second cause of cancer-related deaths worldwide[1]. Hepatocellular carcinoma (HCC) is a primary liver cancer and constitutes 80%-90% of all primary tumors of the liver[2,3]. It has a high incidence and mortality rate, with approximately 662.000 deaths every year[4]. Most HCC patients are diagnosed at advanced stages, contributing to the high rates of recurrence and development of metastasis[5]. In addition, the commonly used biomarker for HCC screening and diagnosis is alpha-fetoprotein (AFP), which has modest sensitivity and specificity[6] and is actually influenced by tumor size and cancer stage[7]. Due to these factors, it is crucial to identify more efficient biomarkers that can be used for the early diagnosis and prognosis of HCC.

MicroRNAs (miRNAs) are short, non-coding RNAs that regulate the transcription or degradation of certain target mRNAs[8-10]. They mediate important physiological processes such as cell differentiation, proliferation, and survival[11,12]. Aberrant regulation of miRNAs is associated with the initiation and progression of numerous cancers, including HCC[13]. Moreover, survival and response to chemotherapeutic drugs have been found to be linked to miRNAs[14-16]. Experimentally, a number of miRNAs have been proven to be related to HCC, and have been proposed as diagnostic and prognostic markers of HCC[17,18].

Aberrant expression of miRNA-424 has been documented in HCC tissues and cell lines[19,20]. It plays a tumor suppressor role[19]. A similar correlation was found for miRNA-326[21] and miRNA-511 [22], and both were significantly associated with survival[23]. Using the Cancer Genome Atlas, Lu and colleagues studied the prognostic and diagnostic roles of 33 miRNA signatures and found 11 down-regulated and 22 upregulated miRNAs by comparing cancerous and non-cancerous samples. They

observed the good role of miRNA-326, -424, and -511[24].

Most miRNA studies in HCC are experimental and are based on HCC tissues and cell lines. They were mainly performed to identify the etiopathogenetic role of miRNAs in HCC. However, few studies have shown their fundamental role in the diagnosis and prognosis of HCC. Hence, this cross-sectional study with prospective follow-up of HCC patients was performed to search for three miRNAs (326, 424, and 511) in blood to highlight their potential role in the diagnosis and prognosis of HCC.

MATERIALS AND METHODS

Patients and study design

This prospective observational study included 70 adult Egyptian patients who developed HCC on top of hepatitis C virus (HCV)-related liver cirrhosis and 25 healthy age- and sex-matched participants who were seronegative for HCV and HBV, and served as controls. HCC was diagnosed according to the American Association for the Study of Liver Diseases updated practice guidelines[25], or was histopathologically confirmed. All the patients were recruited from the multidisciplinary HCC clinic, the Endemic Medicine Department, Kasr al Ainy School of Medicine, Cairo University, and were HCC-treatment naïve at the time of enrollment. Exclusion criteria included; HBV co-infection, any cause of chronic liver disease other than HCV, any associated malignancies other than HCC, and prior treatment for HCV or HCC.

Data collection

All patients were subjected to clinical assessment, laboratory investigations (complete blood count, prothrombin time and concentration, liver and kidney function tests, AFP, hepatitis markers), ultrasound examination, and triphasic CT with documentation of HCC site, size, and number as well as the presence of portal vein thrombosis (PVT). At the time of enrollment into the study, blood samples were obtained from each participant, and Child-Pugh-score[26] and Barcelona Clinic Liver Cancer (BCLC) stage[27] were assessed based on the clinical, laboratory, and imaging data obtained.

Collection of blood samples

Whole blood samples were collected from each participant in 5-mL sterile RNAase-free vacutainer tubes containing EDTA. Blood samples were collected on ice and processed within 30 min of collection. To separate the plasma, each blood sample was centrifuged for 10 min at 1900 g at 4 ℃ (Heraeus, Labofuge 400 R; Germany). Plasma samples were carefully transferred into sterile RNase-free tubes, and then centrifuged for 10 min at 12000 g at 4 ℃ to remove cellular nucleic acid contamination, and haemolysed plasma samples were excluded. Samples were separated into aliquots and stored at 80 ℃ until processed.

RNA extraction

Total RNA was extracted from 200 μL plasma using the miRNeasy Serum/plasma cell lysates kit (Qiagen, Germany), according to the manufacturer's instructions. RNA concentration and purity were monitored using a Nanodrop spectrophotometer device (ThermoScientific, Wilmington, DE, United States).

Quantitative RT-PCR

The reverse transcription reaction was performed using the TaqMan™ MicroRNA Reverse Transcription Kit, Cat number # 4366596, (Applied Biosystems, Foster City, CA, United States) according to the manufacturer's instructions. miRNA-326, miRNA-511, and miRNA-424 quantification was carried out using quantitative real-time PCR (qRT-PCR) (Stratagene Mx3000p; Agilent Technologies, Germany). The qRT-PCR for each sample was carried out in duplicate using TaqMan 2x universal master mix II (Applied Biosystems, Foster City, CA, United States) and TaqMan microRNA Assay Mix containing PCR primers and TaqMan probes for each miRNA. The expression level of RNU6B was used as an endogenous control for normalization. To determine miRNA relative expression, it was reported as a fold change (ΔCt and $\Delta\Delta$Ct calculations).

Patient management and follow-up

After blood sampling, all patients were managed according to the BCLC guidelines[27] after a case-by-case discussion. All HCC patients were followed up for a period of 24 mo. HCC response to treatment was evaluated according to the Modified Response Evaluation Criteria in Solid Tumors (mRECIST)[28]. The overall survival time of the patients was defined as the period from the initial presentation to the last follow-up or death.

Statistical analysis

Microsoft Excel 2016 and the Statistical Package for Social Science (SPSS; Windows, version 26, IBM

Corp., Armonk, N.Y., United States) were used to analyze the data. Continuous, normally distributed variables were presented as mean ± SD, with a 95% confidence interval (CI), while non-normally distributed variables were summarized as the median and interquartile range (IQR). Categorical data were presented as frequencies and percentages. A *P* value of less than 0.05 was considered statistically significant. The Student *t*-test was performed to compare the means of normally distributed variables between groups, and the Mann-Whitney *U* test was used for non-normal variables. The Chi-square test and Fisher's exact test were used to determine the distribution of categorical variables between groups. The diagnostic performance of the studied miRNAs was assessed by receiver operating characteristic (ROC) curves. The area under the ROC (AUROC) was used as an index to compare the accuracy of tests. The optimal cut-off point value was taken from the maximum combined sensitivity and specificity. The sensitivity and specificity of relevant cut-offs are also displayed. The survival analysis was conducted using the "Log Rank (Mantel-Cox) Kaplan-Meier test".

RESULTS

Demographic results

The mean age of the studied cohort was 62.0 ± 7.6 years. Most of the patients were male (68.8%), non-diabetic (74.3%), non-smokers (80%), and Child A (82.9%). According to the BCLC staging system, most patients were in the early stage (48.6%). Most of the HCC lesions were single (70%), present in the right hepatic lobe (84.3%), and not associated with PVT (92.9%). That is why most of the patients were subjected to hepatectomy and microwave ablation (MWA), and most of them showed complete response according to mRECIST. All HCC patients were followed up for a period of 24 mo or until death. The mean survival time was 367.1±173.9 days. The median values of miRNA-326, miRNA-511, and miRNA-424 were 35, 1.2, and 5.1, respectively (Table 1).

Correlation of miRNAs with different parameters in HCC patients

There was no significant association between the studied miRNAs levels and gender, smoking, or the patients' performance status. The serum level of miRNA-424 was significantly elevated in diabetic patients. There was a significant difference in miRNA-326 between Child grades A and B. Moreover, there was a significant difference in miRNA-424 between early and intermediate HCC (Table 2).

Diagnostic efficiency of miRNAs in our patients

On comparing the miRNAs levels between healthy participants and HCC patients, it was found that HCC patients showed significantly higher levels of miRNA-326 ($P = 0.001$) and significantly lower levels of miRNA-511 ($P = 0.02$) (Table 3).

ROC analysis revealed the diagnostic performance of the studied miRNAs. miRNA-326 showed the best diagnostic performance, diagnosing HCC at a cut-off value of 1.165 with a sensitivity of 97.1%, specificity of 52%, positive predictive value (PPV) of 85%, and negative predictive value (NPV) of 86.7%. The area under curve (AUC) was 78.4% (67.7- 89.1), and the overall accuracy was 85.3% ($P < 0.001$) (Table 4, Figure 1).

Association of miRNAs with response to treatment and survival

Despite finding no statistically significant differences in the studied miRNAs between the surviving and non-surviving HCC patients, we found that miRNA-326 >1.165 was significantly associated with overall survival ($P = 0.001$) (Table 5, Figure 2). Moreover, there was a significant association between the BCLC stage as well as the response to treatment according to mRECIST and overall survival (Figure 2). It was also found that miRNA-511 was significantly associated with the response to treatment according to mRECIST (Table 6).

DISCUSSION

Several experimental studies have shown that miRNAs play a regulatory role in the progression of HCC by controlling cell cycle progression, cell growth, and apoptosis. Our study focused on three important miRNAs: miRNA-326, miRNA-424, and miRNA-511. miRNA-326 upregulation is known to inhibit cell proliferation and colony formation, and influence the invasiveness and migratory properties of HCC. It has a tumor suppressor role, and its low expression was found to be associated with tumor, node, metastasis (TNM) staging, tumor differentiation, and lymph node metastases in HCC patients. Regarding miRNA-424, experimental studies found that it is downregulated in HCC and has a role in inhibiting tumor migration, proliferation, and invasion. It was associated with AFP, TNM, multinodularity, vascular invasion, and intrahepatic metastases, as well as poor survival[29,30] and predicted tumor recurrence in HCC patients following liver transplantation[31]. Few studies have mentioned the role of miRNA-511 in HCC proliferation and invasiveness.

Table 1 Descriptive data of the studied hepatocellular carcinoma patients

			HCC
			N = 70
Demographic data	Age (yr)		62.0 ± 7.6
	Gender (%)	Female	22 (31.4)
		Male	48 (68.6)
	Smoker (%)	No	56 (80.0)
		Yes	14 (20.0)
	DM	No	52 (74.3)
	(%)	Yes	18 (25.7)
	BMI (kg/m^2)		27.7 (23.6- 31.9)
Laboratory investigation	Hb (g/dL)		13.2 ± 1.9
	WBC (× 10^3/μL)		6.0 (4.6-8.0)
	Platelets (× 10^3/μL)		169.5 ± 77.9
	ALT (U/mL)		52.5 (32.0- 74.3)
	AST (U/mL)		60.5 (38.0- 83.3)
	Alb (g/dL)		3.7 ± 0.5
	Bil.T (mg/dL)		0.9 (0.6- 1.4)
	AFP (ng/dL)		38.8 (10.0- 370.0)
	Creatinine (mg/dL)		0.9 ± 0.2
BCLC (%)	Early		34 (48.6)
	Intermediate		23 (32.9)
	Late		13 (18.6)
Performance status (%)	0		43 (61.4)
	1		24 (34.3)
	2		2 (2.9)
	3		1 (1.4)
Child grade (%)	A		58 (82.9)
	B		11 (15.7)
	C		1 (1.4)
Child score			5.8 ± 1.0
Number of HCCs (%)	Single		49 (70.0)
	Two		12 (17.1)
	≥ 3		9 (12.9)
Site of HCC (%)	Right lobe		59 (84.3)
	Left lobe		7 (10.0)
	Both lobes		4 (5.7)
HCC size (cm)			4.0 (2.7- 5.0)
Portal vein	Patent		65 (92.9)
(%)			
	Thrombosed		5 (7.1)
Abdominal lymphadenopathy	No		65 (92.9)
(%)	Yes		5 (7.1)

Decision of treatment	Best supportive care	7 (10.0)
	Combined therapy	1 (1.4)
(%)	Hepatectomy	3 (4.3)
	Microwave ablation	20 (28.5)
	Radioembolization	3 (4.3)
	Sorafinib	3 (4.3)
	TACE	29 (41.4)
Response to treatment according to mRECIST (%)	Stationary	4 (7.8)
	Partial response	7 (13.7)
	Complete response	34 (66.7)
	Progressive disease	6 (11.8)
Clinical decompensation (%)	Yes	9 (12.9)
	No	61 (54.5)
Alive or dead (%)	Dead	34 (48.6)
	Alive	36 (51.4)
Survival (days)		367.1 ± 173.9
miRNA-326		35.0 (12.5- 162.3)
miRNA-511		1.2 (0.3- 3.2)
miRNA-424		5.1 (2.9- 10.3)

DM: Diabetes mellitus; Hb: Hemoglobin; WBCs: White blood cells; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; INR: International nationalized ratio; AFP: α-fetoprotein; HCC: Hepatocellular carcinoma; BCLC: Barcelona clinic liver cancer; MWA: Microwave ablation; TACE: Trans-arterial chemoembolization; mRECIST: Modified response evaluation criteria in solid tumors; miRNA: MicroRNA.

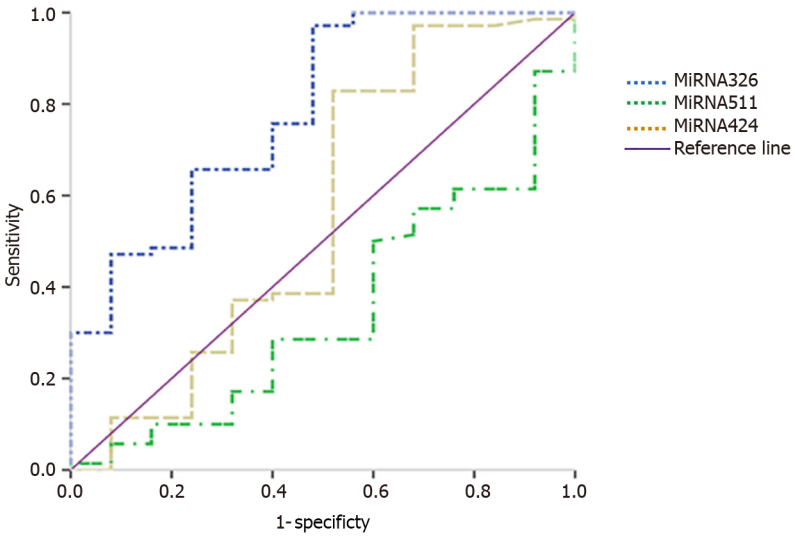

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Figure 1 Receiver operating characteristic curve of the studied miRNAs.

In our study, miRNA-326 was upregulated, in contrast to the aforementioned studies in which miRNA-326 was down-regulated. Importantly, these studies were on HCC tissues and cell lines, while our study adopted an easier methodology of testing miRNA-326 in the blood. It seems that blood tests could give paradoxical results. The study by Moya *et al*[32] assessed different miRNAs as biomarkers for prostate cancer and found a similar paradox. This difference is attributed to the possibility of preferential retainment of oncomirs (these are miRNAs that are overexpressed in cancers) and the release of tumor suppressor miRNAs into the circulation to promote oncogenesis. Added to this, the authors highlighted the physiological cancer conditions that can cause leakage of molecules.

Table 2 Studied miRNA associations with different parameters in patients with hepatocellular carcinoma

	miRNA-326	miRNA-511	miRNA-424
Gender			
Male 48 (68.6%)	33.6 (11.3-157.4)	1.1 (0.4-3.4)	5.4 (3.2-10.4)
Females 22 (31.4%)	42.3 (11.9-168.2)	1.1 (0.4-3.4)	4.4 (1.9-8.3)
P value	0.7	0.8	0.3
Smoker			
No 56 (80.0%)	27.4 (8.4-159.0)	1.1 (0.3-2.7)	5.4 (2.9-10.6)
Yes 14 (20.0%)	67.0 (28.0-177.0)	1.2 (0.7-5.9)	4.1 (2.7-7.0)
P value	0.3	0.3	0.3
Diabetes mellitus			
No 52 (74.3%)	29.4 (9.1- 119.1)	0.9 (0.3-2.5)	4.6 (2.6-9.0)
Yes 18 (25.7%)	70.4 (16.6- 218.4)	1.5 (0.9-5.1)	6.5 (4.1-13.7)
P value	0.1	0.08	0.04[a]
Child grade			
A 58 (82.9%)	64.4 (15.3-177.0)	1.2 (0.4-3.8)	5.1 (3.0-10.3)
B 11 (15.7%)	16.1 (4.1-110.7)	0.9 (0.0-1.4)	5.2 (2.6-11.9)
C 1 (1.4%)	5.7 (5.7-5.7)	0.03 (0.03-0.03)	0.9 (0.9-0.9)
P value			
B *vs* A			
C *vs* A	0.03[a]	0.1	0.7
C *vs* B	0.2	0.1	0.1
	0.7	0.3	0.3
HCC stages			
Early	32.0 (7.5-121.0)	1.0 (0.3-2.6)	4.5 (2.3-8.0)
Intermediate	69.8 (16.1-185.0)	1.4 (0.6-4.6)	5.3 (3.9-13.2)
Late	26.2 (14.5-179.8)	0.9 (0.1-2.9)	5.9 (2.5-10.1)
P value			
Intermediate *vs* Early	0.2	0.1	0.04[a]
Late *vs* Early	0.6	0.8	0.5
Late *vs* Intermediate	0.7	0.2	0.6
Performance status			
0; 43 (61.4%)	64.4 (15.8-153.3)	1.2 (0.5-3.5)	5.7 (3.1-10.2)
1; 24 (34.3%)	18.9 (7.1-184.5)	0.6 (0.0-2.5)	4.0 (1.5-10.4)
2; 2 (2.9%)	7.2 (0.5-7.8)	3.1 (0.4-3.5)	5.3 (4.8-5.5)
3; 1 (1.4%)	160.9 (160.9- 160.9)	9.6 (9.6-9.6)	22.0 (22.0-22.0)
P value	0.2	0.2	0.2

Studied miRNAs are represented as Median with Interquartile range (25% -75%), the data were analyzed by Mann-Whitney *U* test except for the Studied miRNAs associations with Performance status which is analysed by the Kruskal Wallis Test. HCC: Hepatocellular carcinoma.
[a]*P* value < 0.05 is significant.

In our study, a statistically significant difference was noted while revising the miRNA signature of miRNA-326 and miRNA-424 between our HCV-related HCC patients and healthy controls. A clear diagnostic role was identified for both markers. We would like to document another crucial issue. We found no single miRNA to correlate with the different HCC parameters. For example, miRNA-424

Table 3 Studied miRNAs in healthy controls *vs* hepatocellular carcinoma

	Controls	HCC patients	*P* value
	N = 25	*N* = 70	
miRNA-326	1.2 (0.3- 30.8)	35.0 (12.5- 162.3)	0.001[b]
miRNA-511	2.1 (0.9- 7.7)	1.2 (0.3- 3.2)	0.02[a]
miRNA-424	6.1 (0.7- 12.4)	5.1 (2.9- 10.3)	0.4

Studied miRNAs are represented as median with interquartile range (25% -75%), the data were analyzed by the Mann-Whitney *U* test. HCC: Hepatocellular carcinoma.

[a]*P* value < 0.05 is significant.

[b]*P* value < 0.01 is highly significant.

Table 4 Diagnostic performance of the studied miRNAs

	Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	95%CI	*P* value
miRNA-326	> 1.165	0.971	0.52	0.85	0.867	0.853	0.784	67.7-89.1	< 0.001[b]
miRNA-511	< 2.063	0.714	0.6	0.833	0.429	0.684	0.654	53.1-77.7	0.01[a]
miRNA-424	> 2.462	0.829	0.48	0.817	0.5	0.737	0.559	40.4-71.5	0.4

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under curve; 95%CI: 95%Confidence interval.

[a]*P* value < 0.05 is significant.

[b]*P* value < 0.01 is highly significant.

differentiated early *vs* intermediate HCC significantly, miRNA-326 was a major factor related to survival, and miRNA-511 significantly correlated with response to treatment. miRNA-326 cut-off level > 1.165 showed statistically significant sensitivity and specificity when upregulated, while miRNA-511 cut-off < 2.063 showed statistically significant sensitivity and specificity when downregulated. This provides the main clue that no single miRNA can be used for the diagnosis and prognosis of HCC. Wang and Lei identified an eleven long non-coding RNA (lncRNA) signature for predicting HCC prognosis and incorporated seven miRNAs (including miRNA-326 and -424)[33]. In another study, the authors mentioned seven miRNAs that were found to have different expressions between tumors and in-vicinity non-tumorous tissues and found that they were significantly associated with survival (including miRNA-326 and -511)[23]. Similarly, a multidimensional signature stratified the HCC patients into low-risk and high-risk groups ($P < 0.001$) in the training set, validation set, and independent set, and all were statistically significant. It showed better survival prediction power when compared to TNM staging and included three miRNAs (-149, -424, and -579)[34]. In the study by Lu *et al* [24], five biomarkers (among 33 miRNAs) significantly correlated with patient survival. These markers were miRNA-326, -421, -511, -3677, and -424. They divided patients into low-risk and high-risk groups according to the miRNA signatures. None of the high-risk patients survived for 5 years. Thus, it seems that using a combination of miRNAs gives better results than using one biomarker.

The mechanism of action of miRNA in HCC has been proposed in several experimental studies. Concerning miRNA-326, different mechanisms of action were proposed, including NF-kB expression [35], targeting MAPK1 and CSF1 as regulated by circASAP1[21], and targeting LIM and SH3 protein 1 (LASP1)[36]. Also, it acts by suppression of PDK1 in the PDK1/AKT/C-myc axis[37]. It acts as a sponge for circular RNA circ-0000517[38] and a regulator for the SMAD 6 axis[39]. HCC proliferation was found to be promoted *via* CircRNA-PTN that also sponges miRNA-326 and affects ErbB/PI3K in HCC cells [40]. Finally, HOXD-AS1 binds directly to miRNA-326, thereby targeting gene SLC27A4, which also plays a role in HCC progression and metastasis promotion[41]. Proposed mechanisms of action of miRNA-424 include the direct targeting of C-Myb[19], AKT3 and E2F3[30], E2F7 expression[42], and acts as a sponge to different lncRNAs such as lncRNA CASC9[43], LINL 00657[44], lncRNA CDKN2B-AS1[45], and lncRNA LINC00511[34]. All these targets are correlated with poor prognosis, promotion of cell viability, and migration. Also, it affected angiogenesis by activating the VEGFR-2 signaling pathway [46]. Serum clinical samples from HCC patients and healthy volunteers showed that miRNA-424 significantly decreased in HCC patients and correlated with poor overall survival and disease-free survival[47]. With regard to miRNA-511, studies proposed its action through sponging LINC01559[22], regulation of the AKT1 axis[48], and targeting PIK3R3 in the PIK3R3/AKT/mTOR signaling pathway [49].

Table 5 Survival analysis

		Dead, *N* = 34	Median (95%CI)of the EstimatedSurvival Time	Log rank (Mantel-Cox)	*P* value
Gender	Female (%)	9 (26.5)	315.0 (134.4- 495.6)	0.094	0.8
	Male (%)	25 (73.5)	441.0 (329.7- 552.3)		
Smoker	No (%)	29 (85.3)	395.0 (299.9- 490.1)	1.595	0.2
	Yes (%)	5 (14.7)	581.0 (336.4- 825.6)		
DM	No (%)	27 (79.4)	413.0 (341.7- 484.3)	0.259	0.6
	Yes (%)	7 (20.6)	429.0 (257.4- 600.6)		
BCLC stages	Early (%)	12 (35.3)	532.0 (401.2- 662.8)	5.594	0.05[a]
	Intermediate (%)	14 (41.2)	393.0 (225.7- 560.3)		
	Late (%)	8 (23.5)	284.0 (196.6- 371.4)		
Performance status	0 (%)	21 (61.8)	395.0 (314.6 - 475.4)	1.188	0.6
	1 (%)	12 (35.3)	516.0 (404.0- 628.0)		
	2 (%)	1 (2.9)	278.0 (278.0- 278.0)		
	3	0 (0.0)	-		
Child score	A (%)	26 (76.5)	429.0 (316.6- 541.4)	0.574	0.8
	B (%)	7 (20.6)	375.0 (241.9- 508.1)		
	C (%)	1 (2.9)	516.0 (516.0- 516.0)		
Response to treatment mRECIST	Stationary	4 (11.8%)	284.0 (215.4- 352.6)	2.9	0.4
	Partial response	1 (2.9%)	412.0 (412.0- 412.0)		
	Complete response	19 (55.9%)	456.0 (346.7- 565.3)		
	Progressive disease	5 (14.7%)	128.0 (0.0- 285.2)		
miRNA-326 >1.165			441.0 (336.7- 545.3)	17.1	0.001[b]
miRNA-511<2.063			441.0 (329.5- 552.5)	0.626	0.4
miRNA-424>2.462			395.0 (300.7- 489.3)	2.707	0.1

mRECIST: Modified Response Evaluation Criteria in Solid Tumors; BCLC: Barcelona Clinic Liver Cancer; DM: Diabetes mellitus; 95%CI: 95%Confidence interval.

[a]*P* value < 0.05 is significant.

[b]*P* value < 0.01 is highly significant.

Finally, we would like to highlight the aggressive behavior of HCC. Our patients were predominantly BCLC-A, Child-Pugh score A, performance status 0-1, and the majority had single lesions without evidence of vascular invasion or lymph node metastases. Moreover, we had good overall response rates (complete and partial response rates were nearly 60%). Nearly half of patients died during the follow-up period despite all these facts. This indicates the importance of primary prevention of HCV-related HCC through early management of its risk factors rather than managing HCC after its development.

Nonetheless, our study has limitations. We could not study more miRNAs that are correlated with HCC, and we could not correlate blood tests with tissue tests. However, our study is a clinical (not an experimental) one, and this helped to better correlate miRNA with different co-existing potentials and factors that could be present in actual life scenarios. In conclusion, we found a good diagnostic and prognostic role for our studied biomarkers (miRNA-326, -424, and -511) in HCC patients.

CONCLUSION

We conclude that miRNA-326, miRNA-424, and miRNA-511 have diagnostic and prognostic roles in Egyptian patients with HCV-related HCC and should be considered for better disease management.

Table 6 Studied miRNA associations with response to treatment according to modified response evaluation criteria in solid tumors

Response to treatment mRECIST	Stationary, *n* = 4 (5.7%)	Partial response, *n* = 7 (10.0%)	Complete response, *n* = 34 (48.6%)	Progressive disease, *n* = 6 (8.6%)	*P* value
miRNA-326	11.5 (2.1- 142.6)	31.1 (6.9- 209.4)	27.4 (10.0- 155.2)	69.6 (17.7- 197.9)	0.5
miRNA-511	3.8 (0.8- 10.5)	1.2 (0.3- 4.2)	1.0 (0.2- 1.5)	0.6 (0.1- 1.3)	0.05[a]
miRNA-424	4.9 (3.0- 8.6)	4.8 (2.1- 5.1)	5.4 (2.6- 13.1)	4.6 (3.1- 15.2)	0.8

mRECIST: Modified Response Evaluation Criteria in Solid Tumors.
[a]*P* value < 0.05 is significant.

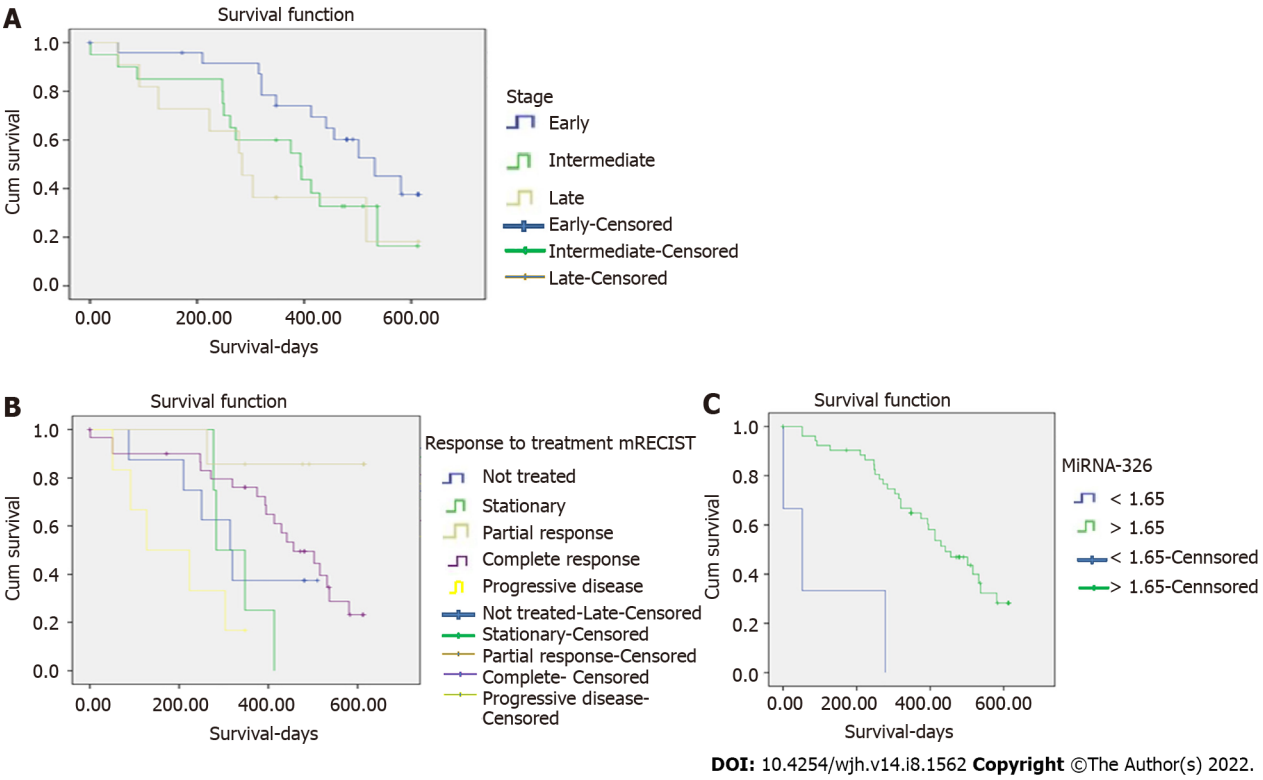

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Figure 2 Survival curve among the studied parameters. A: Survival curve in relation to stage; B: Survival curve in relation to response to treatment according to mRECIST; C: Survival curve in relation to miRNA-326.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is lethal and is the fifth most common cancer. Differential expression of microRNAs (miRNAs)-326, miRNA-424, and miRNA-511 has been associated with the diagnosis and prognosis of HCC in different populations. However, limited information is available regarding their expression in Egyptian HCC patients.

Research motivation

This study aimed to assess the role of circulating miRNAs-326, miRNA-424, and miRNA-511 from plasma as a non-invasive route of detection and to explore the impact of these miRNAs in Egyptian HCC patients.

Research objectives

The study objectives were to assess the role of circulating miRNAs-326, miRNA-424, and miRNA-511 in Egyptian HCC patients by a non-invasive method using plasma samples.

Research methods

This prospective observational study included 70 adult Egyptian patients who developed HCC on top

of HCV-related liver cirrhosis and 25 healthy age- and sex-matched participants who were seronegative for HCV and HBV, and served as controls. HCC was diagnosed according to the American Association for the Study of Liver Diseases updated practice guidelines, or was histo-pathologically confirmed. All patients were recruited from the multidisciplinary HCC clinic, the Endemic Medicine Department, Kasr al Ainy School of Medicine, Cairo University, and were HCC-treatment naïve at the time of enrollment. Exclusion criteria included; HBV co-infection, any cause of chronic liver disease other than HCV, any associated malignancies other than HCC, and prior treatment for HCV or HCC. Data collection: All patients were subjected to clinical assessment, laboratory investigations [complete blood count, prothrombin time and concentration, liver and kidney function tests, alpha-fetoprotein (AFP), hepatitis markers], ultrasound examination, and triphasic CT with documentation of HCC site, size, and number as well as the presence of PVT. At the time of enrolment into the study, blood samples were obtained from each participant, and Child-Pugh-score and BCLC stage were assessed based on the clinical, laboratory, and imaging data obtained. Whole blood samples were collected from each participant in 5-mL sterile RNAase-free vacutainer tubes containing EDTA. Blood samples were collected on ice and processed within 30 min of collection. To separate the plasma, each blood sample was centrifuged for 10 min at 1900g at 4 ℃. Plasma samples were carefully transferred into sterile RNase-free tubes. Plasma samples were then centrifuged for 10 min at 12000g at 4 ℃ to remove cellular nucleic acid contamination, and haemolysed plasma samples were excluded. Samples were separated into aliquots and immediately stored at 80 ℃ until processed. RNA extraction: Total RNA was extracted from 200 μL plasma using the miRNeasy Serum/plasma cell lysates kit, according to the manufacturer's instructions. RNA concentration and purity were monitored using a Nanodrop spectrophotometer device. Quantitative RT-PCR: The reverse transcription reaction was performed using TaqMan™ MicroRNA Reverse Transcription Kit, according to the manufacturer's instructions. miRNA-326, miRNA-511, and miRNA-424 quantifications were carried out using quantitative real-time PCR. The qRT-PCR for each sample was carried out in duplicate using TaqMan 2x universal master mix II and TaqMan microRNA Assay Mix containing PCR primers and TaqMan probes for each miRNA. The expression level of RNU6B was used as an endogenous control for normalization. To determine miRNA relative expression, it was reported as a fold change (ΔCt and $\Delta\Delta$Ct calculations). Patient management and follow-up: After blood sample withdrawal, all the patients were managed according to the BCLC guidelines after a case-by-case discussion. All HCC patients were followed up for a period of 24 mo. The response of HCC to treatment was evaluated according to mRECIST. The overall survival time of the patients was defined as the period from the initial presentation to the last follow-up or death.

Research results

The mean age of the studied cohort was 62.0 ± 7.6 years. Most of the patients were male (68.8%), non-diabetic (74.3%), non-smokers (80%), and Child A (82.9%). According to the BCLC staging system, most patients were in the early stage (48.6%). Most of the HCC lesions were single (70%), present in the right hepatic lobe (84.3%), and not associated with PVT (92.9%). That is why most of the patients were subjected to hepatectomy and microwave ablation (MWA), and most of them showed complete response according to mRECIST. All HCC patients were followed up for a period of 24 mo or until death. The mean survival time was 367.1 ± 173.9 days. The median values of miRNA-326, miRNA-511, and miRNA-424 were 35, 1.2, and 5.1, respectively. Correlation of miRNAs with different parameters in HCC patients: There was no significant association between the studied miRNAs levels and gender, smoking, or the patients' performance status. The serum level of miRNA-424 was significantly elevated in diabetic patients. There was a significant difference in miRNA-326 between Child grades A and B. Moreover, there was a significant difference in miRNA-424 between early and intermediate HCC. Diagnostic efficiency of miRNAs in our patients: On comparing the miRNAs levels between healthy participants and HCC patients, it was found that HCC patients showed significantly higher levels of miRNA-326 (P = 0.001) and significantly lower levels of miRNA-511 (P = 0.02). ROC analysis revealed the diagnostic performance of the studied miRNAs. miRNA-326 showed the best diagnostic performance, diagnosing HCC at a cut-off value of 1.165 with a sensitivity of 97.1%, specificity of 52%, PPV of 85%, and NPV of 86.7%. The AUC was 78.4% (67.7-89.1), and the overall accuracy was 85.3% (P < 0.001). Association of miRNAs with response to treatment and survival: Despite finding no statistically significant differences in the studied miRNAs between surviving and non-surviving HCC patients, we found that miRNA-326 > 1.165 was significantly associated with overall survival (P = 0.001). Moreover, there was a significant association between the BCLC stage as well as the response to treatment according to mRECIST and overall survival. It was also found that miRNA-511 was significantly associated with response to treatment according to mRECIST.

Research conclusions

We conclude that miRNA-326, miRNA-424, and miRNA-511 have diagnostic and prognostic roles in Egyptian patients with HCV-related HCC and should be considered for better disease management.

Research perspectives

miRNA-326, miRNA-424, and miRNA-511 can be detected from plasma and have diagnostic and

prognostic roles in Egyptian patients with HCV-related HCC and should be considered for better disease management.

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FOOTNOTES

Author contributions: Youssef SS conceived the study; Youssef S and ElFiky A performed the experiments, data analysis, and statistical analysis; Elbaz T and Omran D wrote the first draft; Shousha HI performed clinical examinations, conceptualization, and patient data curation; Nabeel MM, Shousha HI, Marie MS, Elzahry MA, Hashem A, Guda MF, and Abdelaziz AO performed clinical examinations; all authors contributed to manuscript reviewing and editing.

Institutional review board statement: The study was approved by the local research ethics committee of the Endemic Medicine Department, Faculty of Medicine, Cairo University, and it was conducted according to guidelines of the Declaration of Helsinki 1975. Informed written consent was obtained from each participant. All patients signed a written informed consent before inclusion in the study.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Missed opportunities for hepatitis C treatment at a tertiary care hospital in South Australia

Sreecanth Sibhi Raja, Suzanne Edwards, Jeffrey Stewart, Dep Huynh

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Abstract

BACKGROUND

Hepatitis C is a global epidemic and an estimated 230 000 Australians were living with chronic hepatitis C in 2016. Through effective public health policy and state commitment, Australia has utilised the advent of direct acting antiviral (DAA) therapy to transform the therapeutic landscape for hepatitis C virus (HCV). However, treatment rates are falling and novel public health approaches are required to maintain momentum for HCV elimination. Contemporary discourse in cascades of care have focused on expanding testing capabilities but less attention has been given to linking previously diagnosed patients back to care. Our simple and focused study rests on the premise that hospital admissions are an excellent opportunity to identify and refer previously diagnosed patients for HCV treatment.

AIM

To assess whether inpatients with HCV are appropriately referred on for treatment.

METHODS

We conducted a retrospective single centre cohort study that examined all patients with HCV presenting to The Queen Elizabeth Hospital (QEH) inpatient service between January 1 and December 31, 2017. QEH is a tertiary care hospital in South Australia. The main inclusion criteria were patients with active HCV infection who were eligible for DAA therapy. Our study cohort was identified using a comprehensive list of diagnosis based on international classification of diseases-10 AM codes for chronic viral hepatitis. Patients were excluded from the analysis if they had previously received DAA therapy or spontaneously cleared HCV. Patients presenting with decompensated liver cirrhosis or other systemic

medical conditions conferring poor short-term prognosis were also excluded from the analysis. The primary outcome of our study was referral of patients for HCV treatment. Secondary outcomes included assessment of factors predicting treatment referral.

RESULTS

There were 309 inpatients identified with hepatitis C as a principal or additional diagnosis between January 1 and December 31, 2017. Of these patients, 148 had active HCV infection without prior treatment or spontaneous clearance. Overall, 131 patients were deemed eligible for DAA treatment and included in the main analysis. Mean patient age was 47.75 ± 1.08 years, and 69% of the cohort were male and 13% identified as Aboriginal or Torres Strait Islander. Liver cirrhosis was a complication of hepatitis C in 7% of the study cohort. Only 10 patients were newly diagnosed with HCV infection during the study period with the remainder having been diagnosed prior to the study.

CONCLUSION

Under 25% of hepatitis C patients presenting to an Australian tertiary hospital were appropriately referred for treatment. Advanced age, cirrhosis and admission under medical specialties were predictors of treatment referral.

Key Words: Hepatitis C; Viral hepatitis; Treatment cascade; Hepatology; Public health; Missed opportunities

Core tip: Hepatitis C virus (HCV) treatment in Australia has undergone a major paradigm shift since the advent of direct acting anti-viral (DAA) therapy. Uptake of DAA therapy for HCV is falling despite universalisation of access through pharmaceutical benefit scheme listing. In our study, 26% of chronic hepatitis C patients presenting to a tertiary hospital were referred for treatment. Hospital admissions constitute an excellent opportunity to identify and treat patients with chronic hepatitis C. Extrapolating this study, both nationally and internationally, would serve to supplement treatment numbers in the goal of HCV eradication.

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INTRODUCTION

Globally, over 70 million people are infected with hepatitis C virus (HCV)[1]. While usually asymptomatic initially, chronic HCV infection can result in the development of cirrhosis and hepatocellular carcinoma over the course of a few decades[2,3]. HCV is primarily spread through use of shared needles in either healthcare or recreational drug use settings. Viral transmission can also occur through exchange of bodily fluids and blood transfusions. Contemporary therapeutic advances in the treatment of HCV have rendered the virus essentially curable within 30 years of its first identification. This has been lauded as a major scientific landmark[4].

Historically, HCV treatment had revolved around lengthy interferon-based regimens. These treatment courses were poorly tolerated and had limited efficacy. The major shift in the treatment paradigm for HCV has been fuelled by the development of highly effective oral direct acting antiviral (DAA) agents. Sustained virological response (SVR) rates have continued to improve with the introduction of pan-genotypic DAA regimens such as sofosbuvir/velpatasvir and glecaprivir/pibrentasvir. These oral treatment regimens have excellent side-effect profiles and SVR rates > 95% across all genotypes in both trial and real world settings[5,6]. The emergence of DAAs has resulted in the World Health Organization ambitiously targeting 2030 for the elimination of HCV as a public health threat. This ambitious target encompasses a 90% decline in new infections, a reduction in HCV-related mortality by 65% and treatment provision for 80% of those infected[7].

While uptake of DAAs around the world has been variable, Australia has been in the vanguard of nations on the path to HCV elimination. DAA therapy is expensive with a 12-wk course of sofosbuvir/velpatasvir costing over $US 75000. Australia's initial success was facilitated by the landmark decision to provide DAA therapy to individuals through the pharmaceutical benefit scheme (PBS) scheme[8]. The Australian PBS scheme is a state sponsored programme subsidising prescription medications for

Australian citizens and permanent residents. PBS subsidisation ensures that patients with HCV only pay a dispensing fee for DAA prescriptions which equates to $AUD 38.30 for general patients and $AUD 6.20 for concessional patients. This has significantly reduced financial barriers to accessing DAA therapy for Australians with HCV. Universalisation of DAA therapy in March 2016 resulted in > 44 000 Australians, approximately 20% of the total estimated population with chronic HCV, being initiated on treatment by June 2017.

However treatment uptake has consistently fallen in Australia since the introduction of DAAs[9]. Eliminating barriers to treatment has supplanted improving SVR as the dominant issue in the current HCV therapeutic landscape[10]. Novel approaches are required to ensure that Australia can meet the aforementioned elimination targets. Modelling has suggested that testing needs to be increased by 50% to achieve this[11]. To this end, recent studies have advocated programmes aimed at identifying and treating HCV in prisons and needle and syringe exchange programmes[12,13].

One area that is often overlooked is the opportunistic identification of untreated patients within hospital inpatient cohorts. Patients with HCV often have complex medical and social situations that necessitate frequent presentations to hospital. Additionally, a significant proportion of patients diagnosed with HCV prior to DAA therapy have been lost to follow up. Hospital admissions thus represent an excellent opportunity to identify both newly and previously diagnosed HCV patients. Our study aims to assess the extent to which current practices ensure that patients with HCV presenting to tertiary hospitals are referred on for DAA therapy.

MATERIALS AND METHODS

Setting

The Queen Elizabeth Hospital (QEH) is a tertiary hospital in Northwest Adelaide with 35 000 inpatient admissions *per* annum. Northwest Adelaide has the highest prevalence of HCV in South Australia[14].

Study cohort

Our single-centre retrospective study included all patients with a principle or secondary diagnosis of HCV presenting to any QEH inpatient service between January 1and December 31, 2017. The study cohort was identified using a comprehensive list of diagnosis based on international classification of diseases-10 AM codes for chronic viral hepatitis. These codes included B17.1, B18.2, B18.8, B18.9 as either principal or additional diagnosis.

Data ascertainment/collection

De-identified data for our patients were collated from electronic medical records and stored on secure hospital databases. Data collated included demographic data, HCV antibody and RNA status, unit of admission, prior treatment information, presence of complications such as liver cirrhosis or, referral to specialist service, attendance at specialist clinic, initiation of DAA therapy and completion of DAA therapy.

We excluded patients who had previously been successfully treated for HCV and those who had spontaneously cleared the virus. Patients were defined as having active HCV on the basis of their most recent polymerase chain reaction study. Successful treatment was defined as achievement of SVR at 12 wk post-treatment (SVR12).

The primary study outcome was referral of patients to gastroenterology or infectious diseases services for treatment of HCV by the admitting team. For the purposes of this study, admitting hospital teams were divided into four subdivisions: Medical, Surgical, Mental Health and Emergency Departments. Referral to specialist service for the purpose of HCV treatment had to be clearly documented in electronic medical records to satisfy our inclusion criteria. Attendance at a clinic and initiation and completion of treatment were determined through electronic medical records and cross-referencing with local HCV treatment databases.

Statistical analysis

Statistical analysis was performed using GraphPad Prism for Windows. Parametric data are presented as mean ± SEM. Categorical variables were compared using the χ^2 and Fisher's exact tests. Quantitative variables were analysed by *t*-test and, when the assumption for normality was not met, the Mann–Whitney *U* test. Univariate binary logistic models were performed to investigate the association between HCV treatment referral and five predictors: clinical unit, age, gender, Aboriginal or Torres Strait Islander status and cirrhosis status.

Ethics

This study was completed in accordance with the guidelines set by the SA Health Research Governance Policy Directive.

RESULTS

A total of 309 inpatients were identified as having HCV as a principal or additional diagnosis between January 1 and December 31, 2017. On further inspection, 96 of these patients had previously received treatment and had successfully been cleared of the virus. Forty-two patients had successfully cleared the virus spontaneously without evidence of treatment. Twenty-five patients had been erroneously labelled as having HCV despite negative serological results. Of the 148 patients with active HCV, 17 were either admitted under palliative care or died within 6 mo of admission. Consequently, 131 patients of our study cohort were deemed eligible for treatment and included in the main analysis (Table 1).

The mean age of eligible patients was 47.75 ± 1.08 years, and 69% of the cohort were male and 13% identified as Aboriginal or Torres Strait Islander. Liver cirrhosis was a complication of HCV in 7% of the study cohort. Only 10 patients were newly diagnosed with HCV during the study period with the remainder of patients having been diagnosed prior to the study. In terms of admitting unit, the majority of patients were admitted under medical specialties (41/131). A substantial proportion of patients were also admitted under surgical specialties (37/131) and mental health (38/131). The remaining 15 patients were admitted as short stay patients by the Emergency Department.

Overall, 32 patients (24%) were referred on for treatment of their HCV and 51% of patients admitted by medical teams were referred. Mental health and surgical teams referred 18% and 11% of patients, respectively. None of the patients admitted under the Emergency Department were referred. The odds ratio (OR) for appropriate referral for medical specialties *versus* nonmedical specialties was 7.20 [95% confidence interval (CI): 3.0–17.1; $P < 0.0001$]. There was a significant association between referral status and age [odds ratio (OR): 1.05, 95% CI: 1.01–1.08, $P = 0.0097$). Those with liver cirrhosis were also significantly more likely to be referred on for treatment (OR: 19.0, 95% CI: 3.7–96.3, $P = 0.0004$). Neither gender (male *vs* female, OR: 1.3, 95% CI: 0.5–3.0, $P = 0.62$) nor Aboriginal status (OR: 1.1, 95% CI: 0.3–3.7, $P = 0.85$) were predictors of treatment referral.

Referral was necessary but not sufficient to ensure successful treatment of HCV in the context of our study. Four patients were referred to their GP for treatment with none of these patients commencing treatment. Thirteen of the 28 patients referred to the gastroenterology or infectious diseases clinic commenced treatment. Eight patients were documented to have completed treatment and achieve SVR12.

DISCUSSION

Our study found that 76% of inpatients with chronic hepatitis C were not referred on for treatment. This suggests that current hospital practices are not adequately addressing the issue of HCV elimination. Low referral rates can be traced back to two main factors.

Firstly, HCV can often be overlooked in the face of more acutely pressing medical and social issues that precipitated hospital admission in the first place. This is unsurprising given that, until late stage hepatological complications arise, HCV is often asymptomatic. This notion would appear to be supported by the fact that presence of cirrhosis was a strong predictor for treatment referral. Secondly, knowledge of HCV treatment advances may be limited outside of gastroenterological and physician spheres[15]. This assertion would appear to be supported by the significantly higher referral rates for patients admitted under the care of physicians. Succinctly, the insidious nature of HCV and limitations of professional awareness constituted the major treatment barriers in the context of our study. Thus, proactive measures are required to ensure that HCV patients are first identified and subsequently treated. Comprehensive multifaceted approaches are required to ensure that opportunities for inpatient HCV treatment are taken advantage of. These approaches should focus on educational initiatives for healthcare professionals and patients, optimisation of electronic medical records notifications and ensuring community outreach initiatives for HCV patients on discharge.

Only 10% of our cohort were newly diagnosed with HCV during the study period. The majority of our patients had been diagnosed with HCV in the pre-DAA era and had been lost to follow-up. This finding is in keeping with a recent population level Swedish study demonstrating that 61% of patients diagnosed with hepatitis C in 2013 were lost to follow-up[16]. Contemporary hepatitis C cascade of care discourse has focused on expanding testing capabilities as a means of targeting resistant pockets of the virus[11]. Linking previously diagnosed patients back with treatment pathways should also form an integral component of the multifaceted public health approach to hepatitis C elimination. Performing this study provided our gastroenterology and infectious diseases units with a database of additional HCV patients to contact and initiate on treatment. Our viral hepatitis nurses were able to successfully treat 27% of patients in whom earlier treatment opportunities were missed. Moreover, our study methodology provides a template for systematically identifying HCV patients from inpatient cohorts in order to link patients to care. Extrapolating this study to other health networks across Australia, and indeed internationally, would supplement overall treatment numbers in the pursuit of HCV elimination.

Table 1 Baseline characteristics

Total number of patients	131
Age, yr	47.75 ± 1.08
Gender, *n* (%)	
Male	90 (69)
Female	41 (31)
Aboriginal or Torres Strait Islander, *n* (%)	17 (13)
Presence of cirrhosis, *n* (%)	
Cirrhosis	9 (6.9)
No clinical evidence of cirrhosis	122 (93.1)
New diagnosis of HCV, *n* (%)	
Yes	10 (7.6)
No	121 (92.4)
Unit of admission, *n* (%)	
Medical	41 (31)
Surgical	37 (28)
Mental health	38 (29)
Emergency department	15 (11)
Referral for DAA treatment, *n* (%)	
Yes	32 (24.4)
No	99 (75.6)
Outcome after referral, *n* (%)	
Attendance at specialist clinic	14 (10.6)
Initiation of DAA treatment	13 (9.9)
Achievement of SVR12	8 (6.1)

HCV: Hepatitis C virus; DAA: Direct acting anti-viral.

It is important to note that appropriate identification and referral of patients is necessary but not sufficient for treatment of hepatitis C. In the context of our study, 50% of referred patients did not attend clinic appointments and were subsequently lost to routine follow-up. Moreover, only 25% of patients referred for treatment of hepatitis C achieved SVR12 by the end of the study period. This equates to 6% of the total cohort of hepatitis C patients presenting to the QEH being cleared of the virus during the study period. Low clinic attendance rates of referred patients can be traced back to a combination of institutional and patient-related factors. Institutional factors included variable levels of patient counselling and deficits in community engagement with either patients or primary care providers after hospital discharge. Patient-related factors contributing to loss to follow-up included poor health literacy, low prioritisation of health needs, and adverse socioeconomic circumstances that may have predisposed them to HCV infection.

Our findings highlight the limitations of centralised specialist services in treating poorly engaged patients and the need for greater emphasis on community treatment. From our experience, viral hepatitis nurses were ideally placed to identify these hitherto missed opportunities for treatment and ensure sustained linkage of patients to HCV care. Furthermore, primary health physicians play a vital role in achieving HCV elimination given their unparalleled access to patients. These realities have been recognised in Australia with updated guidelines permitting both experienced hepatitis nurse practitioners and primary care physicians to prescribe DAA therapy. Early successes of these policy updates are evidenced by recent data from the Kirby Institute, Sydney showing that primary care prescribers had overtaken their specialist counterparts[9]. Nevertheless, specialist hepatitis treatment centres have an important role in treating complex HCV patients as well as providing training for and longitudinal capacity strengthening of community DAA prescribers.

Our study had a number of limitations. Firstly, our study was limited by a small sample size and being a single centre study. Although the study number was small, it still highlighted an area of concern. We identified a large number of missed opportunities for hepatitis C treatment in our inpatient cohort. The sample size was adequate to identify the subspecialties to target additional education and protocols for referral and treatment. Secondly, the use of hospital coding to identify hepatitis C patients introduced a sampling bias by systematically excluding from the analysis patients with HCV without known or recorded diagnosis. Our study did not specifically address assess the extent to which current hospital practices ensure that at-risk patients are investigated for and diagnosed with HCV. Furthermore, given that PBS listing, and thus universalisation, of DAA therapy was only initiated in March 2016, we were only able to study a short time-frame.

CONCLUSION

Hepatitis C remains an important public health issue. The advent of state subsidised DAA therapy has transformed the therapeutic landscape of hepatitis C in Australia. Despite this, patient engagement and social issues remain important barriers to the elimination of hepatitis C. Our study found that 24% of hospital inpatients with hepatitis C were referred for DAA therapy. Less than half of referred patients initiated DAA therapy. Our study methodology provides a template for systematically identifying HCV patients from inpatient cohorts in order to link patients to care. Extrapolating this study to other health networks across Australia, and indeed internationally, would supplement overall treatment numbers in the pursuit of HCV elimination.

ARTICLE HIGHLIGHTS

Research background

An estimated 230 000 Australians were living with hepatitis C virus (HCV) in 2006. The advent of direct acting antiviral (DAA) therapy has revolutionized treatment paradigms and greatly improved rates of sustained virological response. Nevertheless, several challenges remain in striving for the goal of HCV elimination by 2030.

Research motivation

Multifaceted interventions and approaches are required to maintain momentum in order to achieve HCV elimination by 2030. Contemporary discourse in cascades of viral hepatitis care focus on expanding testing as the primary means for identifying and treating remaining HCV patients. Enhancing testing infrastructure and introducing systematic viral assessments in correctional facilities, needle exchange programmes, homeless shelters and in high-risk communities are examples of initiatives currently being undertaken. Less attention has been given to linking patients with pre-existing diagnoses of HCV back to care. Inpatient hospital admissions represent an excellent opportunity to identify and treat both newly and previously diagnosed HCV patients.

Research objectives

To assess whether patients with HCV admitted to a tertiary Australian hospital were appropriately referred on for treatment. Our study was designed to assess the extent to which current hospital practices maximise opportunity for identifying and treating patients with HCV.

Research methods

Our study constituted a retrospective cohort study that assessed patients with HCV admitted to The Queen Elizabeth Hospital, Adelaide in 2017. The primary outcome of our study was referral of patients for HCV treatment. Secondary outcomes included assessment of factors predicting treatment referral.

Research results

There were 148 patients with active hepatitis C. Overall, 131 patients of our study cohort were deemed eligible for DAA treatment and included in the main analysis. Thirty-two patients (24%) were referred on for treatment of their HCV infection. The odds ratio (OR) for appropriate referral for physician specialties *versus* nonphysician specialties was 7.2 (95% CI: 3.0–17.1, $P < 0.0001$). Older patients (OR: 1.05, 95% CI: 1.05–1.08, $P = 0.097$) and those with liver cirrhosis (OR: 19.0, 95% CI: 3.7–96.3, $P = 0.0004$) were significantly more likely to be referred on for treatment. Thirteen patients referred to gastroenterology or infectious diseases clinics commenced treatment.

Research conclusions

Hepatitis C remains an important public health issue. The advent of state subsidised DAA therapy has

transformed the therapeutic landscape of hepatitis C in Australia. Despite this, patient engagement and social issues remain important barriers to the elimination of hepatitis C. Our study found that 76% of chronic hepatitis C patients presenting to the inpatient services of our tertiary hospital were not referred on for treatment. Furthermore, DAA treatment was initiated in less than half of referred patients. This suggests that current hospital practices are not adequately identifying patients with HCV. Hospital admissions constitute an excellent opportunity to identify and treat patients with chronic hepatitis C. Extrapolating this study across tertiary healthcare institutions in Australia and overseas would facilitate re-engagement of previously diagnosed HCV patients with care cascade and supplement overall treatment numbers.

Research perspectives

Our study has internationally relevant implications as our methodology provides a template for systematically identifying HCV patients from inpatient cohorts. Extrapolating this across other national and international tertiary healthcare institutions will serve to supplement treatment rates of HCV as we strive to achieve goals of HCV elimination. Our findings also demonstrate that identification of HCV patients is necessary but not in itself sufficient to achieve cure of HCV. Proactive measures are required to ensure that identified patients successfully commence and complete treatment courses. Hospitals thus require comprehensive multifaceted approaches to ensure opportunities for treatment are taken advantage of.

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We acknowledge that the land on which our study was conducted is the traditional lands for the Kaurna people and that we respect their spiritual relationship with their country. We also acknowledge the Kaurna people as the custodians of the Adelaide region and that their cultural and heritage beliefs are still as important to the living Kaurna people today.

FOOTNOTES

Author contributions: Raja SS contributed to study conception and design, also contributed to data collection and analysis as well as drafting and revision of the manuscript; Huyn D contributed to study design, supervision, data analysis and manuscript preparation; Stewart J contributed to data collection and analysis; Edwards S performed statistical analysis.

Institutional review board statement: This retrospective cohort study was completed in accordance with the guidelines set by the South Australian Health Research Governance Policy Directive.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous de-identified data.

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at sreecanth.raja@sa.gov.au. Individual consent was not obtained but the presented data is de-identified without risk of identification.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Survival outcomes and predictors of mortality, re-bleeding and complications for acute severe variceal bleeding requiring balloon tamponade

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Abstract

BACKGROUND

Acute severe variceal bleeding (AVB) refractory to medical and endoscopic therapy is infrequent but associated with high mortality. Historical cohort studies from 1970-1980s no longer represent the current population as balloon tamponade is no longer first-line therapy for variceal bleeding; treatments including vasoactive therapies, intravenous antibiotics, endoscopic variceal band ligation are routinely used, and there is improved access to definitive treatments including transjugular intrahepatic portosystemic shunts. However, only a few studies from the current era exist to describe the practice of balloon tamponade, its outcomes, and predictors with a requirement for further updated information.

AIM

To describe current management of AVB requiring balloon tamponade and identify the outcomes and predictors of mortality, re-bleeding and complications.

METHODS

A retrospective multi-centre cohort study of 80 adult patients across two large tertiary health networks from 2008 to 2019 in Australia who underwent balloon tamponade using a Sengstaken-Blakemore tube (SBT) were included for analysis.

Patients were identified using coding for balloon tamponade. The primary outcome of this study was all-cause mortality at 6 wk after the index AVB. Secondary outcomes included re-bleeding during hospitalisation and complications of balloon tamponade. Predictors of these outcomes were determined using univariate and multivariate binomial regression.

RESULTS

The all-cause mortality rates during admission and at 6-, 26- and 52 wk were 48.8%, 51.2% and 53.8%, respectively. Primary haemostasis was achieved in 91.3% and re-bleeding during hospitalisation occurred in 34.2%. Independent predictors of 6 wk mortality on multivariate analysis included the Model for Endstage Liver disease (MELD) score (OR 1.21, 95%CI 1.06-1.41, $P = 0.006$), advanced hepatocellular carcinoma (OR 11.51, 95%CI 1.61-82.20, $P = 0.015$) and re-bleeding (OR 13.06, 95%CI 3.06-55.71, $P < 0.001$). There were no relevant predictors of re-bleeding but a large proportion in which this occurred did not survive 6 wk (76.0% *vs* 24%). Although mucosal trauma was the most common documented complication after SBT insertion (89.5%), serious complications from SBT insertion were uncommon (6.3%) and included 1 patient who died from oesophageal perforation.

CONCLUSION

In refractory AVB, balloon tamponade salvage therapy is associated with high rates of primary haemostasis with low rates of serious complications. Re-bleeding and mortality however, remain high.

Key Words: Balloon tamponade; Acute variceal bleeding; Sengstaken-Blakemore tube; Mortality; Complications; Haemostasis

Core Tip: Acute severe variceal bleeding requiring balloon tamponade remains associated with high mortality rates of approximately 50%. Sengstaken-Blakemore tube achieves excellent primary haemostasis rates in > 90% however re-bleeding is common at approximately 30% with subsequent death in approximately 75%. Predictors of all-cause mortality at 6 wk included a greater Model for Endstage Liver disease score, re-bleeding and advanced hepatocellular carcinoma. The most commonly reported complication from SBT was mucosal trauma, which was conservatively managed, with only a small proportion resulting in serious complications (6.3%). There was significant variability amongst technical aspects of balloon tamponade insertion which may result from the infrequent need to perform this procedure.

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INTRODUCTION

Acute severe variceal bleeding (AVB) refractory to endoscopic variceal band ligation (EVBL) and injection therapy occurs infrequently in 10%-20% of variceal haemorrhage but is associated with significant mortality rates of over 30%[1]. In this situation, the main salvage strategy has traditionally involved balloon tamponade with various devices including the Sengstaken-Blakemore tube (SBT)[2], the Minnesota tube and the Linton-Nachlas tube, which are similar devices that differ in terms of the number of balloons and ports[3]. While covered self-expandable metallic oesophageal stents have more recently become available, with potential advantages of improved safety and efficacy over balloon tamponade[1,4], oesophageal stents are still not routinely available in many treating centres. Both these rescue techniques serve a temporising role while awaiting further definitive options including transjugular intrahepatic portosystemic shunt (TIPS), balloon-occluded retrograde transvenous obliteration (BRTO) or liver transplantation[5-7].

Previous retrospective cohort studies published in the 1970s and 1980s demonstrated that balloon tamponade successfully achieved primary haemostasis in 40-98% of cases, however it was associated with a high risk of both re-bleeding (35%-70%) and procedural complications[8-12]. Importantly, the management of AVB has evolved significantly since this time and thus these studies are not reflective of

current practice. For example, balloon tamponade is no longer employed as a first-line management option and endoscopic sclerotherapy has long been superseded by EVBL. In addition, the therapeutic armamentarium for AVB has significantly expanded and now encompasses vasoactive treatment, empiric antibiotics, endoscopic therapies and radiologic procedures such as TIPS and BRTO. Finally, expert opinion-based consensus guidelines for variceal bleeding are also now available[5,6]. Currently there is a paucity of literature examining the clinical outcomes of patients treated with current standards of care, who require balloon tamponade for AVB[13,14]. Subsequently this study aims to: (1) Describe the current clinical practice surrounding management of endoscopically uncontrollable AVB requiring balloon tamponade; (2) Identify the outcomes; and (3) Predictors of mortality, re-bleeding and complications of balloon tamponade.

MATERIALS AND METHODS

Study design

A multi-centre retrospective cohort study was undertaken across Monash Health and Eastern Health, two large metropolitan tertiary health care services in Victoria, Australia. All consecutive adult patients (> 18 years) who underwent balloon tamponade using a SBT for refractory AVB between 1st January 2008 until 31st December 2019 were included. Patients were identified by the International Classification of Diseases-10 procedure code for gastro-oesophageal balloon tamponade. Data extracted from medical records included baseline demographic information, liver disease severity indicators, clinical and biochemical data relating to variceal bleeding, practice surrounding insertion and monitoring of balloon tamponade devices and clinical outcomes including re-bleeding, survival up to 52 wk and complications of both variceal bleeding and balloon tamponade. All patients were risk stratified using the AIMS65, Rockall, pre-endoscopy Rockall (pre-Rockall), Child-Pugh and Model for Endstage Liver disease (MELD) scores on admission prior to index gastroscopy.

Acute variceal bleeding management protocols

AVB was managed according to published United Kingdom and United States guidelines[5,6]. Patients with suspected variceal bleeding received intravenous (IV) antibiotics (ceftriaxone or piperacillin-tazobactam) and vasoactive therapy with either an octreotide infusion (50 microgram (mcg) bolus, followed by a 25-50 mcg/hour infusion) or IV terlipressin (0.85-1.7 mg 6 hourly). A restrictive blood transfusion policy is standard at the treating centres and patients typically receive packed red cells if their haemoglobin is < 70 g/L (or < 80 g/L in the presence of ischaemic heart disease) with a target haemoglobin level of 80-90 g/L. Endoscopy was performed in either a dedicated endoscopy suite or operating theatre with sedation administered by an anaesthetist in all cases. Bleeding oesophageal varices were treated with EVBL and bleeding gastric varices were treated with variceal obturation using histoacryl and lipiodol or thrombin. In cases of AVB not amenable to endoscopic therapy, both interventional radiology (including TIPS or BRTO) and upper gastrointestinal surgery services were available.

Study outcome measures

The primary outcome measure of this study was all-cause mortality after AVB requiring balloon tamponade which was assessed at 6 wk and followed up at 26 and 52 wk. Secondary outcomes assessed included re-bleeding after insertion of SBT and complications of balloon tamponade during the hospital admission. Primary haemostasis was defined as the clinical cessation of variceal bleeding after balloon tamponade during the index hospitalisation and re-bleeding defined as further bleeding after primary haemostasis was achieved upon removal or balloon deflation of the SBT. Patients without cirrhosis (non-cirrhotic portal hypertension) who required balloon tamponade for AVB were excluded from the predictors of mortality analyses but included in remaining analyses surrounding balloon tamponade practice.

Ethics approval

The Monash Health Human Research Ethics Committee assessed this study as low risk (RES-21-0000-218Q-70254) and did not require participant informed consent.

Statistical analysis

Descriptive statistics was used to analyse continuous variables expressed as median and interquartile range (IQR) for continuous non-parametric variables, and absolute frequencies between groups for categorical variables. Analysis was performed on factors potentially contributing to death, re-bleeding and balloon tamponade complications using Mann-Whitney U test for continuous variables and Fisher's Exact Test for dichotamous variables. Univariate binomial regression was used to identify potential clinically relevant variables predictive of death, re-bleeding and complications and those that reached statistical significance ($P < 0.10$) were then included in a multivariate binomial regression analysis. Missing data was excluded from multivariate analysis. Statistical analysis was performed using licensed

SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Figures for survival analysis were prepared using licensed GraphPad Prism software (GraphPad Software for Windows, Version 9.0.0, Sand Diego, California).

RESULTS

Baseline characteristics

Overall, there were 81 adult patients who required balloon tamponade with SBT for endoscopically uncontrollable AVB. Insufficient information was available for 1 patient who was subsequently excluded from the analysis (n = 80). Cirrhosis was diagnosed in 75 (93.8%) patients but 5 (6.3%) had non-cirrhotic portal hypertension and were not included in the predictors of mortality analyses.

Most of the patients were male (61, 76.3%) with a median age of 56 years (range 34 to 80 years). Most patients with cirrhosis had advanced cirrhosis with a median Child-Pugh score 9 (IQR 8-11) and median MELD score 17 (IQR 13-21). The most common aetiology of cirrhosis was alcohol-related liver disease (54, 72.0%) of which 34 (63.0%) were actively still consuming alcohol, followed by chronic hepatitis B or C (30, 40.0%) and then non-alcoholic steatohepatitis (7, 9.3%). Eleven (14.7%) patients with hepatocellular carcinoma (HCC) had Stage C (Advanced) or Stage D (Terminal) staging as per the Barcelona Clinic Liver Cancer classification[15].

The presence of varices had been documented in 51 (63.8%) patients prior to the index bleed. Of these patients, 47.1% of these had prophylactic EVBL prior to the index AVB at a median duration of 3 wk prior (IQR 2-12 wk). Non-selective beta blocker use was documented in 23 (28.8%) patients at the time of variceal haemorrhage.

The baseline characteristics of the patients are presented in Table 1 which compares characteristics of those who survived and those who died at 6 wk after the index variceal bleed. Compared to patients who survived, patients who died were noted to have significantly higher Child-Pugh and MELD scores (P = 0.004 and P < 0.001, respectively), international normalised ratio (P < 0.001), albumin (P = 0.034), bilirubin (P = 0.003), sodium (P = 0.025), creatinine (P = 0.014) and lactate levels (P = 0.007) at the time of presentation to hospital. In addition, the diagnosis of HCC was significantly more prevalent in patients who died within 6 wk (P = 0.019).

Emergency and endoscopic management of index variceal bleed

Including all patients who required balloon tamponade (n = 80), at presentation of the index variceal bleed, 48.8% (39) of patients were tachycardic with a heart rate over 100 beats/min and 28.7% (23) were hypotensive with a systolic blood pressure < 90 mmHg. A reduced Glasgow Coma Scale score was recorded in 24 (30%) patients and 30% (24) required oxygen supplementation at concentrations of at least FiO2 35% for hypoxia. Almost all patients received vasoactive agent therapy with either terlipressin or octreotide (79, 98.8%) and IV antibiotics (77, 96.3%) in the emergency department. Most patients received vitamin K (65, 81.3%) and 16.5% (13 patients) received human prothrombin complex concentrate (Prothrombinex®) in an attempt to correct coagulopathy.

Table 2 summarises the medical and endoscopic management of the index variceal bleed and the clinical practice surrounding insertion of SBT for salvage therapy. The median time to initial endoscopy after AVB was 6.8 h (IQR 4.2-19.0 h). The source of bleeding was noted to be oesophageal varices in 80.0% (64 patients) with 20.0% (16 patients) due to gastric varices. Initial endoscopic therapy was performed in 45 patients (56.4%). Insertion of balloon tamponade devices were performed by specialist endoscopists in all cases, most commonly during the initial gastroscopy. The indications for balloon tamponade with SBT were incomplete haemostasis (39, 48.8%), poor endoscopic views (26, 32.5%) or both (15, 18.8%). The SBT insertion approach was documented to be oral in 49 (61.3%) and nasal in 22 (31.0%), while no documentation was available in 9 (11.3%). Confirmation of SBT position by either direct endoscopic vision or chest X-ray was documented in 80.5% of procedures. The gastric balloon was inflated in all cases with a median volume of 285 mL air (range 50-500 mL), while the oesophageal balloon was inflated in 22 (27.5%) cases with a median volume of 100 mL air (range 20-500 mL) (Table 2). Documentation of devices used to maintain traction on the inflated SBT was very inconsistent. Repeat gastroscopy was performed in 61 (76.3%) patients and generally occurred in the following 24 to 48 h after the index gastroscopy with repeat endoscopic therapy performed in 33.8% (27 patients). Patients that did not undergo repeat gastroscopy had rapidly deteriorated and died.

Mortality, re-bleeding and balloon tamponade complication outcomes

The outcomes of mortality, re-bleeding and complications from balloon tamponade are summarised in Figure 1 and Table 3. Inpatient mortality was 48.8% (39 deaths), and the mortality rates at 6-, 26- and 52 wk were 48.8% (39 deaths), 51.2% (41 deaths) and 53.8% (43 deaths), respectively. The causes of death during the index inpatient hospitalisation included refractory bleeding with failure to achieve haemostasis (20, 51.3%), sepsis with multiorgan failure (14, 35.9%), aspiration pneumonia (3, 7.7%) and 1 patient died from an oesophageal perforation due to SBT (2.6%). This patient had his initial gastroscopy and SBT inserted in a regional hospital prior to transfer, where a chest X-ray revealed the gastric balloon

Table 1 Baseline characteristics of patients with cirrhosis requiring balloon tamponade for acute severe variceal bleeding comparing death and survival at 6 wk

Variable (*n* = 75)	Survived (*n* = 40)	Death (*n* = 35)	*P* value
Male sex	30 (75.0)	28 (80.0)	0.783
Age in years	58 (48, 65)	54 (49, 65)	0.629
Cirrhosis aetiology: Alcoholic liver disease, Chronic viral hepatitis, NASH	26 (65.0), 16 (40.0), 6 (15.0)	28 (80.0), 14 (40.0), 1 (2.9)	0.199, 1.00, 0.113
Child-Pugh score	8 (7, 10)	11 (8, 12)	0.004
MELD score	14 (11, 18)	19 (16, 24)	< 0.001
AIMS65 score	2 (1, 2.5)	3 (2, 3)	0.004
Glasgow-Blatchford score	12 (9, 14)	12 (9, 15)	0.908
Complete Rockall score	8 (7, 8)	8 (7, 8)	0.159
HCC	2 (5.0)	9 (25.7)	0.019
Portal vein thrombosis	6 (15.0)	4 (11.4)	0.742
Antiplatelets or anticoagulants	7 (17.5)	2 (5.7)	0.162
Glasgow Coma Scale	15 (14, 15)	15 (14, 15)	0.503
Systolic blood pressure	101 (91, 123)	96 (76, 124)	0.328
Heart rate	95 (82, 114)	104 (91, 118)	0.304
Significant hypoxia (FiO2 > 35%)	10 (25.0)	13 (37.1)	0.319
Haemoglobin g/L	86 (74, 103)	78 (63, 111)	0.538
Platelets × 10^9/L	96 (72, 123)	110 (75, 150)	0.204
Albumin g/L	26 (22, 28)	22 (19, 28)	0.034
INR	1.5 (1.3, 1.7)	1.9 (1.5, 2.3)	< 0.001
Bilirubin μmol/L	29 (14, 54)	51 (29, 119)	0.003
Serum sodium mmol/L	139 (135, 141)	135 (129, 140)	0.025
Serum creatinine μmol/L	74 (62, 92)	95 (73, 124)	0.014
Serum pH	7.36 (7.26, 7.44)	7.30 (7.10, 7.41)	0.072
Lactate mmol/L	3.10 (1.65, 5.35)	5.20 (2.65, 9.80)	0.007

Categorical variables presented as number, (percentage) and continuous variables as median, (interquartile range). NASH: Non-alcoholic steatohepatitis; MELD: Model for end-stage liver disease; HCC: Hepatocellular carcinoma; FiO2: Fraction of inspired oxygen; INR: International normalised ratio.

was either inflated or migrated into the oesophagus and caused perforation and mediastinitis.

The insertion of SBT successfully achieved primary haemostasis in 73 (91.3%) patients, with no survivors amongst those where this was not achieved. Re-bleeding occurred in 34.2% (25) after achieving primary haemostasis, of which further balloon tamponade was performed in 16 of these patients. Of the 25 patients who had experienced re-bleeding, the inpatient mortality rate was 76.0%. TIPS was performed in 17 (21.3%) patients at a median of 2.95 d from balloon tamponade insertion, of which 5 patients died. One patient underwent liver transplantation and survived.

Complications associated with SBT insertion were documented in 19 (23.8%) patients. The most common complication (17, 89.5%) was superficial mucosal trauma without perforation which was managed conservatively. Only a few serious complications occurred in 5 patients (6.3%) and included aspiration pneumonia recorded in 4 patients (of which 2 died during the index hospitalisation) and 1 patient died from oesophageal perforation as mentioned above.

Predictors of mortality, re-bleeding and complications of balloon tamponade

As most patients who survived their hospital admission continued to survive to 52 wk after the index variceal bleed, the mortality rates and thus the predictors on univariate and multivariate analyses are very similar for all study time points. Subsequently results for predictors will be presented for the primary endpoint of 6 wk mortality after index variceal bleed for cirrhotic patients only (n = 75).

Table 2 Management of index variceal bleed and characteristics around insertion of Sengstaken-Blakemore tube

Variable	Value (*n* = 80)
Vasoactive agent	79 (98.8)
Terlipressin	30 (37.5)
Octreotide	49 (61.3
Empiric antibiotics	77 (96.3)
Time to endoscopy from bleed, h	6.8 (4.2, 19.0)
Site of variceal bleed	
Oesophageal varices	64 (80.0)
Gastric varices	16 (20.0)
Initial endoscopic therapy	
Variceal band ligation	43 (53.8)
Cyanoacrylate injection	2 (2.5)
Indication for SBT insertion	
Incomplete haemostasis	54 (67.5)
Poor endoscopic views	41 (51.2)
Approach for SBT insertion (*n* = 71)	
Nasal	22 (31.0)
Oral	49 (69.0)
Confirmation of SBT position	
X-ray	44 (55.0)
Direct endoscopic vision	18 (22.5)
No documentation	15 (18.8)
Gastric balloon inflation (*n* = 76)	
Volume, mL	285 (250, 300)
Time inflated, h	26.8 (16.9, 44.7)
Oesophageal balloon inflation (*n* = 22)	
Volume, mL	100 (39, 100)
Time inflated, h	20.5 (10.2, 36.8)
Therapy post-SBT deflation (*n* = 62)	
Repeat endoscopic therapy	27 (43.5)
Further balloon tamponade	16 (20.0)

Categorical variables presented as number, (percentage) and continuous variables as median, (interquartile range). SBT: Sengstaken-Blakemore tube.

Upon univariate analyses, variables that significantly predicted 6 wk mortality included: Markers of liver disease severity (Child-Pugh score, MELD score, international normalised ratio, bilirubin, serum creatinine and sodium), pH and serum lactate, the presence of HCC, the AIMS65 score and re-bleeding. Of the validated upper gastrointestinal bleeding risk scoring algorithms used to predict outcomes, only the AIMS65 score[16] reached significance at univariate analysis (OR 1.96, 95%CI 1.15- 3.35, P = 0.014) while the Glasgow-Blatchford score (GBS) (OR 0.98, 95%CI 0.86-1.15, P = 0.767), pre-endoscopy and complete Rockall scores (both OR 1.44, 95%CI 0.86-2.43, P = 0.168) were not significant[17-19]. Results of the univariate analyses are detailed in Table 4.

To avoid collinearity, the only liver disease severity indicator used in the multivariate analysis was the MELD score. MELD scores of > 19 have been shown to predict 6 wk mortality of > 20% for AVB[20]. Predictors of 6 wk mortality on multivariate analysis in this cohort showed that the MELD score, the presence of HCC and re-bleeding were statistically significant independent predictors.

Table 3 Patient outcomes following Sengstaken-Blakemore Tube insertion

Variable	Value (*n* = 80)
Length of stay, d	
Total hospital LOS	11.0 (7.0, 19.5)
ICU LOS	4.90 (2.3, 9.1)
Duration of mechanical ventilation, h	89.5 (39.3, 153.8)
Blood product transfusion	
Packed red cells, units	6 (4, 10)
Platelets, units	1 (0, 3)
Fresh frozen plasma, units	2 (1, 6)
Achieved primary haemostasis	73 (91.3)
Re-bleeding	25 (34.2)
Complications of SBT	19 (34.5)
Mucosal ulceration and tears (without perforation)	17 (89.5)
Aspiration pneumonia	4 (21.0)
Perforation	1 (5.3)
TIPS	
Underwent TIPS	17 (21.3)
Time to TIPS from SBT, d	2.95 (1.4, 4.1)
Survival	
Survived hospital admission	41 (51.2)
Alive at 6 wk	41 (51.2)
Alive at 26 wk	39 (48.8)
Alive at 52 wk	37 (46.3)

Categorical variables presented as number, (percentage) and continuous variables as median, (interquartile range). LOS: Length of stay; SBT: Sengstaken-Blakemore tube; TIPS: Transjugular intrahepatic portosystemic shunt.

The survival curves over 52 wk for MELD score >19, HCC and re-bleeding are shown in Figures 2-4, respectively.

On univariate analysis, there were no relevant predictors for re-bleeding after salvage therapy using SBT for AVB. When comparing 6 wk outcomes in those that re-bled after primary haemostasis to those that did not, re-bleeding was resulted in significantly greater mortality (76.0% *vs* 27.1%, $P < 0.001$), a longer duration of mechanical ventilation ($P = 0.026$) and higher transfusion requirements for packed red cells ($P = 0.001$) and fresh frozen plasma ($P = 0.001$) as shown in Table 5.

Non-serious mucosal trauma which was conservatively managed was not thought to be a significant complication in the life-threatening context of refractory AVB requiring balloon tamponade. Given that the incidence of serious complications from SBT insertion were uncommon and occurred in only 5 patients, no further analyses was performed to identify predictors.

DISCUSSION

AVB represents a life-threatening emergency in patients with liver cirrhosis and portal hypertension. However, with current treatment paradigms, 6 wk mortality has improved to 10%-15%[21]. Variceal bleeding refractory to first-line therapy requiring salvage therapy with balloon tamponade reflects a serious life-threatening condition in advanced liver disease that is associated with significant mortality. We demonstrate a 6 wk mortality rate of 48.8% in this cohort of patients despite current standards of care. Balloon tamponade with a SBT was found to be a very effective rescue therapy in refractory AVB, achieving primary haemostasis in 91.3% of patients with a low serious complication rate of 6.3%. On multivariate analysis, increasing MELD score, the presence of HCC and re-bleeding were all associated with a significantly increased odds of mortality.

Table 4 Predictors of 6 wk mortality after acute severe variceal bleeding requiring balloon tamponade

Variable (*n* = 75)	Univariate analysis		Multivariate analysis (*n* = 68)	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age	0.99 (0.95-1.04)	0.669	-	-
Male sex	1.33 (0.45-3.98)	0.606	-	-
Haemodynamic instability	2.01 (0.75-5.41)	0.167	-	-
INR	8.19 (2.10-31.89)	0.002	-	-
Albumin	0.924 (0.85-1.01)	0.063	-	-
Bilirubin	1.02 (1.01-1.030)	0.009	-	-
Creatinine	1.02 (1.01-1.035)	0.024	-	-
Sodium	0.93 (0.86-0.99)	0.045	-	-
pH	0.02 (0.01-0.68)	0.030	-	-
Lactate	1.16 (1.02-1.31)	0.019	-	-
AIMS65 score	1.96 (1.15-3.35)	0.014	-	-
GBS	0.98 (0.86-1.15)	0.767		
Rockall score	1.44 (0.86-2.43)	0.168		
Child-Pugh score	1.40 (1.01-1.80)	0.007	-	-
MELD score	1.22 (1.09-1.37)	< 0.001	1.21 (1.06-1.41)	0.006
HCC	6.58 (1.31-32.95)	0.022	11.51 (1.61-82.20)	0.015
Re-bleeding	8.76 (2.77-27.73)	< 0.001	13.06 (3.06- 55.71)	< 0.001
SBT complications	1.40 (0.44-4.51)	0.573	-	-
TIPS performed	0.30 (0.09-1.04)	0.058	-	-

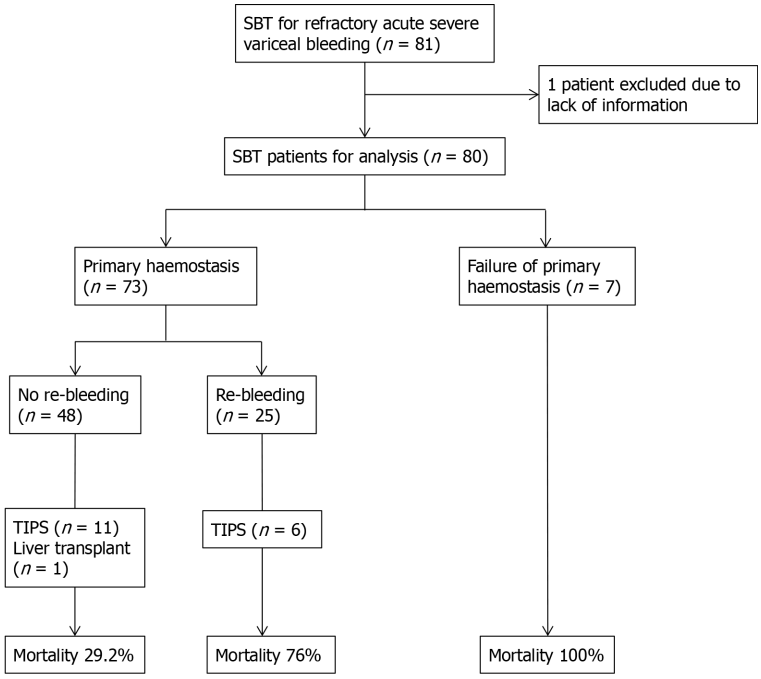
INR: International normalised ratio; GBS: Glasgow-Blatchford score; MELD: Model for end-stage liver disease; HCC: Hepatocellular carcinoma; SBT: Sengstaken-Blakemore tube; TIPS: Transjugular intrahepatic portosystemic shunt.

Table 5 Outcomes for patients who re-bled after Sengstaken-Blakemore tube insertion for acute severe variceal bleeding

Variable (*n* = 73)	No Re-bleeding (*n* = 48)	Re-bleeding (*n* = 25)	*P* value
Duration of mechanical ventilation (h)	86.0 (46.0, 134.5)	138.0 (79.0, 210.0)	0.026
No. of packed RBC transfused	5 (3, 7)	10 (5, 16)	0.001
No. of platelets transfused	1 (0, 2)	1 (0, 4)	0.430
No. of FFP transfused	2 (0, 4)	6 (2, 14)	0.001
6 wk mortality	13 (27.1)	19 (76.0)	< 0.001

Categorical variables presented as number, (percentage) and continuous variables as median, (interquartile range). RBC: Red blood cells; FFP: Fresh frozen plasma.

This study represents one of the largest series to examine the efficacy of SBT in patients presenting with AVB treated with current standards of care; an era where nearly all patients routinely receive vasoactive therapy and IV antibiotics, timely access to emergency endoscopic therapies and access to early TIPS. Balloon tamponade now represents a rescue therapy utilised in the 10-20% of patients with AVB in whom haemostasis cannot be achieved with vasoactive therapy and endoscopic techniques such as EVBL. Our 6 wk mortality rate of 48.8% is comparable to other modern cohorts at 41%-60%[4,13,14]. In comparison with older cohorts from 1970-1980s with pooled 30-day to 6 wk mortality rates of 32.5% [1], the modern studies counterintuitively demonstrate a higher mortality rate. However, the historical cohorts often used balloon tamponade as a first-line treatment option and thus the cohorts are not readily comparable. Interestingly, in 2017 Nadler *et al*[13] reported similar survival rates to our study even though the rate of TIPS performed was much higher than in our cohort at 55.9% overall (19 of 34



DOI: 10.4254/wjh.v14.i8.1584

Figure 1 Schematic for mortality outcome at 52 wk for patients requiring SBT for acute severe variceal bleeding. SBT: Sengstaken-Blakemore tube; TIPS: Transjugular intrahepatic portosystemic shunt.

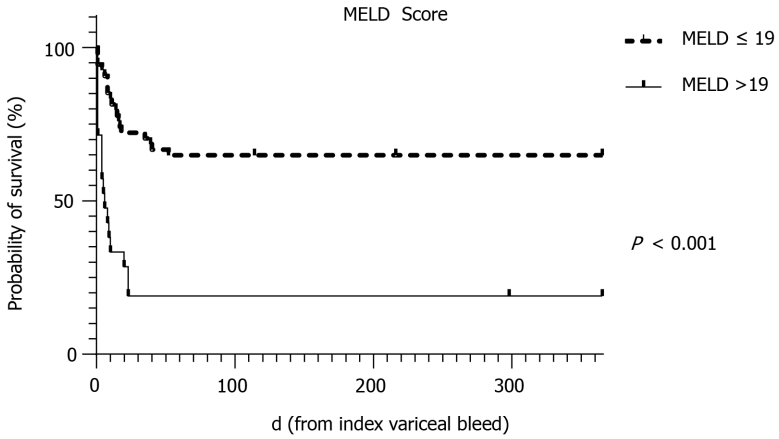


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Figure 2 Survival curve for Model for Endstage Liver disease score > 19 over 52 wk. MELD score: Model for end-stage liver disease score.

patients). In our cohort, only 21.3% underwent TIPS at a median of 70.8 h (IQR 34.3-97.4 h) although variability in both expertise and availability of this radiological procedure throughout the years in our health services may have existed and the proportion of patients in whom TIPS may have been contraindicated remains unclear. Consideration of early TIPS insertion is currently recommended in all Child Pugh C patients and Child Pugh B patients with active bleeding who present with AVB[22]. TIPS placement is generally performed within 72 h (but ideally within 24 h) due to a high risk of treatment failure[7]. The early re-bleeding rate of 34.2% and high associated mortality found in our cohort highlights the propensity for serious complications in patients with AVB refractory to first-line treatments. Thus, if TIPS is considered in this cohort of patients, it should ideally be performed as soon as possible after primary haemostasis is achieved while the patient remains haemodynamically stable.

This study supports previous evidence that balloon tamponade with a SBT remains very effective at achieving primary haemostasis in 91.3%. Of the 7 patients who did not achieve primary haemostasis, all had clinical evidence of ongoing bleeding despite SBT placement and rapidly deteriorated with haemodynamic instability and death within h despite maximal vasopressor and inotropic support. Apart from 1 patient where the gastric balloon was inflated to 100 mL, all others had inflation of the gastric balloon to adequate volumes (250-400 mL) with the oesophageal balloon also documented to be

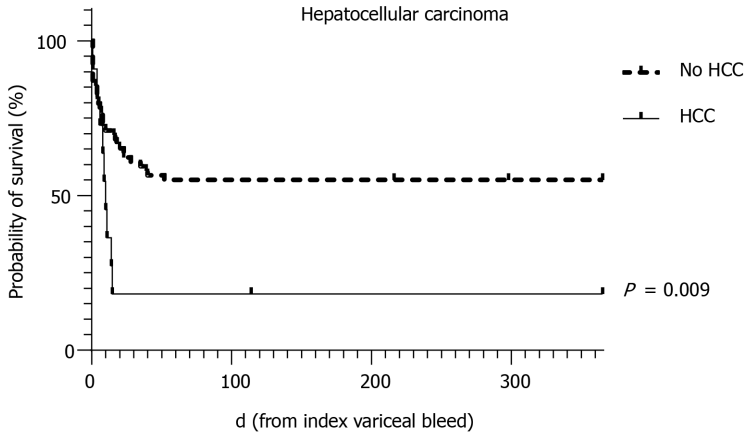

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Figure 3 Survival curve for hepatocellular carcinoma over 52 wk. HCC: Hepatocellular carcinoma.

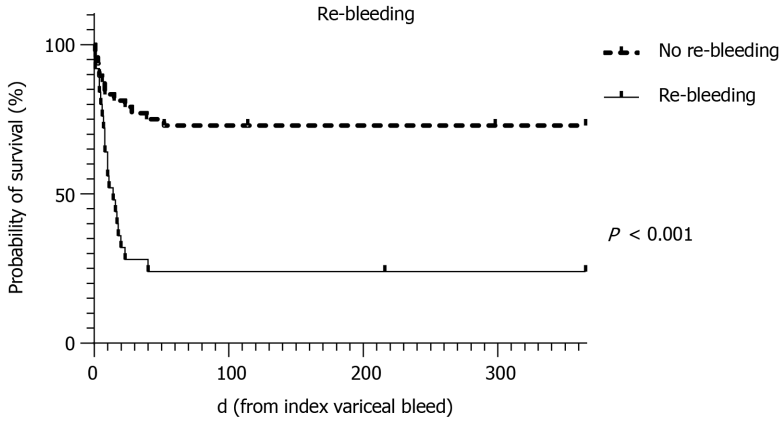

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Figure 4 Survival curve for re-bleeding over 52 wk.

inflated in 2 patients. Our rates of primary haemostasis are comparable with historical larger cohorts published in the 1970 and 1980s at 90.7% and 88.5%[11,23]. However, compared to the other current studies, our rates of primary haemostasis are higher than those reported by Choi *et al*[14] and Escorsell *et al*[4] at 75.8% and 47%, respectively. Our re-bleeding rates lie between that of the 1970-1980s cohort (43%)[11] and Choi *et al* (22%)[14], and similarly we did not identify any significant relevant predictors for re-bleeding. We have showed that re-bleeding was also associated with greater mortality (76.1% *vs* 27.1%, $P = 0.001$) and required greater use of resources including blood products and mechanical ventilation. However, the serious complication rates of 6.3% we observed from SBT insertion was significantly lower than studies from the 1970s-1980s (approximately 32%)[1,11].

In our study, the main predictors of 6 wk mortality on univariate and multivariate analysis were similar to those previously reported for AVB in cirrhosis and largely reflect liver disease severity eg. Child-Pugh and MELD scores (and its components) or severe biochemical systemic disturbance eg. pH and lactate[14,24]. In terms of validated tools for prognostication of upper gastrointestinal bleeding, we identified that the AIMS65 score significantly predicted 6 wk mortality but not the GBS or Rockall scores. A previous study has also demonstrated superiority of the AIMS65 score over the GBS and pre-endoscopy Rockall scores[25]. In addition, we also found advanced HCC and re-bleeding independently predicted 6 wk mortality. With regards to advanced HCC, 9 of 11 patients with SBT for acute severe variceal bleeding died during the admission suggesting that the utility of this SBT in this patient subgroup needs to be considered in context of the futility of the situation, particularly as it is inevitably resource-heavy, requiring invasive monitoring and intensive care admission.

We also identified significant variability amongst several aspects of clinical practice around SBT insertion at our centres, particularly around the inflation volumes of air into the gastric and oesophageal balloons. General guidelines[3] have recommended approximately 250-400 mL insertion of air into the gastric balloon based on clinical assessment, however 20.0% used < 250 mL with several of these noting migration of the SBT on confirmation chest X-ray due to under-filling. The oesophageal balloon is generally inflated to 25-40 mmHg or approximately 150 mL however 45.5% of oesophageal balloons

were inflated to < 70 mL which is likely inadequate. Varying degrees of experience are expected with SBT insertion as most centres may only encounter this situation a few times every year, and formalised training is likely beneficial to optimise survival rates by appropriate tamponade technique and to prevent complications of oesophageal perforation, which may occur with balloon migration into the oesophagus from under-filling the gastric balloon. A previous survey of United States gastroenterologists and hepatologists from the American Association for the Study of Liver Diseases found that most respondents had not received training for balloon tamponade over the last 2 years and no trainees at that time were comfortable with balloon tamponade[26].

This cohort study has certain limitations, particularly its retrospective nature and that identification of the study population relies on accurate coding. However, due to the infrequent need for this procedure, prospective data collection remains challenging. While there was variation in SBT balloon volume inflation, which may result in suboptimal use of this technique, this is the only study that attempts to provide the technical information surrounding this procedure in a real world cohort. Also, none of the patients at any of our health centres had oesophageal stents inserted for haemostasis in the study time period, which have more recently been shown to be superior to balloon tamponade[4]. Nonetheless, to our knowledge this is the largest cohort study available in the current era with most patients treated according to clinical practice guidelines. Other modern cohort studies of acute severe variceal bleeding requiring balloon tamponade remain scarce and the SBT insertion was often not performed by trained specialist gastroenterologists.

CONCLUSION

In conclusion, in the modern era of standardised medical and endoscopic therapies to treat AVB, salvage techniques such as balloon tamponade remain relevant for the time being. Overall, this condition remains associated with a high mortality of approximately 50% and although rates of primary haemostasis remain excellent, rates of re-bleeding occur in around one third of cases with high rates of subsequent death. These outcomes have not significantly changed when compared with the 1970-1980s even with improved therapies. However, rates of serious complications are low. Patients who survived the admission were likely to survive until at least 52 wk. Independent predictors for mortality include a higher MELD score, re-bleeding and advanced HCC which may assist in further stratification of at-risk individuals for either early definitive therapy with TIPS or early palliation.

ARTICLE HIGHLIGHTS

Research background

Salvage treatment using balloon tamponade techniques such as Sengstaken-Blakemore tubes (SBT) represents the most severe end of the spectrum of acute variceal bleeding (AVB), where failure to achieve primary haemostasis inevitably results in death. However, few studies report on the clinical practice and outcomes of this procedure in the current era, and only include small study populations where balloon tamponade is often performed by non-specialists in the emergency department setting. This retrospective multi-centre cohort study is the largest study including 80 patients over a decade who have undergone SBT for salvage therapy performed by gastroenterologists during endoscopy in tertiary hospitals. This study provides detailed technical aspects of the SBT insertion procedure and provides insight into the success rate, clinical outcomes of patients who undergo SBT insertion for refractory AVB and predictors of mortality, re-bleeding and complications from SBT.

Research motivation

The main topics of this study include detailed descriptions regarding the real-world practice of SBT performed by gastroenterologists in tertiary hospitals, and the clinical outcomes and predictors of short- and long-term mortality after SBT for AVB, the success rate of balloon tamponade in achieving primary haemostasis and the rate of re-bleeding and complications arising from SBT insertion. Information regarding these topics are not currently available for the current era which significantly differs from historical cohorts from the 1970-1980s due to a very different patient population where balloon tamponade was often first-line therapy. Currently, there are clear expert opinion-based consensus guidelines using a range of medical and endoscopic therapies and definitive treatment with radiologic procedures or liver transplantation for AVB. Furthermore, performing salvage technique with SBT is highly resource-intense and thus appropriate risk stratification to optimise outcomes for patients is required.

Research objectives

To assess the primary outcome which was all-cause mortality of AVB requiring SBT in the short-term (6 wk) as well as long-term (52 wk) and the secondary outcomes of re-bleeding and complications after

SBT insertion. The predictors of these outcomes were also analysed. These objectives were all achieved apart from the predictors of complications from SBT as serious complications were infrequent.

Research methods

Due to the infrequent need to perform SBT for AVB, an appropriate method to undertake this study resulted in a multi-centre retrospective cohort study including 80 adult patients with SBT for refractory AVB from 2008 to 2019. The study population was identified using International Classification of Diseases-10 codes and clinical data was collected from medical records. Descriptive statistics, univariate and multivariate binomial regression and survival analyses were used to analyse the data collected.

Research results

SBT salvage for refractory AVB is a life-threatening condition with high mortality rates of 48.8% at 6 wk and 53.8% at 52 wk. The SBT procedure was highly successful in achieving primary haemostasis in 91.3% of patients but re-bleeding was common at 34.2% and associated with very high mortality of 76.0%. The predictors of mortality after SBT insertion included increased severity of liver disease, severe metabolic disturbance, presence of hepatocellular carcinoma (HCC) and re-bleeding. Serious complications from SBT insertion were uncommon at 6.3% and the main complications were superficial mucosal trauma without perforation which was managed conservatively. Despite this procedure being performed by specialist gastroenterologists in this study, there was still significant variation amongst technical aspects of the SBT procedure particularly amongst gastric and oesophageal balloon inflation volumes.

Research conclusions

In the current era, SBT as a salvage therapy for refractory AVB continues to be associated with high short and long-term mortality rates. The utilisation of this temporising procedure remains relevant and is associated with high rates of primary haemostasis over 90%. As the mortality rate exceeds 75% after re-bleeding, this highlights the importance of prompt treatment with definitive therapies such as transjugular intrahepatic portosystemic shunts to optimise clinical outcomes. Furthermore, as SBT is associated with intense use of resources with even greater mortality in the presence of advanced HCC, this study suggests early palliation may be more appropriate in this futile setting.

Research perspectives

Future directions of this research should focus on strategies to optimise the clinical outcomes for this cohort of severe refractory AVB including prevention, the use of covered self-expandable oesophageal stents and prompt transition to definitive treatments before re-bleeding occurs. Further studies into risk stratification for optimal outcomes is required as well to assist clinicians in decision making regarding whether or not salvage therapy should be performed at all.

FOOTNOTES

Author contributions: Keung C designed the study, collected and analysed data and wrote the manuscript; Morgan A collected data and wrote the manuscript; Le ST reviewed the statistical analysis and performed critical revisions of the manuscript; Robertson M performed critical revisions of the manuscript; Urquhart P performed critical revisions of the manuscript; Swan M designed and supervised the study and performed critical revisions of the manuscript.

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ORIGINAL ARTICLE

Retrospective Study

Simple diagnostic algorithm identifying at-risk nonalcoholic fatty liver disease patients needing specialty referral within the United States

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Abstract

BACKGROUND

There is an urgent need to risk stratify patients with suspected nonalcoholic fatty liver disease (NAFLD) and identify those with fibrotic nonalcoholic steatohepatitis. This study aims to apply a simple diagnostic algorithm to identify subjects with at-risk NAFLD in the general population.

AIM

To apply a simple diagnostic algorithm to identify subjects with at-risk NAFLD in the general population.

METHODS

Adult subjects were included from the National Health and Nutrition Examination Survey database (2017-2018) if they had elevated alanine aminotransferase (ALT) and excluded if they had evidence of viral hepatitis or significant alcohol consumption. A fibrosis-4 (FIB4) cutoff of 1.3 differentiated patients with low risk *vs* high risk disease. If patients had FIB4 > 1.3, a FAST score < 0.35 ruled out advanced fibrosis. Patients with FAST > 0.35 were referred to a specialist. The same algorithm was applied to subjects with type 2 diabetes mellitus (T2DM).

RESULTS

Three thousand six hundred and sixty-nine patients were identified who met all inclusion and exclusion criteria. From this cohort, 911 (28.6%) patients had elevated ALT of which 236 (22.9%) patients had elevated FIB4 scores ≥ 1.3. Among patients with elevated FIB4 score, 75 (24.4%) had elevated FAST scores, ruling in advanced fibrosis. This accounts for 2.0% of the overall study population. Applying this algorithm to 737 patients with T2DM, 213 (35.4%) patients had elevated ALT, 85 (37.9%) had elevated FIB4, and 42 (46.1%) had elevated FAST scores. This accounts for 5.7% of the population with T2DM.

CONCLUSION

The application of this algorithm to identify at-risk NAFLD patients in need for specialty care is feasible and demonstrates that the vast majority of patients do not need subspecialty referral for NAFLD.

Key Words: Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis; Hepatology; Diabetes; Endocrinology; Primary care

Core Tip: Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis represent a major public health threat as the incidence and prevalence continues to rise. This patient population has the potential to overwhelm hepatology clinics if not appropriately triaged by those physicians making referrals. This manuscript presents a simple diagnostic algorithm that outlines how physicians can approach an undifferentiated patient with findings concerning for NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) undoubtedly presents a significant public health crisis in the coming years. The estimated global prevalence of NAFLD is about 25% with higher prevalence in middle-aged American adults at 38%[1,2]. As projected by Census.gov, the population of the United States is expected to surpass 350 million by 2030[3]. While the demand will continue to increase for specialty referral, it is likely that the triaging of these patients into low-risk and high-risk will increasingly fall on the shoulders of primary care physicians (PCPs).

NAFLD has a spectrum ranging from nonalcoholic fatty liver (NAFL) or simple steatosis to nonalcoholic steatohepatitis (NASH) which is characterized by hepatic inflammation and hepatocyte ballooning leading to progressive fibrosis (F1-F3) and eventually cirrhosis (F4)[4-7]. Several studies have shown that patients with NASH and significant fibrosis (^{3}F2) have increased risk for developing major adverse liver outcomes such as ascites, encephalopathy, and variceal bleeding[8,9].

There are several different methods for determining the presence and severity of NAFLD in the general and at-risk population. Checking alanine aminotransferase (ALT) levels has been recommended by several professional societies as a simple screening tool for NAFLD in at-risk populations such as those with metabolic syndrome and type 2 diabetes mellitus (T2DM)[10] {, 2016 #538}{, 2016 #538}. The fibrosis-4 (FIB4) index was developed and validated to identify patients with advanced fibrosis. Its use has been validated in patients with NAFLD; however, there has been some debate about the optimal cutoff[11-13]. By choosing a cutoff of 1.3 to differentiate low risk from high risk patients, our aim was to optimize the sensitivity to ensure patients with advanced fibrosis are not excluded prematurely. This cutoff point has been validated in patients with NAFLD and NASH and is generally regarded as the optimal benchmark for ruling out patients who are low risk for NASH[14,15].

Vibration controlled transient elastography (VCTE) is a more direct measure of liver stiffness. In a validation cohort of nearly 400 patients, VCTE performed well in identifying patients with advanced fibrosis and cirrhosis with areas under the receiver operating curve of 0.83 and 0.93, respectively[16]. Measuring the liver stiffness measure (LSM) and controlled attenuated parameter (CAP), VCTE or FibroScan® is a relatively inexpensive way to identify patients at risk for advanced liver disease. The

FAST score, which is calculated from combining LSM, CAP and aspartate aminotransferase data, is a noninvasive metric that has improved sensitivity and specificity compared to FibroScan® alone in terms of identifying patients with NASH and fibrosis stage F2 or higher (at-risk NAFLD). In a validation cohort, the cutoff of 0.35 was chosen for its 90% sensitivity in identifying at-risk NAFLD patients[17].

Given that this disease process can take several decades to become clinically significant and that there is significant phenotypic variation, identifying patients who may qualify for therapeutic interventions has become increasingly important. Often, patients are referred to hepatology after their disease has progressed to cirrhosis when there is more limited opportunity to reverse disease course compared to earlier therapy. Conversely, patients can be referred for evaluation extremely early in their disease course at which point they are not candidates for pharmacologic therapy. Currently, there are no FDA-approved therapies for NAFLD or NASH. However, patients with advanced or bridging fibrosis (F2-F3) and cirrhosis (F4) are the targets for several ongoing clinical trials[18]. Catching patients in this therapeutic window presents a significant obstacle in the efficacious management of NASH. Presently, there are no unifying diagnostic algorithms to help these providers differentiate between which patients need referral to hepatology. Several algorithms that combined serologic tests and VCTE have been proposed but their implications for the US healthcare system in terms of resource utilization and need for specialty providers have not been evaluated. The aim of this study was to assess the application of a simple diagnostic algorithm that combined ALT, FIB4 and the FAST score to identify subjects with at-risk NAFLD in the US general population and in those with T2DM.

MATERIALS AND METHODS

Database

The National Health and Nutrition Examination Survey (NHANES) is a deidentified database created in partnership with the Center for Disease Control and Prevention. This database catalogs patient information from patient-completed surveys and objective medical data obtained from physical exams. In addition to demographic, socioeconomic, dietary, and health-related questions, the NHANES database also contains medical, dental, physiologic, and laboratory measurements[19].

Definitions and inclusion criteria

We identified all adult subjects from the NHANES database between 2017 and 2018 who had valid VCTE data. Patients were excluded if they had any history or evidence of viral hepatitis (B or C), or significant alcohol consumption as defined by an average of ≥ 2 alcoholic beverages per day in men and ≥ 1 alcoholic beverage in women. Elevated ALT was defined as having values > 19 IU/L in women and > 30 IU/L in men.

Statistical analysis

Patients were considered for be at risk for NAFLD if they had elevated ALT score (> 19 U/L for female and > 30 U/L for male) in the absence of excessive alcohol consumption or viral hepatitis. FIB4 scores were calculated on all patients. Those with FIB4 < 1.3 were deemed low-risk for at-risk NAFLD while those with FIB4 ≥ 1.3 were subject to further evaluation with the FAST score. Subjects with a FAST score < 0.35 were also deemed low risk. If patients had both an elevated FIB4 and elevated FAST score, they were considered high risk for advanced liver disease and warranted further evaluation by a specialist. The same algorithm was applied to patients with T2DM. Appropriate survey weights were applied for all analyses which were performed using Stata version 17 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.).

RESULTS

Cohort characteristics

A total of 9254 patients were identified in the NHANES database between 2017 and 2018. From this list, 3808 patients (41.1%) were excluded based on the aforementioned exclusion criteria. Another 1018 patients (11.0%) had not completed transient elastography and 759 patients (8.2%) did not have satisfactory record of alcohol consumption. The final study population included 3669 patients meeting all inclusion and exclusion criteria. Among these patients, 737 patients (7.9%) had T2DM (Figure 1).

Applying the diagnostic algorithm to the entire cohort

In the overall study population, 911 patients (28.6%) had elevated ALT per our inclusion criteria. Among these patients with elevated ALT, 236 patients (22.9%) had an elevated FIB4 score ≥ 1.3. Among the 236 patients with an elevated FIB4 score, 75 patients (24.4%) had an elevated FAST score ≥ 0.35. This accounts for 2.0% of the overall study population (Figure 2).

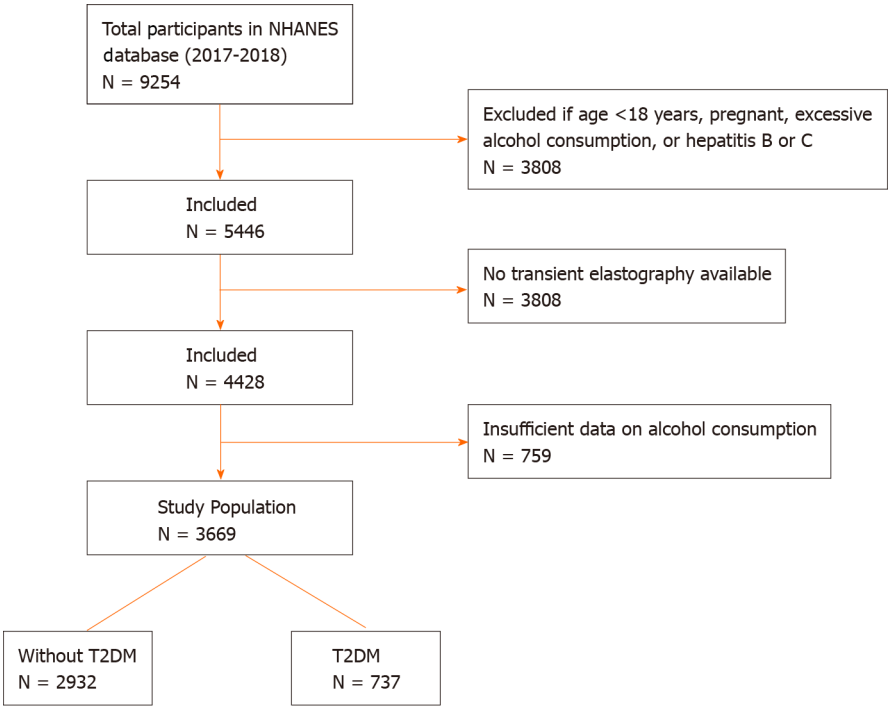

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Figure 1 Flow chart for study population. A total of 9254 participants were identified from the National Health and Nutrition Examination Survey, from which 3,669 patients met all inclusion and exclusion criteria. From this cohort, 737 patients were identified with type 2 diabetes. NHANES: National Health and Nutrition Examination Survey; T2DM: Type 2 diabetes.

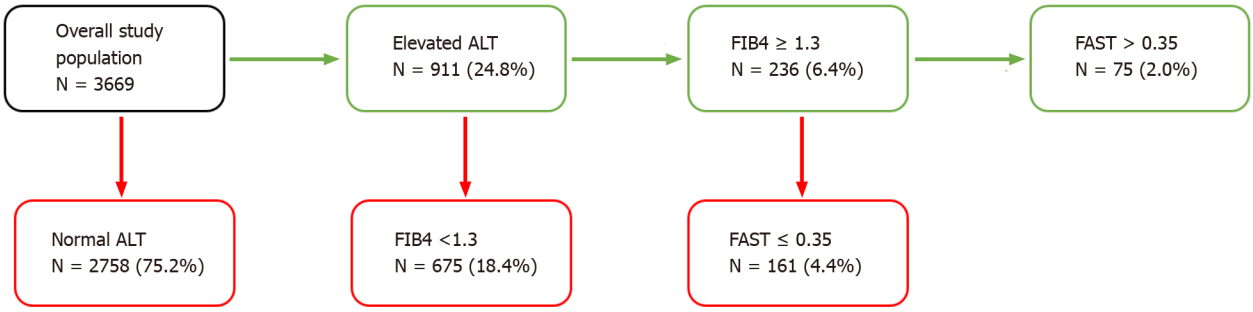

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Figure 2 Triage based on non-invasive tests for overall study population. From the study population of 3669 patients, 75 (2.0%) were identified who satisfied all the parameters of our algorithm: elevated alanine aminotransferase value, elevated fibrosis-4 score ≥ 1.3, and elevated FAST score > 0.35. ALT: Alanine aminotransferase; FIB4: Fibrosis-4.

When comparing the patients at high risk for clinically significant NASH to their low-risk counterparts, several variables were clinically significant. Patients with at-risk NAFLD were more likely to be older (57.6% *vs* 47.3%, $P < 0.01$), of male gender (71.6% *vs* 49.6%, $P < 0.01$), obese (33.1% *vs* 29.6%, $P < 0.01$), and Hispanic (29.2% *vs* 16.6%, $P < 0.01$). When assessing their comorbidities, high risk patients were more likely to have T2DM (55.7% *vs* 14.1%, $P < 0.01$) and hypertension (86.5% *vs* 45.2%, $P < 0.01$). These data are summarized in Table 1.

Applying the diagnostic algorithm to diabetics

In the subset of 737 patients with T2DM, 213 patients (35.4%) had an elevated ALT. Among these patients, 85 patients (37.9%) had an elevated FIB4 score ≥ 1.3. Among these patients, 42 patients (46.1%) had an elevated FAST score. This accounts for 5.7% of the diabetes cohort (Figure 3) being in the category of at-risk NAFLD. Patients with diabetes are nearly three times more likely to have fibrotic NASH compared to the overall study population (OR 2.89 [1.96-4.26, $P < 0.01$]).

Clinical parameters were similarly evaluated in the diabetes cohort and compared between high risk and low risk patients. These data are summarized in Table 2. When compared to their low-risk counterparts, diabetic patients at high risk for clinically significant NASH were not found to be significantly different in any demographic category. In this cohort, high risk patients were found to

Table 1 Demographics and summary statistics of overall study population

	Study population	High risk patients	*P* value[1]
	N = 3669	N = 75	
Age (yr) – mean (95%CI)	47.3 (45.7-48.9)	57.6 (54.6-60.6)	0.005
Male – % (95%CI)	49.6 (46.8-52.5)	71.6 (51.9-85.5)	0.005
BMI (kg/m^2) – mean (95%CI)	29.6 (28.9-30.3)	33.1 (30.5-35.7)	0.003
Race/Ethnicity – % (95%CI)			
Non-Hispanic White	63.6 (58.3-68.6)	45.4 (29.6-62.2)	< 0.001
Non-Hispanic Black	10.8 (7.9-14.6)	10.7 (4.1-25.4)	
Hispanic	16.6 (12.8-21.1)	29.2 (17.3-44.9)	
Non-Hispanic Asian	4.4 (3.1-6.4)	4.2 (1.6-10.7)	
Other	4.7 (3.5-6.2)	10.4 (2.4-35.8)	
Comorbidities – % (95%CI)			
T2DM	14.1 (12.4-15.9)	55.7 (39.2-71.0)	0.001
Hypertension	45.2 (42.0-48.5)	86.5 (75.4-93.1)	0.001
Lab values – mean (95%CI)			
Total bilirubin (mg/dL)	0.47 (0.46-0.49)	0.60 (0.50-0.70)	0.89
AST (IU/L)	21.34 (20.87-21.82)	52.63 (44.03-61.24)	< 0.001
ALT (IU/L)	22.55 (21.77-23.32)	54.88 (46.57-63.18)	< 0.001
GGT (IU/L)	28.05 (26.78-29.32)	92.98 (46.70-139.26)	0.02
Albumin (g/dL)	4.11 (4.07-4.14)	4.10 (4.02-4.18)	0.50
Alkaline (IU/L)	76.04 (74.33-77.76)	89.72 (66.07-113.36)	0.46
LSM (kPa)	5.65 (5.43-5.86)	11.83 (7.99-15.67)	0.002
CAP (dB/m)	261.59 (257.55-265.62)	324.59 (310.78-338.40)	< 0.001

[1]*P* value compares at-risk group (N = 75) *vs* low-risk group (N = 161). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BMI: Body mass index; T2DM: Type 2 diabetes mellitus; CAP: Controlled attenuated parameter; GGT: Gamma-glutamyl transferase; LSM: Liver stiffness measure.

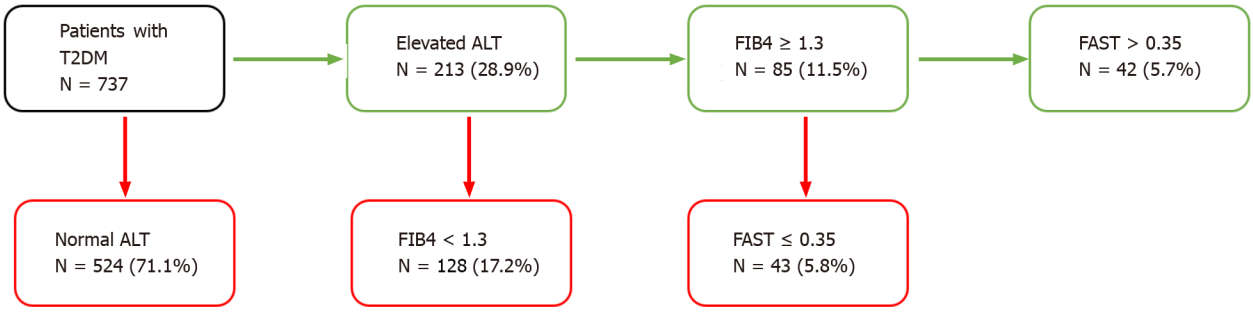

Figure 3 Triage based on non-invasive tests for type 2 diabetes population. From subset of 737 patients with type 2 diabetes, 42 (5.7%) were identified who satisfied all the parameters of our algorithm: elevated alanine aminotransferase value, elevated fibrosis-4 score ≥ 1.3, and elevated FAST score > 0.35. ALT: Alanine aminotransferase; T2DM: Type 2 diabetes; FIB4: Fibrosis-4.

have an elevated GGT value compared to the low-risk patients (69.2 IU/L *vs* 34.6 IU/L, $P < 0.01$).

Table 2 Demographics and summary statistics of the cohort with type 2 diabetes mellitus

	T2DM population	High risk patients	*P* value[1]
	N = 737	N = 42	
Age (yr) – mean (95%CI)	60.3 (58.3-62.5)	62.7 (57.4-68.0)	0.48
Male – % (95%CI)	55.6 (47.2-63.8)	77.1 (55.6-90.0)	0.67
BMI (kg/m^2) – mean (95%CI)	33.5 (32.2-34.8)	34.5 (30.9-38.1)	0.13
Race/Ethnicity – % (95%CI)			
Non-Hispanic White	60.5 (53.6-66.9)	45.7 (25.5-67.4)	0.07
Non-Hispanic Black	13.2 (8.8-19.3)	5.7 (1.8-16.9)	
Hispanic	15.2 (11.5-19.7)	26.5 (14.7-42.9)	
Non-Hispanic Asian	4.9 (3.2-7.4)	3.7 (1.1-12.2)	
Other	6.2 (4.1-9.4)	18.4 (4.3-53.4)	
Comorbidities – % (95%CI)			
T2DM	NA	NA	NA
Hypertension	80.5 (76.6-83.9)	95.7 (80.1-99.2)	0.64
Lab values – mean (95%CI)			
Total bilirubin (mg/dL)	0.48 (0.43-0.53)	0.56 (0.42-0.70)	0.18
AST (IU/L)	21.74 (20.58-22.89)	47.49 (38.36-56.61)	< 0.001
ALT (IU/L)	24.69 (22.83-26.56)	53.58 (43.19-63.98)	0.004
GGT (IU/L)	34.66 (32.51-36.81)	69.21 (47.44-90.98)	0.005
Albumin (g/dL)	3.99 (3.94-4.05)	4.11 (4.01-4.20)	0.46
Alkaline (IU/L)	83.36 (79.97-86.75)	82.85 (71.44-94.26)	0.46
LSM (kPa)	7.32 (6.47-8.18)	14.45 (8.62-20.27)	0.005
CAP (dB/m)	308.21 (302.05-314.37)	333.66 (320.14-347.17)	0.02

[1]*P* value comparing at-risk group (N = 42) *vs* low-risk group (N = 43). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BMI: Body mass index; CAP: Controlled attenuated parameter; GGT: Gamma-glutamyl transferase; LSM: Liver stiffness measure; NA: Not available; T2DM: Type 2 diabetes mellitus.

DISCUSSION

The main findings of the current study are the following: (1) In a nationally representative cohort of adult Americans, the implementation of a simple diagnostic algorithm to identify patients with at-risk NAFLD (fibrotic NASH) in need for pharmacologic intervention was feasible; and (2) by using this algorithm, only 2% of the general adult population and 6% of diabetics would have needed referral to a subspecialist providing reassurance that the implementation of this algorithm on a large scale will not lead to overwhelming subspecialists with unnecessary referrals.

Given a burdened healthcare system with significant logistic constraints, there is room for improvement in the subspecialty referral process to gastroenterology and hepatology for patients with NAFLD. These data add to a growing body of literature that supports that the vast majority of patients who are referred require clinical observation and serial monitoring. In a recent study of an Australian cohort, the authors found that 75% of patients referred to hepatology had low risk of advanced fibrosis and more than 2/3 (68%) of patients could be discharged back to their primary care or referring physician[20].

Our approach provides a more nuanced method of screening and identifying patients with NAFLD. In a survey of more than one hundred primary care physicians, the vast majority (70%) reported they would be unlikely to refer a patient to hepatology unless the patient had abnormal liver enzymes[21]. In a survey of eleven laboratories, it was found that the upper limit of normal for ALT values varied from 35 U/L to 79 U/L for men and 31 U/L to 55 U/L for women[22]. Thus, our more sensitive cutoff of 30 U/L for men and 19 U/L for women could be misinterpreted as within normal range by providers[23]. The circumstance may arise where patients have elevated FAST scores, but normal FIB-4 scores or vice versa. In these situations with equivocal findings in which patients did not follow the stepwise

algorithm, the authors would recommend subspecialty referral for further risk stratification and diagnostics. This algorithm provides a more effective method of triaging patients for subspecialty referral by relying less on one sole biomarker. Rather, it employs a more comprehensive set of tools for assessment that account for other liver specific variables better than transaminases alone.

Notably, several demographic variables lost statistical significance in the cohort of patients with T2DM, for which there may be several explanations. It's possible that our sample size was too small to achieve statistical significance, which necessitates further investigation into this vulnerable population. However, it is more likely that consistent with previous literature, insulin resistance and the associated T2DM is one of the most significant independent risk factors for the development of NAFLD and NASH [24-26]. This association can make triaging patients with T2DM more challenging in the clinical setting. Rather, clinicians should rely on objective metrics such as the FIB4 score and FibroScan® data to better understand these patients' disease states.

A significant challenge for PCPs, endocrinologists, and other non-liver providers is likely access to FibroScan®. As a relatively new technology that has yet to become ubiquitous both in awareness and availability, referring providers rely on subspecialists to distinguish low-risk patients from high-risk patients and subsequently order the appropriate diagnostic imaging. However, even in the absence of Fibroscan®, meaningful triage can be accomplished with the FIB4 score. The FIB4 score is an extremely cost-efficient starting point for providers that requires measurement of a patient's blood count and serum chemistry. The implementation of this inexpensive screening modality alone would increase the specificity of referral for NAFLD significantly.

In a recently published clinical care pathway, the authors generated a similar pathway for categorizing patients at high risk for advanced fibrosis. Their pathway starts with identifying patients at risk for NAFLD, including those with metabolic risk factors, T2DM, or imaging that shows steatosis or fibrosis. Subsequently, these patients are stratified into low, intermediate, or high risk based on FIB-4 cutoffs of < 1.3, 1.3-2.67, and > 2.67 respectively. Low risk patients are referred back to their PCPs for clinical observation, while high-risk patients are referred forward to hepatology. Intermediate risk patients are recommended to undergo FibroScan® and are further stratified in to low, intermediate, or high risk based on LSM cutoffs of < 8, 8-12, and > 12 kPa. Both intermediate and high-risk patients based on LSM value (> 8 kPa) are recommended for referral to hepatology[27]. The authors estimated that roughly 10% of patients in this pathway will have high-risk disease. The implementation of our complementary pathway showed that this may be a slight overestimate given that 2% of the general population and 6% of diabetics were found to be high-risk based on our algorithm. To our knowledge, this is the first study of its kind to implement this type of algorithm on a large scale.

CONCLUSION

Due to a multitude of factors including indolent disease course and phenotypic variation, identifying patients at high risk for advanced fibrosis presents a significant challenge and leads to an excess of referrals to subspecialists. The creation and implementation of a novel diagnostic algorithm that stratifies patients into low-risk and high-risk demonstrates that less than 5% of the general population would need subspecialty referral and the overwhelming majority of patients can be managed with clinical observation and subsequent non-invasive testing by their primary care and referring physicians.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) presents a significant public health crisis to primary care physicians and endocrinologists. This growing need necessitates a simple and efficient algorithm that can streamline the process of subspecialty referral to hepatology.

Research motivation

More than half of all patients with NAFLD are at low risk for advanced fibrosis. Though there are no Food and Drug Administration -approved agents for nonalcoholic steatohepatitis (NASH) presently, the efficient identification of patients with NASH with advanced fibrosis will be paramount in the care of these patients.

Research objectives

This study aims to create and enact a diagnostic algorithm for all patients with suspected NAFLD to identify the patients at high risk for advanced fibrosis.

Research methods

Patients with suspected NAFLD were identified in the NHANES database who had historical FibroScan data. FIB4 and FAST scores were calculated for these patients. Those with FIB4 > 1.3 and/or FAST score > 0.67 were deemed high risk for advanced fibrosis.

Research results

Of the 3669 patients meeting the inclusion and exclusion criteria, only 75 patients had both an elevated FIB4 and an elevated FAST score which represents roughly 2.0% of the overall population. Among the 737 patients with type 2 diabetes mellitus, 42 patients (5.1%) were found to have both elevated FIB4 and FAST scores.

Research conclusions

Given an overwhelming number of patients are referred to hepatology who are most likely at low risk for advanced fibrosis, the utilization of this algorithm by referring providers would help to streamline the process for referrals and eventually more seamlessly identify patients at risk for advanced fibrosis who may need therapy for NASH.

Research perspectives

As novel therapeutic agents are currently being studied in patients with NASH with advanced fibrosis, the creation and implementation of a diagnostic algorithm to efficiently identify patients needing therapy becomes increasingly important. Given the wide range of noninvasive tests, this algorithmic approach using two popular tests helps to capture patients at risk for advanced fibrosis while reassuring low-risk patients.

FOOTNOTES

Author contributions: Alkhouri N, Sakkal C, and Polanco P contributed to the data collection; Le P and Payne J contributed to the data collection; Alkhouri N and Aggarwal P contributed to the drafting and revision of the manuscript; Harrison S and Noureddin M contributed to the revision of the manuscript.

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Institutional review board statement: This study has been reviewed and approved by the Arizona Liver Health IRB (protocol 2020-ALH-NAFLD-01).

Informed consent statement: As this was a retrospective review from a deidentified patient database, individual informed consent of subject participants was not applicable as per the institutional review board.

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ORIGINAL ARTICLE

Retrospective Study

Real-life multi-center retrospective analysis on nivolumab in difficult-to-treat patients with advanced hepatocellular carcinoma

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Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide. The landscape of the systemic treatment for advanced HCC is changing quickly, and recently, the standard of care became either atezolizumab plus bevacizumab or tremelimumab plus durvalumab in the single tremelimumab regular interval durvalumab regimen. Nivolumab monotherapy has proven to be effective sometimes for advanced HCC and could be a valuable treatment option for patients outside current treatment indications and reimbursement criteria for the standard of care. This is a particular population of interest.

AIM
To evaluate the real-world effectiveness of nivolumab monotherapy in patients with advanced HCC who are not eligible for other treatment.

METHODS
We conducted a retrospective, multicentric study including 29 patients with advanced HCC from 3 Belgian tertiary hospitals. All patients had had prior chemotherapy or were intolerant or ineligible for treatments. All study subjects received nivolumab 3 mg/kg in monotherapy, administered once every two weeks intravenously. Treatment continued until disease progression, severe adverse events or death. Data were retrieved from patients' medical records. The outcome parameters such as radiological response according to response evaluation criteria in solid tumors (RECIST) criteria, the biological response through the evolution of the alpha-fetoprotein (AFP) level, and clinical response considering both the Child–Pugh (CP) score and the World Health Organization (WHO) performance status (PS) were reported. A safety profile was also reported. Statistical analysis was performed using the SPSS Statistics 27 statistical software package.

RESULTS
The radiological overall response rate (defined as complete or partial response according to the immune RECIST and modified RECIST criteria) to nivolumab monotherapy was 24.1%. The biological overall response rate (defined as a decrease of ≥ 25% in AFP blood level) was 20.7%. Radiological and biological responses were significantly associated both with each other (P < 0.001) and with overall survival (P < 0.005 for radiological response and P < 0.001 for biological response). Overall survival was 14.5 mo (+/- 2.1), and progression-free survival was 10.9 mo (+/- 2.3). After 4 mo of treatment, 78.3% of patients remained clinically stable or even showed improvement in WHO PS. Grade 3 adverse events occurred in 17.2% of patients, none had grade 4 adverse events, and no patients ceased nivolumab due to adverse events.

CONCLUSION
Nivolumab monotherapy is a good treatment choice in frail patients with HCC who are ineligible for the standard of care or other validated systemic treatments.

Key Words: Advanced hepatocellular carcinoma; Systemic treatment; Immunotherapy; Nivolumab; Difficult-to-treat patients; Real-life setting

Core Tip: We conducted a study on the real-world effectiveness of nivolumab (immunotherapy) in patients with advanced liver cancer who were ineligible for the standard of care or other validated treatments, including patients with impaired liver function and a poor general condition, a population that is usually not included in studies. We showed a reduction of tumor mass in 24.1% of patients, with a disappearance of tumor mass in 13.9% of patients, which is better than that reported in the literature. Furthermore, we confirmed the favorable safety profile of nivolumab. Hence, nivolumab should be considered as a valuable treatment option in selected patients who are otherwise not eligible for treatment.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and a leading cause of cancer-related death worldwide, with over 826000 deaths in 2020. The worldwide incidence is 14.1 and 5.2 per 100000 men and women, respectively, and the incidence is still increasing[1]. HCC develops mainly in the context of underlying chronic liver disease, mostly in the cirrhotic stage. Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, excessive alcohol consumption and metabolic syndrome are the most important risk factors[1-3].

Over the past years, therapeutic options for advanced HCC have changed remarkably. Advanced HCC is defined as a liver tumor not eligible for local therapies given the extent of disease or liver tumors that recurred after local therapies[4]. Sorafenib, a tyrosine kinase inhibitor (TKI) that showed survival benefits as a first-line systemic treatment for advanced HCC, was the only available therapy for more than a decade. Thereafter, other TKIs have become available as first-and second-line treatments for advanced HCC. The introduction of immunotherapy, however, has caused a major shift in the therapeutic landscape of advanced HCC. Immune checkpoint inhibitors (ICIs), such as anti-programmed cell death protein 1 (PD1), anti-PD-ligand 1 (L1), and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which enhance the patient's antitumor immune response by countering the inhibitory signals from cancer cells that block the natural antitumor lymphocyte response[5], have been extensively studied. Atezolizumab (anti-PD-L1) plus bevacizumab anti-vascular endothelial growth factor (VEGF) has recently become the first-line treatment of choice for advanced HCC, since recent results from the IMbrave150 trial have shown superiority in both coprimary outcomes [median overall survival (OS) of 19.2 mo and progression-free survival (PFS) of 6.8 mo) over sorafenib (median OS 13.4 mo and PFS 4.3 mo) as first-line treatment[6]. Since January 2022, the phase III HIMALAYA trial proposed single tremelimumab regular interval durvalumab (STRIDE) as a novel, first-line standard of care systemic therapy for advanced HCC, since this has shown superior efficacy and a favorable benefit-risk profile *vs* sorafenib[7].

Recent and ongoing trials of ICI in advanced HCC show encouraging results, with some excellent responders among the treated patients. However, there is great heterogeneity in response to treatment [5,8]. Recently, the Keynote-394 trial presented positive results at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium for pembrolizumab as a second-line monotherapy treatment in advanced HCC; pembrolizumab, an anti-PD-1 blocker equal to nivolumab, showed significant improvements in OS, PFS and overall response rate (ORR) compared with placebo[8].

Nivolumab as monotherapy for advanced HCC has shown promising results in the phase I/II trial Checkmate 040[9] with some durable responses but failed to show statistically significant benefit *vs* sorafenib in the primary endpoint of overall survival in the phase III trial Checkmate 459[10]. Both studies confirmed the good tolerability and favorable safety profile of nivolumab. The studies enrolled patients with unresectable HCC who were naïve to any systemic treatment and limited to CP class A liver function and World Health Organization (WHO) performance status (PS) 0 or 1[9,10].

In the current retrospective study, we analyzed a population of patients with advanced HCC who showed disease progression under one or more TKIs or were intolerant or ineligible for TKIs. Some of these patients also had decreased liver function with a CP score of B. This study aims to assess the effect of nivolumab monotherapy in a difficult-to-treat patient population with advanced HCC after multiple lines of treatment and for whom best supportive care was the only alternative option. In contrast to the IMbrave150[6] and HIMALAYA trials[7], our study patients were ineligible for the standard of care because they were no longer in a first-line setting because of the more advanced stage of disease, more severely impaired liver function, worse WHO PS and/or underlying comorbidities.

MATERIALS AND METHODS

Study design and patient selection

This is a retrospective, multicentric study including 29 patients with advanced-stage Barcelona clinic liver cancer stage C (BCLC-C) or intermediate stage (BCLC-B) HCC for whom no other validated therapeutic option at that time was available[11]. All study subjects were diagnosed with HCC between September 2014 and February 2019. Follow-up and data collection continued until May 2021. Patients were ineligible for curative options, locoregional or systemic TKI therapies, due to disease progression, more advanced cirrhosis, worse WHO PS, underlying comorbidities (mostly cardiovascular) or intolerability to TKIs because of side effects. All patients received nivolumab 3 mg/kg as monotherapy. Nivolumab was administered once every 2 wk intravenously. Treatment continued until progression (as defined below), severe adverse events or death. Patients were enrolled in 3 Belgian centers as follows: Hôpital Erasme, Université Libre de Bruxelles (ULB), Brussels (18 patients), University Hospital of Antwerp (UZA, 6 patients) and Maria Middelares Hospital Ghent (MMG, 5 patients). Approval from the ethics committee was obtained (EC number 21/06/080).

Data collection

Baseline characteristics and demographics were retrospectively retrieved from the patients' medical records. The tumor stage was defined by the BCLC staging system. Hepatic function was expressed by the CP system in case cirrhosis was present. Response to nivolumab was evaluated through 3 different outcome parameters. First, the radiological response was evaluated according to the RECIST1.1 (Response Evaluation Criteria In Solid Tumors) criteria in all three centers and additionally iRECIST in ULB and mRECIST in UZA and MMG. Second, the biological response was measured through the evolution of the alpha-fetoprotein (AFP) level, with response defined as a ≥ 25% decrease in the AFP blood level and disease progression defined as a ≥ 25% increase in the AFP blood level. Third, clinical

response was assessed considering both the CP score and the World Health Organization PS at baseline and after 2 and 4 mo of therapy.

The primary endpoints were overall and progression-free survival. OS was counted in months from the start of nivolumab treatment until the patient died or until the last follow-up. Seven patients were still alive at the time of enrollment of this study. Progressive disease leading to the decision of treatment cessation was defined by the treating physician considering radiological progression or progression of the AFP level, and patients with progression of one of both were considered as progressive. One patient died before progression was reached; in this case, PFS was equal to overall survival. Adverse events were recorded and graded according to the National Comprehensive Cancer Network severity scale (grades 1-4).

Statistical analysis

Statistical analysis was performed using the SPSS Statistics 27 statistical software package. Descriptive statistics were used to express categorical variables in numbers and percentages and continuous variables in the mean and standard error of the mean. Survival (OS and PFS) was calculated and graphically represented using a Kaplan–Meier survival curve, and comparative chi-square analysis was performed. Comparative analyses were performed with chi-square testing; Breslow, long rank and Pearson chi-square coefficients were used for correlations. Conventionally, the cutoff for statistical significance was a P value < 0.05.

RESULTS

Patient characteristics at baseline

The patient population consisted of 29 patients (see Table 1). The male/female ratio was 21/8 (72.4%/27.6%), which is a good representation of the real-life HCC context[12]. The mean age was 69.1 (2.1) years, and the mean body mass index was 26.6 (+/- 1.0) kg/m².

One patient had an HCC stage BCLC-B, and all 28 others were advanced stage BCLC-C. The disease was bilobar in 64.3% of patients, multifocal in 57.7% and macrovascular invasion in 27.6%. Up-to-7 criteria were met in 55.2% of patients[13]. Thirteen patients (44.8%) had metastatic disease, of which 8 patients (27.6%) had only one metastatic location. Seventy-five percent of patients had increased levels of AFP at baseline, and the other 25% of patients were AFP negative. The mean baseline AFP was 4375.6 ± 2566.6 μg/L.

Eighteen patients had underlying cirrhosis, 10 patients had no underlying cirrhosis, and in 1 patient, there were no data recorded about underlying liver disease. Of the 18 cirrhotic patients, 10 had CP score A, and 8 had CP score B at baseline. The origin of cirrhosis in our study cohort was heterogeneously divided into HBV-, HCV-, alcoholic- and nonalcoholic steatohepatitis-induced cirrhosis.

Before the start of nivolumab, the WHO PS was 0 in 5 patients (17.2%), 1 in 21 patients (72.4%) and 2 in 3 patients (10.3%). Twenty-seven patients (93.1%) had received 1 or more previous treatments, and 2 patients (6.9%) had not received previous treatments because of contraindications due to comorbidities. For a detailed overview of the different previous treatment lines for each of the study subjects, we refer to Supplementary Table 1.

Response rate

Radiological response: Serial radiological evaluation was performed according to the criteria RECIST 1.1, iRECIST and mRECIST; to calculate the response rate, we used iRECIST (ULB) and mRECIST (UZA and MMG), as these criteria are more specific in the current context. While the RECIST 1.1 criteria are the most commonly used criteria to evaluate response to conventional chemotherapies in solid tumors, iRECIST criteria are developed specifically to evaluate the response of novel immunomodulating agents, and mRECIST criteria are developed specifically for evaluation of HCC treatment[14].

Four patients (13.9%) showed a complete response, 3 patients (10.3%) showed a partial response, and 6 patients (20.7%) showed stable disease following nivolumab therapy. As a result, the radiological overall response rate (defined as complete or partial response) was 24.1%. The radiological disease control rate (defined as complete or partial response or stable disease) was 44.8% (Figure 1).

One patient died before the radiological evaluation was possible.

Biological (AFP) response: Of the 29 patients, 7 had no increased AFP level at diagnosis, of whom 2 developed AFP progression during nivolumab therapy. Overall, 16/29 study subjects experienced AFP progression (55.2%). AFP remained stable in 7 patients (24.1%), including the 5 AFP-negative patients at baseline. There was a 25% decrease without normalization of AFP in 1 patient (3.4%) and a normalization of AFP in 5 patients (17.2%). In our results, we differentiated between AFP decrease without normalization and complete AFP normalization.

Altogether, this accounts for a biological disease control rate (defined as AFP normalization, AFP decrease of ≥ 25% or stable AFP, including those with negative AFP from baseline until last follow-up) of 44.8% and a biological overall response rate (defined as AFP normalization or AFP decrease of ≥ 25%)

Table 1 patient characteristics

Characteristic	Case subjects (*n* = 29)
Sex	
Male (*n*)	21
Female (*n*)	8
Age at diagnosis, yr (mean ± SEM)	69.1 ± 2.1
BMI, kg/m² (mean ± SEM)	26.6 ± 1.0
BCLC stage (*n*)	
BCLB-B	1
BCLB-C	28
HCC characteristics (*n*)	
Bilobar	18/28
Multifocal	15/26
Vascular invasion	8/29
UP-TO-7-Criteria	16/29
Metastasis (*n*)	
No metastases	16
1 meta location	8
2 meta locations	3
4 meta locations	2
AFP at baseline, ng/mL (mean ± SEM)	4375.6 ± 2566.6
Cirrhosis (*n*)	
No Cirrhosis	10
CP A	10
CP B	8
Unknown	1
Origin cirrhosis (*n*)	
HBV	4
HCV	2
Ethyl	7
NAFLD	3
Other	2
Missing	1
WHO performance status (*n*)	
0	5
1	21
2	3
Previous treatment (*n*) (Resection, radiofrequency ablation, transarterial radioembolization, transarterial chemoembolization, selective internal radiation therapy, sorafenib, capecitabine, GEMOX, doxorubicine, FOLFOX, regorafenib, cabozantinib)	
Yes	27
No	2

WHO: World Health Organization; CP: Child-Pugh; BMI: Body mass index; BCLC: Barcelona clinic liver cancer; HCC: Hepatocellular cancer; AFP: Alpha-

fetoprotein; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NAFLD: Nonalcoholic fatty liver disease.

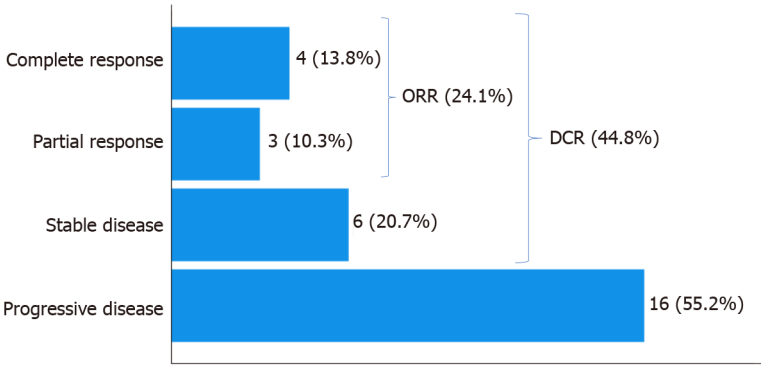

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Figure 1 Radiological response categories by mRECIST and Irecist. The figure shows the number (%) of patients per radiological response category. ORR: Overall response rate; DCR: Disease control rate.

of 20.7% (Figure 2).

Correlation between the radiological and biological response: The radiological and biological responses were significantly correlated (Pearson chi-square coefficient 42.28 with a *P* value < 0.001). All 4 patients with complete radiological remission had AFP normalization. Among the 3 patients with partial response, the results were mixed: 1 showed AFP normalization, 1 a > 25% decrease and 1 remained AFP-negative from baseline until the last follow-up. Conversely, among the 5 patients with AFP normalization, 4 had a complete radiological response, and 1 had a partial radiological response.

Survival

The mean OS after the start of nivolumab was 14.5 mo (+/- 2.1). Seven patients were still alive at the time of completion of this study. The patient who was censored at 3 mo underwent liver transplantation at that time (Figure 3).

Progression-free survival (with progression defined as progression radiologically or progression of AFP level, CFR. Methods) was 10.9 mo (+/- 2.3). There were 6 patients (20.7%) in the study cohort who did not show disease progression and were still alive at the time of study closure; they were censored at 3, 23 (3 patients), 28, and 33 mo (Figure 3).

Overall survival per radiological response category: There was a statistically significant correlation between the overall survival and the radiological response to nivolumab, with a *P* value < 0.005 (Breslow 12.85). In the (radiological) responder group (partial and complete), all 7 patients showed a durable treatment response (1 patient for 20 mo, 1 patient for 26 mo, and 5 patients were still alive at the time of study closure with a sustained tumor response). All 4 patients with complete responses were among those 5 survivors. In the group with stable disease, the mean survival was 10.4 mo (+/- 2.1). One patient in this group received a liver transplant after 3 mo and is still alive without HCC recurrence; the patient was censored at the time of transplantation. Patients with progressive disease had a survival of 8.8 mo (+/- 2.0). One patient in this group was switched to cabozantinib after 6 mo of nivolumab treatment and was still alive at study closure (Table 2, Figure 4A). In Table 2 we presented the survival per radiological response category; total number of patients in each response category, mean overall survival in months, and the number of patients death and alive at study closure are depicted.

Overall survival per biological (AFP) response category: There was a statistically significant correlation (Breslow coefficient 21.5) between overall survival and biological AFP response, with a *P* value < 0.001. If we excluded the group of patients with negative AFP levels at baseline until death or last follow-up from the correlation, it remained significant with a Breslow coefficient of 10.27 (*P* value 0.016).

All 5 patients who showed AFP normalization were still alive at the time of elaboration of this paper, with survival of 100% at 23 mo since the start of nivolumab. One patient with a > 25% AFP decrease died at 26 mo after the start of nivolumab. Two patients with an increased AFP level at baseline showed a stable AFP level during treatment, and both died 2 mo after nivolumab was started. All but 1 of the 16 patients who showed AFP progression died. In this group, survival was 9.6 mo (+/- 1.8). In the group of patients (n = 5) with negative AFP levels at baseline until death or last follow-up, all patients but 1 died; the mean survival was 13.8 mo (+/- 3.9). In Table 3 we presented the survival per biological response category; total number of patients in each response category, mean overall survival in months, and the number of patients death and alive at study closure are depicted. For a visual representation, the

Table 2 Survival per radiologic response category

Radiological response	Total *n*	Overall survival, mo (mean ± SEM)	*n* of deaths (%)	*n* alive at study closure (%)
Progressive disease	16	8.8 ± 2.0	15 (93.7)	1 (6.3)
Stable disease	6	10.4 ± 2.1	5 (83.3)	1 (16.7)
Partial response	3	Not assessable	2 (66.7)	1 (33.3)
Complete response	4	Not assessable	0 (0)	4 (100)
Overall	29	14.5 ± 2.1	22 (75.9)	7 (24.1)

Not assessable: Patients were alive at study closure.

Table 3 Survival per biological response category

Biological (AFP) response	Total *n*	Overall survival, mo (mean ± SEM)	*n* of deaths (%)	*n* alive at study closure (%)
Increase ≥ 25%	16	9.6 ± 1.8	15 (93.7)	1 (6.3)
Stable	2	2	2 (100)	0 (0)
Decrease ≥ 25% without normalization	1	26	1 (100)	0 (0)
Normalization of AFP (< 7 μg/L)	5	Not assessable	0 (0)	5 (100)
AFP remains negative	5	13.8 ± 3.9	4 (80)	1 (20)
Overall	29	14.5 ± 2.1	22 (75.9)	7 (24.1)

Not assessable: Patients were alive at study closure. AFP: Alpha-fetoprotein.

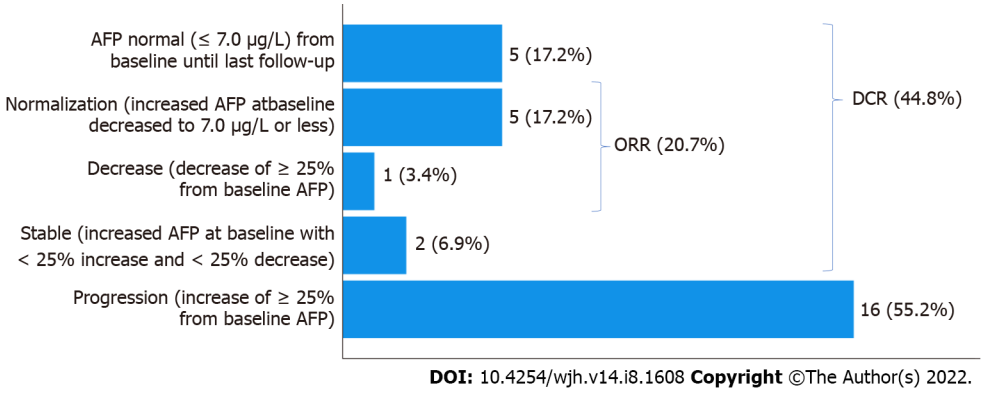

Figure 2 Biological (alpha-fetoprotein) response categories. The figure shows the number (%) of patients per biological response category. AFP: Alpha-fetoprotein; ORR: Overall response rate; DCR: Disease control rate.

categories 'stable AFP' and 'AFP remaining negative from baseline until death/Last follow-up' were merged in the graph (Table 3, Figure 4B).

Overall survival according to baseline AFP comparing patients with an AFP level > 200 ng/mL and patients with an AFP level < 200 ng/mL was not significantly different (P = 0.517).

Clinical response

We evaluated the clinical response using the WHO PS and the CP score at baseline and at 2 mo and 4 mo.

WHO PS: Before the start of nivolumab treatment, the WHO PS was 0 in 17.2% of patients (n = 5), 1 in 72.4% of patients (n = 21) and 2 in 10.3% of patients (n = 3) (Table 1). We looked at the WHO PS before the start of treatment, after 2 mo, and after 4 mo of treatment to examine the evolution of the WHO PS over time for each patient individually.

After 2 mo of nivolumab treatment, 29.6% (8/27) of the remaining patients had a worse WHO PS compared to the start of treatment; 51.9% (14/27) of patients had a stable WHO PS; and 18.5% (5/27) of

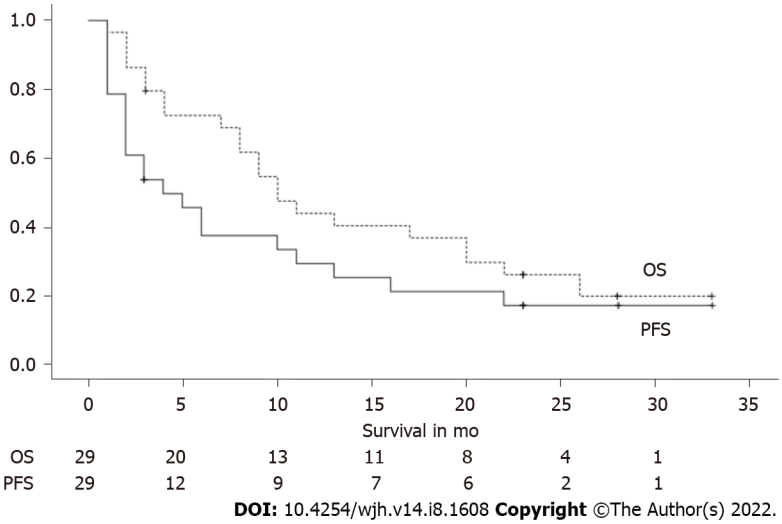

Figure 3 Overall and progression-free survival. The graph shows the Kaplan-Meier survival curves for overall and progression-free survival. Below the graph the number of patients still alive at that time is depicted. OS: Overall survival; PFS: Progression-free survival.

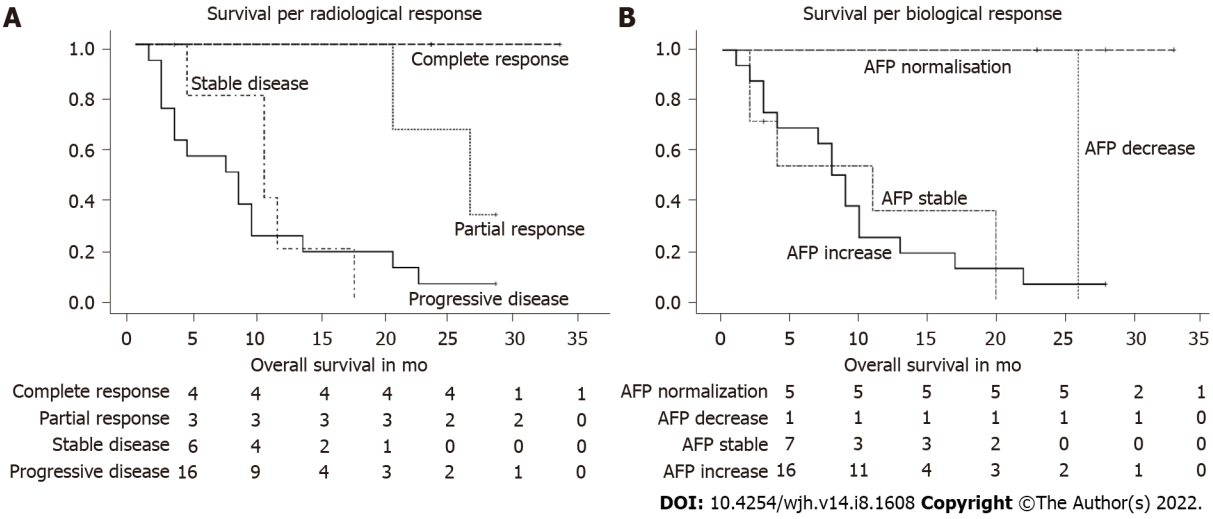

Figure 4 Survival per radiological and biological response. A and B: The graph shows the Kaplan-Meier survival curves for each category of radiological (A) and biological (B) response. Below the graph the number of patients still alive at that time is depicted. AFP: Alpha-fetoprotein; AFP decrease: AFP decrease without normalization.

patients had a better WHO PS (Table 4, Figure 5A).

After 4 mo of treatment, in the 23 remaining patients (6 died), 21.7% (5/23) had a worse WHO PS, 56.5% (13/23) had a stable WHO PS, and 21.7% (5/23) had a better WHO PS compared to the start of treatment (Table 4, Figure 5B).

Thus, we can say that 70.4% of patients at 2 mo and 78.3% of patients at 4 mo remained clinically stable or even showed improvement.

These data illustrate the favorable tolerability of nivolumab and can support the finding that a subgroup of patients responds well to nivolumab clinically.

CP score

Before the start of nivolumab treatment, 18/29 patients had cirrhosis, of which 10 (35.7%) had CP score A and 8 (28.6%) had CP score B.

We looked at the CP before the start of treatment, after 2 mo, and after 4 mo of treatment to examine the evolution of the CP over time for each patient individually. The patients who had no cirrhosis at baseline and did not develop cirrhosis were classified in the category 'stable CP', assuming that their liver function remained stable.

After 2 mo of nivolumab treatment, 36% (9/25) of the remaining patients had a worse CP compared to the start of treatment; 60% (15/25) of patients had a stable CP; and 4% (1/25) of patients had a better CP (Table 5).

Table 4 Evolution of World Health Organization performance status

WHO PS	2 mo	4 mo
Worse PS	8 (29.6%)	5 (21.7%)
Stable PS	14 (51.9%)	13 (56.5%)
Better PS	5 (18.5%)	5 (21.7%)
Total	27 (100%)	23 (100%)
Death	2	6

WHO PS: World Health Organization performance status.

Table 5 Evolution of Child-Pugh score

CP score	2 mo	4 mo
Worse CP	9 (36%)	7 (33.3%)
Stable CP	15 (60%)	12 (57.1%)
Better CP	1 (4%)	2 (9.5%)
Total	25 (100%)	21 (100%)
Death	2	6
Missing	2	2

CP: Child-Pugh score.

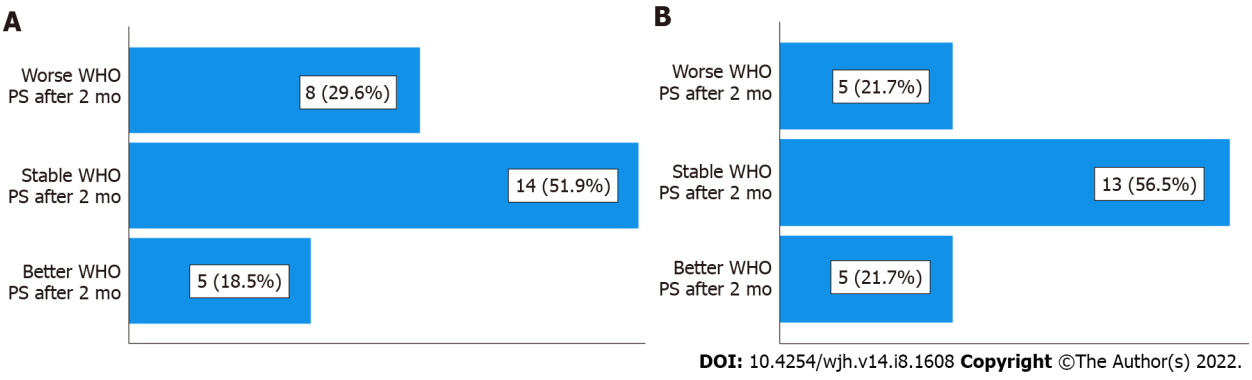

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Figure 5 Evolution of the World Health Organization performance status after 2 mo and 4 mo of treatment, compared to baseline. A and B: The figure shows the proportion of patients with an improved, a stable, and a worse World Health Organization performance status after 2 mo (A) and after 4 mo (B) compared to baseline. WHO PS: World Health Organisation performance status.

After 4 mo of treatment, 33.3% (7/21) had a worse CP, 57.1% (12/21) had a stable CP, and 9.5% (2/21) had a better CP compared to the start of treatment (Table 5).

There were 2 patients for whom there was no information available concerning the evolution of CP scores. Generally, we observed no major impact on liver dysfunction in the first months after the start of nivolumab therapy.

Nivolumab-associated toxicity

Five out of 29 patients (17.2%) had grade 3 adverse events: 2 cases of dyspnea, 1 asthenia, 1 cholestatic hepatitis, and 1 cerebellar ataxia. None of these grade 3 adverse events were judged to be unequivocally related to the use of nivolumab. No side effects of grade 4 were reported. No patients ceased nivolumab due to adverse events.

Correlation between viral vs non-viral etiology of liver disease and response to nivolumab

Previous studies[15] suggested a more favorable outcome in certain etiologies of underlying liver

disease (*e.g.*, viral-mediated) because of improved antiviral immune responses and reduction of viral load after ICI therapy.

In our study cohort, we could not detect an association between a viral *vs* a non-viral etiology of liver disease and the radiological response to nivolumab (Pearson correlation was -0.330 with *P* value 0.086). However, it is important to note that this finding is of limited relevance given the small patient numbers (only 6 patients had an underlying viral liver disease) and hence the lack of power for a robust statistical analysis; therefore, it is impossible to draw conclusions based on this small amount of data (Table 6).

DISCUSSION

The current standard of care for first-line treatment of advanced HCC is either atezolizumab plus bevacizumab, based on the results of the phase III IMbrave 150 trial[6], or tremelimumab plus durvalumab in the STRIDE regimen, based on the results of the phase III HIMALAYA trial[7]. The IMbrave trial included patients with advanced HCC who had an excellent PS, a liver function no worse than CP A, no extended macrovascular invasion, no prior bleeding event due to untreated or incompletely treated esophageal and/or gastric varices within the previous 6 mo and no cardiovascular events in the previous three months[6]. The HIMALAYA trial included patients with advanced HCC who had liver function no worse than CP A and who had not received prior systemic treatment[7].

Our real-world population also included patients with a worse PS and worse liver function (including CP B). Furthermore, our patients had already received (often multiple) previous treatment lines, while the IMbrave150 trial and the HIMALAYA trial took place in a first-line setting[6,7].

In the present study, 6 of 29 patients (20.7%) showed an impressively good and sustained response (radiologically and biologically) to nivolumab monotherapy, with 4 complete responders. All 6 patients were still alive and had an excellent PS at study closure, while before nivolumab treatment, these patients had a very poor prognosis, since no other treatment options were available at the start of treatment.

Compared to the phase III Checkmate 459 trial[10], the results of the present study in a selected patient population are notably better, with an overall response rate of 24.1% and especially a complete response in 13.9% of patients, compared to 15% and 4%, respectively, in the Checkmate459 trial[10].

The phase III Checkmate459 trial included systemic therapy-naïve patients, while most of our patients had already received (multiple) previous systemic treatments. However, in the entire cohort of the Checkmate trial, durable effects of nivolumab were observed in sorafenib-naïve (ORR 23%) and sorafenib-experienced (ORR 16%-19%) patients[9,10].

Due to the long follow-up (of 33 mo) of the present study, we demonstrated that when there was a good response to treatment, this response was sustained, and the patient remained in a very good condition for a long time (our patient with the longest survival received nivolumab monotherapy at the time of study closure (2 years) and 9 mo) and was in excellent condition.

In conclusion, even though nivolumab monotherapy could not demonstrate a significant survival benefit over sorafenib in the phase III Checkmate 459 trial[10], in our specific population of patients with HCC for whom no other validated therapeutic option is available, this treatment has been shown to be effective and associated with a sustained response and an excellent PS in a subset of patients.

Recently, pembrolizumab (an anti-PD 1 blocker such as nivolumab) showed positive results as a second-line treatment for advanced HCC in the Keynote-394 trial compared with placebo[8].

In the HIMALAYA trial[7], durvalumab (an anti-PD-L1 blocker) in monotherapy showed noninferiority to sorafenib as a first-line treatment for advanced HCC with favorable safety.

These results confirm that blocking the pathophysiologic pathway of PD-1 is an effective strategy in the treatment of advanced HCC.

Interestingly, nivolumab plus ipilimumab (= anti-CTLA-4) as a second-line treatment for advanced HCC demonstrated an ORR of 33% (per RECIST 1.1)[16]. In the HIMALAYA trial[7], on which the latest standard of care was based, the same combination of dual antibody therapy was used, durvalumab, a PD-L1 blocker, plus tremelimumab, a CTLA-4 blocker, and this combination showed good efficacy and safety.

However, dual antibody therapy implies a higher cost and a higher potential of (life-threatening) adverse events, but compared to TKIs in second-line therapy, ICIs might be preferable because of the higher response rate (including some complete responders) and the more favorable safety profile[7,17].

Especially in a second- or third-line setting, in a more frail or ill patient population who have worse liver function or intolerability or contraindications for TKIs (*e.g.*, patients with a higher risk of bleeding varices) or who have already progressed under TKI treatment and for whom there is no validated treatment available (yet), nivolumab in monotherapy is a valuable treatment option[18,19].

Radiological (tumor mass) and biological (AFP) evolution were used as outcome parameters to define the response to nivolumab therapy. They are strongly associated both mutually and with survival, supporting their value as good outcome parameters.

Our study confirmed that nivolumab is generally well tolerated and has a favorable safety profile even in patients with impaired liver function and/or poor PS. Generally, there was no major impact on

Table 6 Viral *vs* non-viral liver disease and response to nivolumab

Radiological response	Non-viral liver disease	Viral disease	Total
Progressive disease	10	5	15
Stable disease	5	1	6
Partial response	3	0	3
Complete response	4	0	4
Total	22	6	28

The numbers represent the patient count.

the WHO PS or on the CP scores following the start of nivolumab treatment. Nevertheless, clinicians should always be aware of potentially life-threatening immune-mediated toxicity when administering ICIs[16].

Today, the field of systemic therapy of advanced HCC is in full development. Future research is warranted to determine the best standard of care in the first-line setting and to develop evidence-based recommendations concerning the treatment sequence after first-line therapy, strategies to select patients who will benefit from one of the currently available treatment modalities, and an adequate therapeutic approach in patients with CP B and/or a poor PS[18].

CONCLUSION

Nivolumab monotherapy in this real-world retrospective multicenter case series of difficult-to-treat HCC cases – deemed ineligible for other systemic treatments, including patients with impaired liver function and poor PS – is a valuable option with a substantial number of responders with sustained clinical improvement, good OS and PFS and with excellent tolerability and safety.

Nivolumab monotherapy should hence be considered in selected patients otherwise not eligible for systemic treatment, a population that is usually not included in randomized controlled trials.

These results need to be confirmed in a prospective trial targeting the specific population of frail patients with HCC for whom antiangiogenic agents are contraindicated. Further studies are warranted to better define the group of patients who might benefit from this strategy and how to select them.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma is one of the leading causes of cancer-related death worldwide. The landscape of the systemic treatment for advanced hepatocellular carcinoma (HCC) is changing quickly. However, frail patients with impaired liver function and poor performance status, who already received multiple treatment lines, are often excluded from randomized controlled trial's and are ineligible for the standard of care. Nivolumab monotherapy has proven to be effective sometimes for advanced HCC, with a favorable safety profile, and hence nivolumab could be a valuable treatment option for these patients.

Research motivation

Given the recent fast evolutions in the systemic treatment of advanced HCC nowadays, this study is very topical. This article provides interesting information as a starting point for further research, *e.g.*, on patient selection per treatment strategy.

Research objectives

We aimed in this study to evaluate the real-world effectiveness of nivolumab monotherapy in patients with advanced HCC who are not eligible for other treatment.

Research methods

We conducted a retrospective, multicentric study including 29 patients with advanced HCC. All patients had had prior chemotherapy or were intolerant or ineligible for treatments. Data were retrieved from patients' medical records. The outcome parameters that we evaluated were radiological response according to RECIST criteria, the biological response through the evolution of the alpha-fetoprotein level, and clinical response considering both the Child–Pugh score and the World Health Organization

performance status. A safety profile was also reported. Statistical analysis was performed using the SPSS Statistics 27 statistical software package.

Research results

The radiological overall response rate (ORR) to nivolumab monotherapy was 24.1%. The biological ORR was 20.7%. Radiological and biological responses were significantly associated both with each other and with overall survival. Overall survival was 14.5 mo (+/- 2.1), and progression-free survival was 10.9 mo (+/- 2.3). We confirmed the favorable safety profile of nivolumab. We showed notably better results than reported in literature. Hence nivolumab monotherapy should be considered as a valuable treatment option in selected patients otherwise not eligible for systemic treatment. Further research is warranted to confirm these findings.

Research conclusions

Nivolumab monotherapy is a good treatment choice in frail patients with HCC who are ineligible for the standard of care or other validated systemic treatments.

Research perspectives

This article provides interesting information as a starting point for further research. Future research is warranted to confirm the good treatment response to nivolumab in a subgroup of patients with advanced HCC, and furthermore to define this subgroup of patients, to facilitate patient selection per treatment strategy.

FOOTNOTES

Author contributions: De Wilde N merged the dataset, executed the statistical analysis, wrote the manuscript and performed the revision; Verset G, Van Steenkiste C, Vonghia L were the supporting co-promotors in the entire process and provided the data; Francque S and De Somer T did a profound revision of the manuscript; Staub E and Bagdadi A helped with the partial composition of the dataset; Lambrechts J prepared the figures. All authors reviewed the manuscript.

Institutional review board statement: Approval of the ethics committee of the University Hospital of Antwerp, Belgium was obtained (EC number 21/06/080) and is attached. Our study conforms to the recognized standards of the Declaration of Helsinki. All participants were above 16 years of age.

Conflict-of-interest statement: There are no competing interests to declare for all authors.

Data sharing statement: Data available from the first author at nika.dewilde@ugent.be. Consent for data sharing was not obtained from the study participants, but the presented data are anonymized and risk of identification is low.

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ORIGINAL ARTICLE

Clinical Trials Study

Iohexol plasma and urinary concentrations in cirrhotic patients: A pilot study

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Abstract

BACKGROUND

Renal failure is an independent prognostic factor for survival in patients with cirrhosis. Equations to calculate serum creatinine significantly overestimate the glomerular filtration rate (GFR). Plasma clearance of direct biomarkers has been used to improve the accuracy of evaluations of GFR in this population, but no study has simultaneously measured plasma and urinary clearance, which is the gold standard.

AIM

To study calculated plasma and urinary concentrations of iohexol, based on the kinetics of samples collected over 24 h from cirrhotic patients with three different grades of ascites.

METHODS

One dose of iohexol (5 mL) was injected intravenously and plasma concentrations were measured 11 times over 24 h in nine cirrhotic patients. The urinary concentration of iohexol was also measured, in urine collected at 4, 8, 12 and 24 h.

RESULTS

The plasma and urinary curves of iohexol were similar; however, incomplete urinary excretion was detected at 24 h. Within the estimated GFR limits of our population (> 30 and < 120 mL/min/1.73 m²), the median measured GFR (mGFR) was 63.7 mL/min/1.73 m² (range: 41.3–111.3 mL/min/1.73 m²), which was an accurate reflection of the actual GFR. Creatinine-based formulas for estimating

GFR showed significant bias and imprecision, while the Brochner–Mortensen (BM) equation accurately estimated the mGFR (r = 0.93).

CONCLUSION

Plasma clearance of iohexol seems useful for determining GFR regardless of the ascites grade. We will secondly devise a pharmacokinetics model requiring fewer samples andvalidate the BM equation.

Key Words: Cirrhosis; Glomerular filtration Rate; Iohexol; Pharmacokinetics; Brochner-Mortensen

Core tip: Accurately evaluating glomerular filtration rate (GFR) in cirrhotic patients is critical to optimize their management and identify patients who should be prioritized for liver transplantation, and informs discussion of double liver–kidney transplantation. Until now, no formula or direct method for measuring GFR was available. This prospective pilot study is the first to systematically describe the plasma and urinary concentrations of iohexol, based on the kinetics of samples collected over 24 h from cirrhotic patients with three different ascites grades. The next step will be to construct a Bayesian estimator from a limited number of samples.

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INTRODUCTION

Impaired renal function is an independent prognostic factor in patients with cirrhosis, particularly decompensated patients. In addition, chronic renal impairment after liver transplantation (prevalence = 15%) is an independent predictor of mortality[1]. Serum creatinine has been incorporated into the Model for End-stage Liver Disease (MELD) as a prognostic factor. The MELD predicts mortality at 3 mo.

Guidelines recommend double liver–kidney transplantation in cases where the measured glomerular filtration rate (mGFR) is < 30 mL/min/1.73 m²[2]. Accurate evaluation of the GFR is essential to optimize the management of cirrhotic patients and to identify those who should be prioritized for liver transplantation, and can also inform the discussion of double liver–kidney transplantation[3,4].

Serum creatinine and creatinine clearance, calculated using equations such as the Cockcroft and Gault, Modification of Diet in Renal Disease-4 (MDRD-4), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), and MDRD-6 equations, tend to overestimate the GFR of cirrhotic patients by approximately 23 ± 23 mL/min/1.73 m²[5,6], particularly in cases of advanced liver disease. The cystatin-C-based and combined cystatin C and creatinine equations show promise but have not been validated in cirrhotic patients. To date, no equation has shown efficiency for direct measurement of GFR based on inulin, iohexol, or ^{51}Cr-EDTA, which are considered the gold standards in some liver transplantation centers. Iohexol has the advantages of being nontoxic (at the dose used to estimate the GFR), filtered by the glomerulus, and not reabsorbed or secreted by the tubules; it is also inexpensive compared to inulin and Cr-EDTA. However, only a few studies have assessed iohexol in compensated and noncirrhotic populations[7-9]. Moreover, those studies had several limitations, such as use of plasma samples only, or insufficient samples or a small collection time window. Finally, no study has measured iohexol urinary clearance, which remains the gold standard.

Study objectives

Our main aim was to determine the urinary and plasma concentrations of iohexol in a pilot population of nine cirrhotic patients with different grades of ascites, based on full pharmacokinetics (PK) profiles obtained over 24 h. The secondary objectives were to assess whether 24 h was sufficient to recover the entire administered dose from the urine, and to compare the plasma clearance of iohexol among different GFR evaluation methods [CKD-EPI, MDRD-4 and 6, the Royal Free Hospital (RFH) formula, and the Brochner–Mortensen (BM) formula]. Finally, we evaluated the influence of covariates on plasma iohexol clearance measurements, particularly ascites.

MATERIALS AND METHODS

Patients and samples

Eligible patients were > 18 years old and had advanced liver disease with different grades of ascites (three without ascites, three with grade I, and three with grade II or III) and a potential indication for liver transplantation. The inclusion and exclusion criteria are detailed in Supplemental material 1. This study was conducted in full compliance with the European and French guidelines of Good Clinical Practice, the most up-to-date Declaration of Helsinki (Seoul 2008), and the International Conference on Harmonization, Harmonized Tripartite Guideline for Good Clinical Practice in the European Community. This study was approved by the Independent Ethics Committee of Limoges and relevant authorities. All patients provided written informed consent to participate in this study and have their blood samples analyzed. This study was registered at EudraCT (2018-002778-35), and on ClinicalTrials.gov (NCT03769597).

Eligible patients were screened during routine medical consultations in the Hepato-Gastroenterology Department of Limoges University Hospital (V 1 visit). Patients were given time to decide on whether they wanted to participate in the study and, where applicable, consent was obtained. Whether patients met the inclusion and exclusion criteria was checked during a second visit (V0 visit). The inclusion of each patient was finalized during the V1 visit at the Clinical Investigation Center of Limoges. Clinical and biological data were collected before iohexol was injected.

Patients received a single 5 mL bolus of iohexol (Omnipaque®, 5 mL; GE Healthcare, Chicago, IL, USA). Over the next 24 h, 11 blood samples were collected for measurement of iohexol plasma concentrations at 15, 30, 60 and 90 min, and 2, 3, 4, 6, 8, 12 and 24 h. In addition, urine was collected at 4, 8, 12 and 24 h, and voiding volumes were measured. Liquid consumption was quantified and 300 mL water was provided at 3 and 6 h. Liquids were provided by the attending physician according to the clinical condition of the patients. Diuretics were systematically withdrawn during the urine collections.

Iohexol was measured in serum and urine samples, using a sensitive and specific method based on liquid chromatography coupled with tandem mass spectrometry, in the Pharmacology Unit of the University Hospital of Limoges. The internal standard was ioversol and the limit of quantification was 1 mg/L.

Statistical analysis

A noncompartmental analysis was performed using PKanalix (Lixoft, Antony, France) to determine the plasma clearance of iohexol. Urinary clearance was measured using the formula U × V/P, where U is the urinary concentration, V is the urinary output, and P is the plasma concentration of the marker. The mean concentration of iohexol from three urine samples (collected at 0–4, 4–8 and 8–12 h) was used in the formula; the plasma concentration was measured in the middle of each urine collection (2, 6 and 10 h). As no sample was available at 10 h, it was calculated using the first-order process from samples taken at 8 and 12 h: $C_{10} = C_8 \times e^{-kt}$ with k being calculated using the same formula based on t8 and t12.

The reference iohexol clearance was analyzed using linear correlation and a Bland–Altman plot. The relationships between covariates and the reference clearance were studied using linear regression and a scatter plot (for continuous covariates) or the Mann–Whitney test and boxplots (for categorical covariates). The covariates of interest were the ascites grade, age, weight (at inclusion and 24 h), albumin, natriuresis, diuretics (type and dose) and other drugs that could affect the GFR, and biological markers of liver failure or portal hypertension (bilirubin, albumin, international normalized ratio, platelet count, Child–Pugh class, and MELD score).

RESULTS

Patients

Nine male patients were included in our study. Three other patients were screened and signed the informed consent form at the V0 visit, but subsequently withdrew their consent. The characteristics of the nine patients are shown in Table 1.

PK of the plasma and urinary iohexol concentrations

The results of noncompartmental analysis of iohexol blood concentrations and the urinary clearance results are presented in Table 2.

Overall, the plasma concentration decay was similar in all patients, with two distinct phases: a rapid distribution phase (phase 1; first 2 h) and a slower phase (phase 2; elimination phase) (Figure 1). Iohexol was not detected in the plasma after 24 h, which allowed us to extrapolate the area under the curve (AUC) for 0–24 h to AUC 0-∞.

Cumulative urinary curves were similar, and showed the opposite pattern to the plasma curves. However, the dose administered was not fully recovered from the urine after 24 h; the volume collected varied from 60% to 90% of the dose injected (Figure 1).

Table 1 Patient's main characteristics median (min-max)

Characteristics	Median (min-max)
Age (yr)	60 (47-70)
Sex ratio (M:F)	1
Etiology of cirrhosis	Alcohol 78% - mixed[1] 22%
MELD	17 (8-33)
Child Pugh	7 (5-12)
Serum creatinine (µmol/L)	87 (53-142)
CKD-EPI (mL/min/1.73 m²)	83 (46-120)
MDRD 4 (mL/min/1.73 m²)	78 (44-144)
MDRD 6 (mL/min/1.73 m²)	86 (46-134)
RFH (mL/min/1.73 m²)	55 (32-105)
BM formula (mL/min/1.73 m²)	70 (40-139)
mCl plas (mL/min)	64 (41-111)
mCl urin (mL/min)	59 (42-100)
Serum albumin (g/L)	34.2 (26-37.7)
SBP (mmHg)	120 (101-140)
DBP (mmHg)	70 (60-89)
Weight (kg)	96 (70-143)
BMI (kg/m²)	32.8 (22.3-48.3)
Natremia (mmol/L)	132 (118-139)
Diuretics	56%
Bilirubin (µmol/L)	29.9 (10.2-159)
ALKP (UI/L)	136 (73-323)

[1]Mixed = alcoholic and non-alcoholic steatohepatitis. RHF: Royal Free Hospital Formula; BM: Brochner-Mortensen formula, mCl plas: Measured plasmatic clearance; mCl urin: Extrapolated urinary clearance; SBP: Systolic Blood Pressure; DBP: Diastolic blood pressure; BMI: Body mass index; ALKP: Alkaline phosphatase.

Notably, iohexol was measured in the ascites of Patient #1 at 24 h, who had grade 2–3 ascites and needed paracentesis: A low concentration (14 mg/L) was observed, suggesting negligible accumulation of iohexol in the ascites.

The median plasma and urinary clearance iohexol concentrations over 24 h were 64 mL/min (range: 41–111 mL/min) and 59 mL/min (range: mL/min) respectively. Relative to the body surface area of each patient, the median plasma clearance was 61 mL/min/1.73 m².

The Bland–Altman plots of the urinary clearance by plasma clearance of iohexol are shown in Figure 2. The correlation between urinary clearance and iohexol plasma clearance was strong (r = 0.84). The mean ± standard deviation (SD) difference between plasma clearance and urinary clearance was 3.46 ± 11.5 mL/min.

Comparison of iohexol-measured plasma clearance with other formulas estimating GFR

The Bland–Altman plots of the plasma clearance of iohexol according to the creatinine-based equations, RFH equation and BM equation are presented in Figure 2. The BM formula produced the lowest mean ± SD difference from the measured plasma clearance of iohexol (−2.48/12.90 mL/min/1.73 m²).

The regression matrix and scatterplot for the plasma clearance and urinary clearance of iohexol, for the creatinine-based equations, RFH equation and BM equation, are presented in Figure 3. A weak correlation was detected between plasma and urinary iohexol clearance for the creatinine-based equations, while a strong correlation was observed for the BM formula (r = 0.93).

Influence of covariates on iohexol plasma clearance

None of the clinically or biologically relevant covariates significantly affected the measured plasma clearance of iohexol (Figure 4).

Table 2 Non compartmental analysis: iohexol pharmacokinetic parameters

Patient no.	Cl plas (mL/min)	Cl urin (mL/min)	K plas (min-1)	Cmax (mg/L)	Vd (mL)	AUC 0-inf (mg/h/L)	AUC 0-24 h (mg/h/L)	%
1	63.71	52.89	0.003211	284	19839.7	846.26	830.171	-1.9
2	55.92	46.33	0.002345	196	23852.5	964.113	924.802	-4.1
3	58.70	48.17	0.002031	148	28897.5	918.45	860.117	-6.4
4	76.25	79.24	0.002644	154	28830.0	707.119	686.323	-2.9
5	111.26	93.53	0.003635	227	30607.4	484.599	480.014	-0.9
6	79.01	74.79	0.004057	202	19475.6	682.396	680.013	-0.3
7	41.27	42.26	0.001994	173	20689.5	1306.38	1226.09	-6.1
8	79.88	99.89	0.003817	247	20924.1	674.929	671.568	-0.5
9	63.39	58.17	0.001947	140	32556.8	850.604	779.724	-8.3

Cl plas: Measured plasma clearance of iohexol; Cl urin: Extrapolated urinary clearance of Iohexol; k plas: Plasmatic elimination constant; C max: Plasma maximal concentration; Vd: Distribution volume; AUC: Area under the curve.

The distribution volume of iohexol estimated based on noncompartmental analysis of the nine patients was compared according to the presence *versus* absence of ascites (grade I *vs* II/III *vs* absence of ascites) (Supplementary Figure 1). A trend was observed graphically, but there was no significant difference among the three groups (Kruskal–Wallis, $P = 0.8359$).

DISCUSSION

This pilot study was the first to confirm the relevance of the plasma clearance of iohexol in a cirrhotic population, by comparison with urinary clearance (the gold standard). In addition, it also confirmed the low correlations between the estimates of creatinine-based equations and the measured GFR, and showed a good performance of the BM equation in cirrhotic patients.

Iohexol is a nonionic contrast agent that is replacing inulin as the marker of choice for determining GFR. Iohexol is characterized by low extrarenal excretion and weak protein binding, and is neither secreted nor reabsorbed by the kidney. Moreover, it is nontoxic and inexpensive. The safety of iohexol has been extensively studied and confirmed in numerous studies. The doses of injected iohexol (5 or 10 mL) are more than 20 times lower than those used for computed tomography (CT); the exclusion of patients with known contrast medium reactions explains the reportedly high safety[10].

We assessed both the plasma (11 samples/patient) and urinary clearance (four separate measurements) of iohexol, based on full PK profiles of a population of cirrhotic patients with different ascites grades. Few data are available regarding the utility of this biomarker for this population[8], and no study has validated the plasma clearance of iohexol as a direct measure of GFR by comparison with urinary clearance, which remains the gold standard. Other methodological limitations of previous studies included blood sample collection over only 5 h, plasma not being sampled before 1 h, and lack of consideration of the third compartment (ascites) in which iohexol may accumulate over time (except in one study, in which ascites samples were not available after 4 h)[8].

Urinary clearance is the most accurate method to determine the filtering capacity of the kidneys, particularly in patients presenting with ascites or voluminous edema. Ideally, urinary samples should be collected every hour, with a plasma sample obtained in the middle part of the measurement period. However, as this is difficult in routine clinical practice, we collected urine at 4-h intervals and extrapolated the plasma concentration at 10 h. This may have led to imprecision in the iohexol urinary clearance estimates, and could partially explain the difference between the plasma clearance and urinary clearance. In the future, we will use a urometer in all patients given the difficulties of collecting urine.

Surprisingly, the total dose of iohexol administered was not fully recovered in the urine at 24 h, regardless of ascites grade. Between 60% and 90% of the initial dose was eliminated from the urine, although only three patients achieved the 90% clearance rate and none had an estimated GFR (eGFR) < 30 mL/min/1.73 m^2 at baseline.

Three hypotheses are proposed based on the iohexol urinary elimination curves. The first is that iohexol is not a good marker to measure GFR. However, many studies have shown that the plasma clearance of iohexol provides similar results to inulin and ^{51}Cr-EDTA measurements[11,12], except in patients with cirrhosis, and it is used as a reference for measuring GFR. Additionally, measurements of

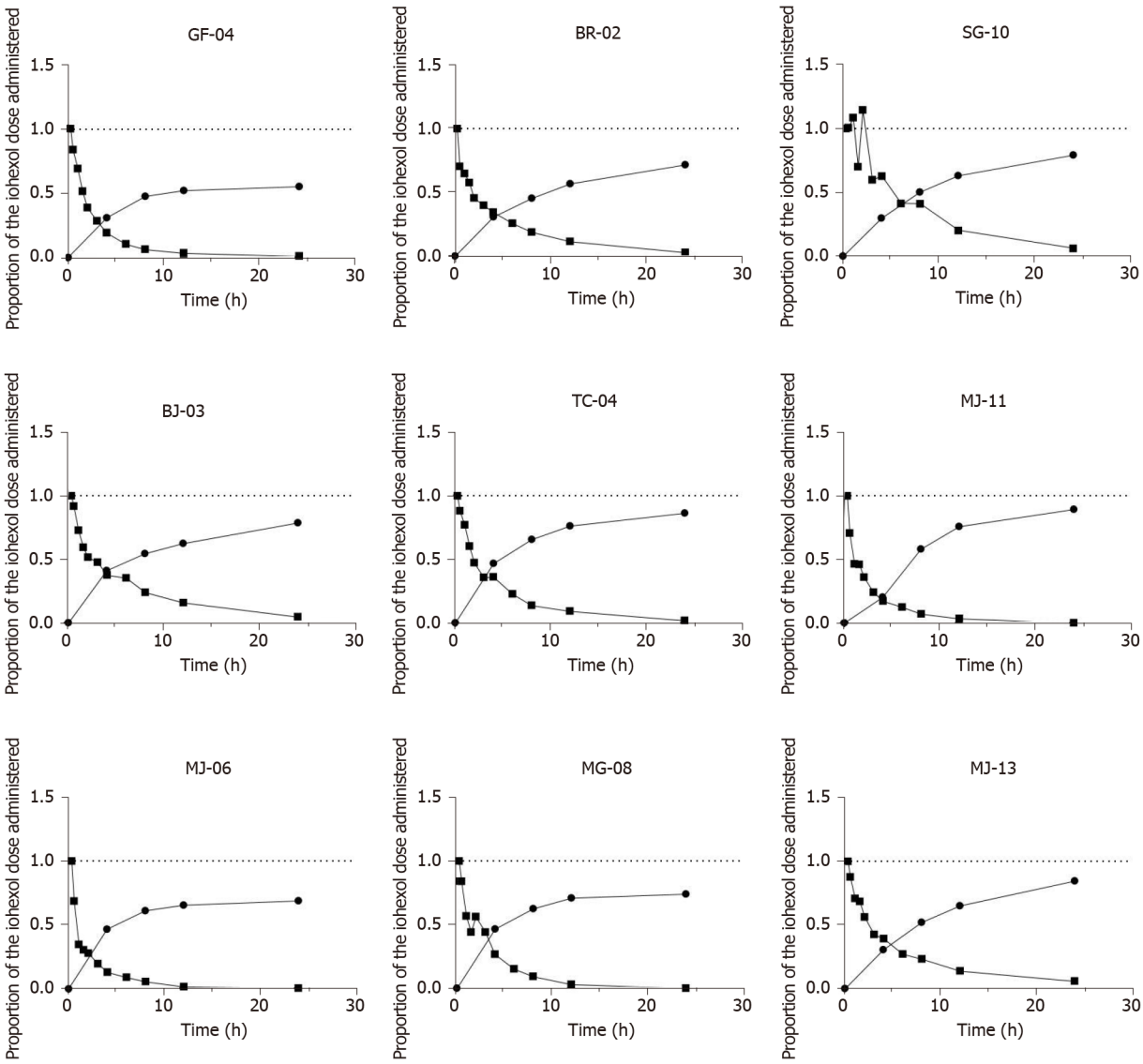

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Figure 1 For each patient studied, proportion of the iohexol administered dose detectable as a function of time in plasma and urine.

plasma clearance of these biomarkers are considered as accurate as urinary clearance measurements[10, 13].

The second hypothesis is that the collection of samples over more than 48 h, *i.e.*, until complete elimination of the iohexol in the urine, provides more accurate data, although this is almost impossible in routine clinical practice. Most studies focusing on the urinary clearance of other markers, such as inulin and Cr-EDTA, have demonstrated that systematic urine collection is difficult and rarely complete, such that interpretations of the results are prone to error. We had difficulty obtaining the entire urine output of Patient #1, and collected only approximately 60% of the initially injected dose of iohexol from this patient's urine at 24 h.

The third hypothesis is that iohexol is mainly stored in ascites or edema. Slack *et al*[8] analyzed iohexol concentrations in the ascites and plasma of three patients. Iohexol equilibrated between the blood and ascites compartments after 4 h, but blood and ascites samples were not available beyond 4 h, limiting the interpretability of their results. Those authors also compared iohexol and Cr-EDTA plasma clearance, and showed a small difference (1.3 mL/min/1.73 m^2). Our study was not designed to analyze iohexol in ascites, but we evaluated the ascites concentration of iohexol in one patient with grade 2–3 ascites who had benefited from paracentesis, immediately after collecting the plasma and urine samples at 24 h. Iohexol was present, albeit at a low concentration (14 mg/L), indicating that ascites was not the main storage location for iohexol. A question raised by the present study is where is the iohexol stored in decompensated cirrhosis patients?

Iohexol plasma clearance was significantly different However, the distribution volume was not significantly affected by ascites (Supplementary Figure 1) within the GFR range estimated in our patients (30–120 mL/min/1.73 m^2). This supports the hypothesis that the difference in plasma iohexol

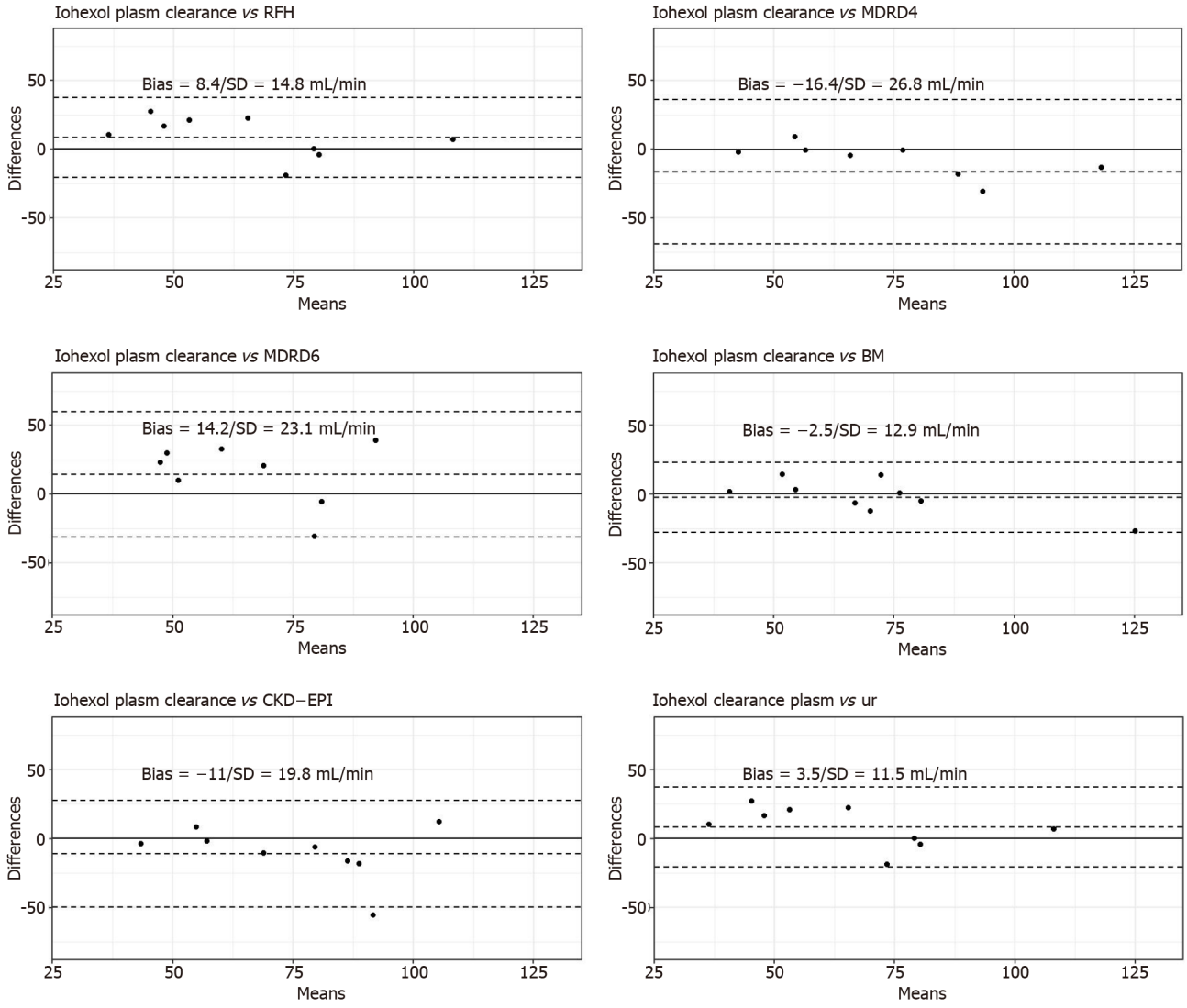

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Figure 2 Bland–Altman plots for creatinine-based formula, Royal Free Hospital formula, Brochner–Mortensen formula and iohexol urinary clearance in comparison to iohexol plasma clearance.

clearance observed between patients with and without ascites is attributable to a true difference in renal function, as opposed to iohexol clearance measurement error.

A strength of this study was that we collected a large number of plasma samples, allowing highly accurate plasma iohexol concentration curves to be constructed. The decrease in plasma iohexol concentrations during phase 2 (elimination phase) followed first-order elimination, and the amount of iohexol detected in blood at 24 h was at or below the detection limit (1 ng/mL). Thus, the half-life of iohexol did not vary significantly among ascites grades, or according to the presence or absence of ascites. This result suggests that the characteristics of cirrhotic patients, such as ascites, have a minor effect on iohexol clearance. The correlation coefficient between the complete plasma clearance of iohexol and estimated urinary clearance was strong (R^2 = 0.846). However, we were unable to draw conclusions regarding GFR values < 30 or > 120 mL/min/1.73 m², as these were not measured in this study. High-precision evaluation of the GFR in this range is useful, as patients with an eGFR < 30 mL/min/1.73 m² are considered as candidates for immediate double transplantation[14].

We conclude that measurements of plasma clearance of iohexol are probably as accurate (and less cumbersome and more feasible) as urinary clearance measurements to estimate the GFR in cirrhotic patients, with consideration of the limitations mentioned above. Therefore, we used plasma clearance as a reference for GFR assessment based on the comparisons conducted in this study.

The creatinine-based equations were not useful, with the possible exception of the RHF formula; the correlation coefficients between the complete plasma clearance of iohexol and GFR estimated by the serum creatinine-based formulas were small. The coefficients ranged from 0.634 (for the CKD-EPI equation) to 0.684 (for the MDRD-6 equation), which agrees with literature data indicating that the MDRD-6 equation is probably the most accurate[15]. The MDRD-6 was proposed as the reference (by

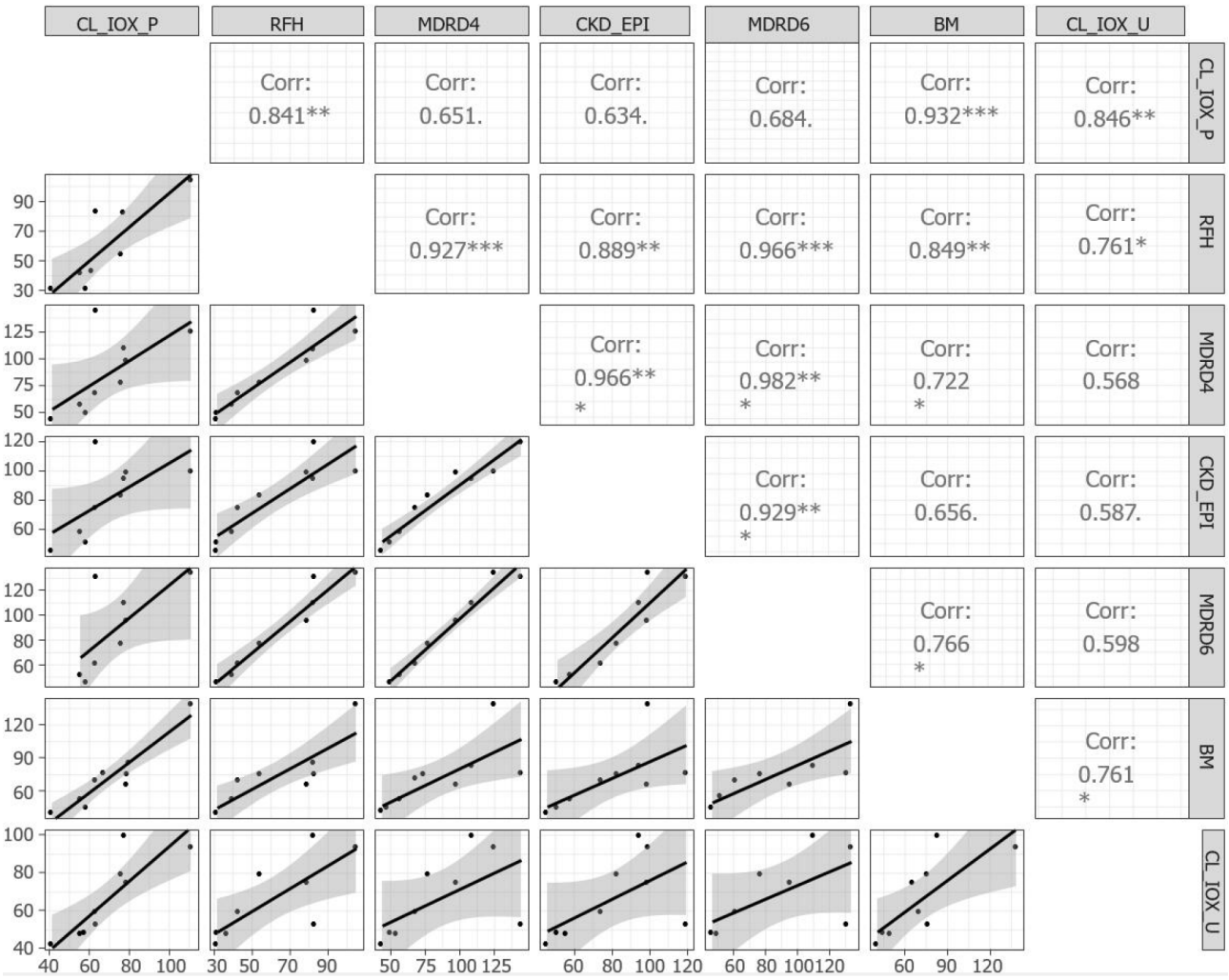

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Figure 3 Matrix correlation and scatter plots for the iohexol plasma and urinary clearance, the creatinine-based equations, RHF and Brochner–Mortensen. CL-IOX-P: Iohexol plasma clearance; CL-IOX-U: Iohexol urinary clearance.

US consensus guidelines) to identify candidates for simultaneous liver and kidney transplantation[14]. The RFH formula, which was introduced more recently[16], was the most accurate creatinine-based equation for evaluating the GFR in our population (R^2 = 0.841) but showed large variability (SD = 8.44/14.79 mL/min/1.73 m²). Moreover, the RFH formula has not been widely validated.

An unexpected result in our cirrhosis patients was the strong correlation between the measured plasma clearance and GFR estimated by the BM equation[17]; the difference between the estimated and measured GFR was lowest for this equation, for which dispersion on the Bland–Altman plot was also the smallest. The BM equation is not affected by the initial rapid phase of plasma clearance of iohexol (< 1 h; distribution phase). The BH equation requires fewer plasma samples (n = 4 in this study), which are obtained during phase 2 (elimination), and provides accurate estimates of the GFR. However, it has not been validated in terms of the third compartment (ascites).

The main limitation of this study was the small number of patients included. However, this was a descriptive pilot study aiming to elucidate the behavior of iohexol in the plasma and urine through full PK profiles (*i.e.*, with early and late samples obtained over a period of at least 24 h), as this has not been explored before in a cirrhotic population. The small number of patients may also explain why variables known to affect GFR were not significant (*e.g.*, diuretic intake).

Patients with different ascites grades were recruited prospectively and consecutively, but unfortunately, as all participants were male, there was a recruitment bias with respect to gender. Although cirrhosis mainly affects men, women are equally affected by overestimates of the GFR by serum creatinine-based formulas, particularly during the pretransplant period. Women have relatively low serum creatinine levels and are therefore likely to be disadvantaged by graft allocation systems based on the MELD score. As an illustration, after the MELD score was adopted to allocate liver transplants, the proportion of male transplant recipients increased, and the waiting list mortality rate for women was higher than for men. Women scored higher when creatinine was replaced with the mGFR in the MELD scoring system. Therefore, this is essential to ensure equal access to liver transplantation between

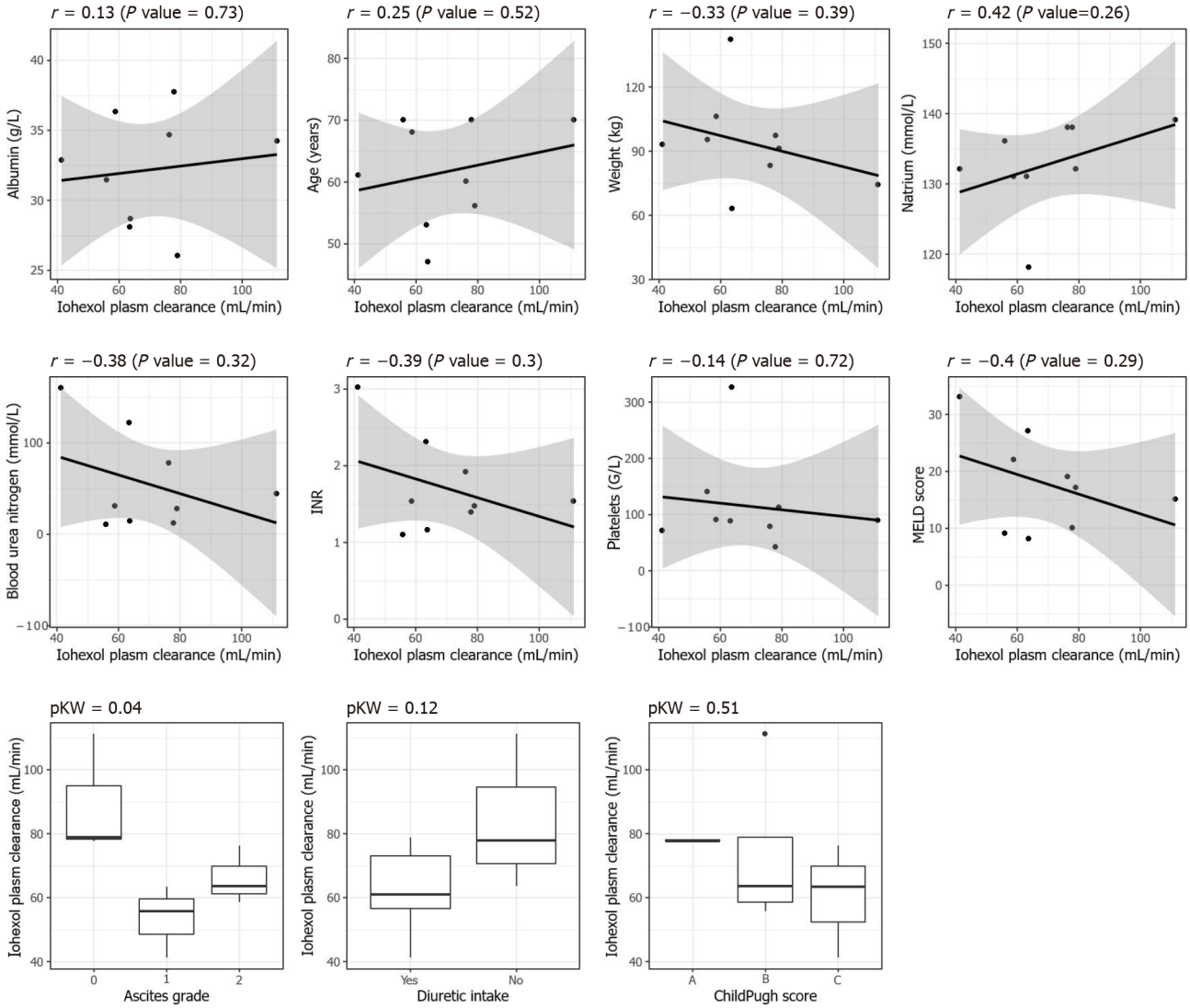

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Figure 4 Scatter plot and boxplots between the different covariables including the grade of ascites (0 = no ascites, 1 = grade I, 2 = grade II or III) and the iohexol plasma clearance, *P* KW = *P* value for the Kruskal–Wallis test.

genders[15,18,19].

Patient #7 had a maximum concentration of iohexol higher than that measured at the end of perfusion. This may have been due to the iodine injection that this patient received for a CT scan 15 d previously. Thus, patients with severe cirrhosis are likely to benefit from radiological examinations involving an iodine injection that may interfere with iohexol plasma clearance.

Finally, no control group of healthy patients or patients without cirrhosis was included in this study. However, two previous studies that compared the performance of iohexol and inulin in terms of estimating plasma and renal clearance in healthy subjects obtained comparable results. If we assume that 100% of inulin is recovered in the urine in a healthy population, and that renal clearance is the same using iohexol, we can further assume that 100% of iohexol will be recovered[20,21].

Obtaining a full PK profile remains difficult in clinical practice, as 11 plasma samples are needed over 24 h. This can only be achieved in a small proportion of patients with highly complex profiles, particularly before liver transplantation. The next steps will be to investigate the performance of the BM equation in a larger cohort of cirrhotic patients, and to construct a PK model for cirrhotic patients for estimating iohexol plasma clearance based on a limited number of samples.

CONCLUSION

Accurate evaluation of GFR in cirrhotic patients is critical, but no formula or direct measurement method has been available until now. Even though urinary clearance is considered the gold standard, we showed that it requires urine to be collected over more than 24 h, which is not feasible in practice.

Our study suggests that the plasma clearance of iohexol is more valuable for determining the GFR in cirrhotic patients than urinary clearance is, and that specific patient characteristics, such as ascites, have a minor effect on mGFR. The next step will be to construct a PK model in a larger cirrhotic cohort that requires fewer samples, to simplify the mGFR estimate. In addition, the validity of the BM equation must be confirmed.

ARTICLE HIGHLIGHTS

Research background

To date, no method for measuring the glomerular filtration rate (GFR) based on either creatinine or an exogenous marker, which is both reliable and applicable in clinical practice in cirrhotic patients with different degrees of decompensation, is available.

Research motivation

We urgently need accurate methods to measure GFR in cirrhotic patients; renal failure being a key prognostic factor in decompensated cirrhosis, particularly in the pre and post-transplant period.

Research objectives

Describing the complete pharmacokinetic (PK) study of iohexol in blood and urine as an appropriate and inexpensive marker is essential to subsequently construct a PK model from a limited number of samples.

Research methods

This pilot study included nine patients with different ascites grades, who received a single 5-mL bolus of iohexol, with the collection of 11 blood samples and all the urine volume (in four samples) over a period of 24 h.

Research results

Iohexol was almost no longer detected in plasma at 24 h that allowed us to extrapolate the area under the curve (AUC) 0–24 h to AUC 0–∞. The dose recovery in urine varied from 60% to 90% of the dose injected. The correlation between urine clearance and iohexol plasma clearance was strong. As expected, a low correlation with the estimated GFR (eGFR) calculated by creatinine-based equations was observed contrary to the Brochner–Mortensen (BM) equation, which exhibited a high correlation.

Research conclusions

This study confirmed the relevance of the plasma clearance of iohexol in the cirrhotic population. It also suggests a high accuracy of the BM equation and confirms the low correlation with eGFR estimated by creatinine-based equations.

Research perspectives

A future study based on a larger cohort of cirrhotic patients with different ascites grades will be performed to devise a PK model allowing the estimation of iohexol plasma clearance from a limited number of samples and to investigate the performance of the BM equation.

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FOOTNOTES

Author contributions: Carrier P, Essig M, Monchaud C, Woillard JB, and Loustaud-Ratti V designed the study; Carrier P, Debette-Gratien M, and Loustaud-Ratti V included the patients; Giguet B collected the data; Destère A and Woillard JB performed the statistical analysis; Carrier P, Destère A, Woillard JB, and Loustaud-Ratti V wrote the manuscript.

Institutional review board statement: The study was reviewed and approved by the French National Review Board and the Independent Ethics Committee of Limoges.

Clinical trial registration statement: This study was registered at EudraCT (2018-002778-35), and on ClinicalTrials.gov (NCT03769597).

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

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

Data sharing statement: No additional data are available.

CONSORT 2010 statement: The authors have read the CONSORT 2010 statement, and the manuscript was prepared and revised according to the CONSORT 2010 statement.

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ORIGINAL ARTICLE

Observational Study

Higher cardiovascular risk scores and liver fibrosis risk estimated by biomarkers in patients with metabolic-dysfunction-associated fatty liver disease

Giovanni Alejandro Salgado Alvarez, Samanta Mayanin Pinto Galvez, Uriel Garcia Mora, Ana Delfina Cano Contreras, Cristina Durán Rosas, Bryan Adrián Priego-Parra, Arturo Triana Romero, Mercedes Amieva Balmori, Federico Roesch Dietlen, Sophia Eugenia Martinez Vazquez, Ines Osvely Mendez Guerrero, Luis Alberto Chi-Cervera, Raúl Bernal Reyes, Leonardo Alberto Martinez Roriguez, Maria Eugenia Icaza Chavez, Jose Maria Remes Troche

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Abstract

BACKGROUND

The definition of metabolic-dysfunction-associated fatty liver disease (MAFLD) allows identification of metabolically complicated patients. Fibrosis risk scores are related to cardiovascular risk (CVR) scores and could be useful for the identi-

fication of patients at risk of systemic complications.

AIM

To evaluate the relationship between MAFLD and CVR using the Framingham risk score in a group of Mexican patients.

METHODS

Cross-sectional, observational and descriptive study carried out in a cohort of 585 volunteers in the state of Veracruz with MAFLD criteria. The risk of liver fibrosis was calculated with aspartate aminotransferase-to-platelet ratio index, nonalcoholic fatty liver disease score and fibrosis-4, as well as with transient hepatic elastography with Fibroscan®. The CVR was determined by the Framingham system.

RESULTS

One hundred and twenty-five participants (21.4%) with MAFLD criteria were evaluated, average age 54.4 years, 63.2% were women, body mass index 32.3 kg/m^2. The Framingham CVR was high in 43 patients (33.9%). Transient elastography was performed in 55.2% of volunteers; 39.1% with high CVR and predominance in advanced fibrosis (F3–F4). The logistic regression analysis showed that liver fibrosis, diabetes and hypertension independently increased CVR.

CONCLUSION

One of every three patients with MAFLD had a high CVR, and in those with high fibrosis risk, the CVR risk was even greater.

Key Words: Fatty liver; Cardiovascular risk; Hepatic steatosis; Fibrosis; Liver disease

Core Tip: Metabolic-dysfunction-associated fatty liver disease (MAFLD) allows identification of metabolically complicated patients. Evaluation of the relationship between hepatic fibrosis and cardiovascular risk (CVR) in patients with MAFLD using the Framingham risk score allows us to identify which patients with MAFLD and liver fibrosis have a higher CVR than patients without fibrosis.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterized by steatosis > 5% in the absence of alcohol consumption and other causes of liver disease[1]. Due to its close relationship with the components of the metabolic syndrome, a consensus of international experts proposed a change of name, resulting in the concept of metabolic-dysfunction-associated fatty liver disease (MAFLD) as the new terminology[2-4].

The presence of diabetes mellitus (DM), obesity and metabolic dysregulation to establish the diagnosis of MAFLD can help identify patients with metabolically complicated fatty liver disease and consequently with higher cardiovascular risk (CVR). We consider that the Framingham score can be a useful tool in the evaluation of CVR in patients with MAFLD because it independently assesses the presence of diabetes as a CVR factor[5,6].

The Hepatic Fibrosis Scoring System is related to CVR scores in patients with MAFLD and can be useful for identifying the risk of systemic complications[7]. However, we do not have Mexican cohorts that consider the new definition of MAFLD and CVR[8]. Therefore, the objective of our work was to evaluate the relationship between MAFLD and CVR in a group of Mexican volunteers using the Framingham scale.

MATERIALS AND METHODS

This was a cross-sectional, observational and descriptive study carried out in the population of a cross-sectional sample evaluated at the Instituto de Investigaciones Medico Biologicas and Centro de Servicios en Salud of the Universidad Veracruzana during February to March 2020. Residents of the State of Veracruz aged > 18 years were invited to participate. After signed informed consent, a medical evaluation was performed, which consisted of anthropometry measurements [weight, height, body mass index (BMI), waist and hip circumference, waist–hip index], biochemical studies [hematic biometry, glucose, creatinine, uric acid, lipids, aspartate aminotransferase (AST), alanine aminotransferase, alkaline phosphatase (AP), bilirubin, albumin and insulin] and liver ultrasound. In addition, blood pressure, personal history of DM, systemic arterial hypertension, dyslipidemia, cardiovascular events, and tobacco use were recorded.

From the studied population, patients older than 40 years with diagnostic criteria for MAFLD were included. Patients with cancer, terminal disease, history of cardiovascular events and pregnant women were excluded.

The risk of liver fibrosis was determined with aspartate aminotransferase-to-platelet ratio index (APRI), NAFLD and fibrosis-4 (FIB-4) scores. The APRI was at high risk of significant fibrosis with a score of > 1.5, indeterminate 0.5–1.5, and unlikely or absent < 0.5; NAFLD score was considered high risk with > 0.675, indeterminate 1.455 to 0.675, and absent < 1.455; FIB-4 score was considered high risk with > 3.25, indeterminate 1.45–3.25 and absent < 1.45[9-11]. Transient elastography (TE) with Fibroscan® was performed in patients with undetermined and high risk of liver fibrosis[12,13]. The CVR was calculated with the Framingham system which evaluates: age, sex, total cholesterol, high-density lipoprotein cholesterol, blood pressure, use of antihypertensive drugs, tobacco consumption, DM, history of vascular disease; classifying patients as low, moderate or high CVR[14,15].

The project was carried out in accordance with the principles of Good Clinical Practices and prior approval of the Ethics Committee with number IIMB-UV 2020/03.

Statistical analysis

The analysis of the results, elaboration of figures and tables was carried out with the IBM SPSS® Statistics version 22.0 . Nominal and ordinal variables were described with frequencies and percentages, continuous and discrete variables with measures of central tendency and dispersion according to their distribution. The comparison between groups was carried out with the χ^2 test and analysis of variance. Nonparametric statistics with Spearman's correlation test were used in the relationship between CVR and fibrosis. Statistical significance was considered when the *P* value was < 0.05.

RESULTS

Population characteristics

Of the 585 volunteers, 125 (21.4%) who met the inclusion criteria were studied, 79 (63.2%) were women, average age 54.4 ± 8.8 years, BMI 32.3 ± 5.3 kg/m^2.

CVR assessment

According to the Framingham score, 46 patients (36.2%) had mild CVR, 36 (28.3%) moderate and 43 (33.9%) high. No differences were found by sex or BMI between the CVR categories. The patients' age with high CVR was 59 ± 8.4 years; higher than in mild and moderate CVR (*P* = 0.028). The presence of DM, hypertension and tobacco use was significantly higher in patients with high CVR. The concentration of glucose and insulin was higher in patients with high CVR; therefore, the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index showed a value > 3.0 compatible with insulin resistance (*P* = 0.000) compared to patients with low and moderate CVR (HOMA-IR 1.96–3). The rest of the biochemical parameters evaluated did not show significant differences (Table 1). Fibrosis scores showed an increasing trend in patients with high CVR; however, this difference was not significant (0.094).

Evaluation of liver fibrosis

The distribution between the fibrosis risk stages by FIB-4, NAFLD and APRI scores is shown in Figure 1. Fifty-two patients (44.8%) with indeterminate and high risk of fibrosis were identified according to FIB-

Table 1 Clinical and biochemical characteristics of patients with metabolic-dysfunction-associated fatty liver disease and cardiovascular risk estimated by the Framingham system (*n* = 125)

	Cardiovascular risk[1]			
***n* (%) / mean ± SD**	**Mild, *n* = 46**	**Moderate, *n* = 36**	**High, *n* = 43**	***P* value**
Sex				
Female	35 (76.1)	23 (63.9)	21 (48.8)	0.028
Male	11 (23.9)	13 (36.1)	22 (51.2)	
Age	50.1 ± 8.1	54.6 ± 7.6	59 ± 8.4	0.000[2]
BMI (average)	32.5 ± 5.8	32.3 ± 5.9	32.1 ± 4.1	0.964
Normal, *n* (%)	-	2 (5.6)	1 (2.3)	-
Overweight, *n* (%)	16 (34.8)	13 (36.1)	12 (27.9)	0.480
Obesity, *n* (%)	30 (65.2)	21 (58.3)	30 (69.8)	
Comorbidities				
Smoking, *n* (%)	10 (21.7)	16 (44.4)	21 (48.8)	0.018
DM, *n* (%)	-	-	28 (65.1)	0.000[2]
Hypertension, *n* (%)	11 (23.9)	16 (44.4)	27 (62.8)	0.000[2]
SBP	120 ± 12	127 ± 13	131 ± 16	0.001[2]
Biochemistry				
PLQ (x 10^3/mm^3)	249 ± 82	229 ± 60	229 ± 68	0.335
Glucose (mg/dL)	93 ± 14	95 ± 16	147 ± 74	0.000[2]
TB (mg/dL)	0.62 ± 0.20	0.65 ± 0.26	0.75 ± 0.4	0.126
AST (UI)	34.0 ± 15.4	36.1 ± 13	37.2 ± 20.4	0.660
ALT (UI)	39.7 ± 28.1	37.6 ± 18.6	40.7 ± 35.1	0.888
AP (UI)	82.1 ± 19.2	96.5 ± 33.2	97.7 ± 36.6	0.030[2]
Albumin (g/dL)	4.0 ± 0.25	4.1 ± 0.32	4.0 ± 0.23	0.626
Insulin	8.9 ± 4.7	8.3 ± 4.3	12.8 ± 9.1	0.004[2]
HOMA-IR	2.0 ± 1.19	1.9 ± 1.0	4.4 ± 3.5	0.000[2]
Creatinine (mg/dL)	0.7 ± 0.1	0.9 ± 0.5	0.9 ± 0.3	0.289
Uric acid (mg/dL)	6.0 ± 1.3	5.9 ± 1.6	6.1 ± 1.6	0.813
TC (mg/dL)	205.9 ± 38.2	201.4 ± 36.4	192.5 ± 38.4	0.240
LLD (mg/dL)	114.9 ± 32.7	115.1 ± 27.9	104.3 ± 40.4	0.261
HDL (mg/dL)	52.7 ± 12.5	53.09 ± 18.3	48.6 ± 12.8	0.296
TG (mg/dL)	225.1 ± 332.6	167.6 ± 58.8	197.6 ± 96.6	0.477
Fibrosis markers				
FIB-4	1.286 ± 0.772	1.569 ± 0.836	1.851 ± 1.744	0.094
APRI	0.345 ± 0.241	0.376 ± 0.215	0.419 ± 0.419	0.526
NAFLD score	-1.166 ± 1.180	-1.151 ± 1.060	-0.677 ± 1.333	0.110

[1]Cardiovascular risk estimated by Framingham system.
[2]Analysis of variance ANOVA.
BMI: Body mass index; DM: Diabetes mellitus; SBP: Systolic blood pressure; PLQ: Platelets,; TB: Total bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AP: Alkaline phosphatase; TC: Total cholesterol; LLD: Lipoprotein low-density; HDL: High-density lipoprotein; TG: Triglycerides; FIB-4: Fibrosis-4; APRI: Aspartate aminotransferase-to-platelet ratio index; NAFLD: Nonalcoholic fatty liver disease.

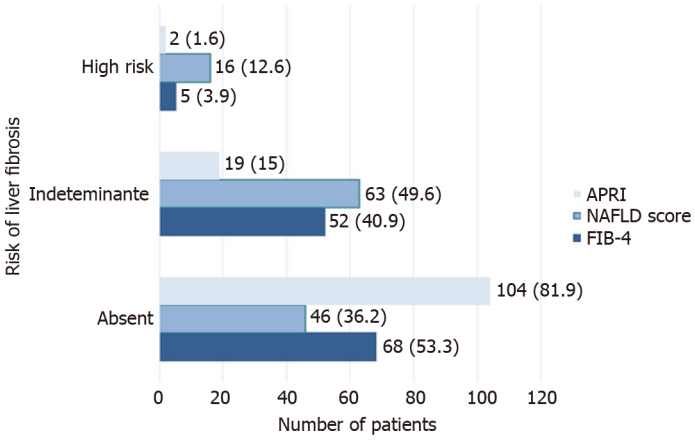

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Figure 1 Frequency of liver fibrosis risk in patients with metabolic-dysfunction-associated fatty liver disease according to fibrosis-4, nonalcoholic fatty liver disease score and aspartate aminotransferase-to-platelet ratio index, *n* (%). APRI: Aspartate aminotransferase-to-platelet ratio index; NAFLD: Nonalcoholic fatty liver disease; FIB-4: Fibrosis-4.

4, 79 (62.2%) according to NAFLD score and 21 (15.6%) with APRI.

The study was performed in 69 patients (55.2%) with indeterminate or high risk of fibrosis. Patients were identified as follows, F0: 19 (27.5%), F1: 12 (17.4%), F2: 11 (15.9%), F3: 13 (18.8%) and F4: 14 (20.3%). In the evaluation of hepatic steatosis by controlled attenuation parameter (CAP) the results were the following, S0: 13 patients (18.8%), S1: 7 (5.5%), S2: 3 (2.4%) and S3: 46 (36.2%).

The age distribution showed a significant difference between the risk of fibrosis due to FIB-4, as it was higher in the group with indeterminate fibrosis and lower in patients with absence of fibrosis (P = 0.000). The BMI and the comorbidities evaluated did not show significant differences between the risk groups. Patients with high risk of fibrosis had decreased platelet and albumin counts, as well as significantly elevated levels of total bilirubin, AST, and phase angle compared to patients without fibrosis or indeterminate risk of fibrosis (P = 0.000).

Liver fibrosis and CVR

The correlation of liver fibrosis risk by FIB-4 and CVR according to the Framingham system showed that 33.4% of patients with MAFLD had a high CVR, predominantly in patients with indeterminate risk of fibrosis (18.2%). 14.4% of the patients with no fibrosis had a high CVR *versus* 19.8% of the patients with an indeterminate or high risk of fibrosis. The risk of fibrosis did not show a significant correlation with the severity of CVR (P = 0.257). The categories of CVR and risk of fibrosis by FIB-4 can be observed in Figure 2A.

In 69 patients evaluated with TE, 18 patients (26.1%) had mild CVR according to the Framingham system, 24 (34.7%) with moderate and 27 (39.1%) with high risk. It was observed that the group with the greatest number of patients with high CVR were those with advanced fibrosis. 39.1% of patients with MAFLD had a high CVR at the time of diagnosis with a predominance of advanced fibrosis (F3–F4). In relation to fibrosis severity, it was noted that in advanced fibrosis, 34.8% had moderate to high CVR, predominantly observing the correlation between advanced fibrosis and high CVR. A statistically significant relationship was reported between the presence of fibrosis and the severity of CVR (P = 0.026). The distribution of the different risk categories in patients with absent or mild-moderate fibrosis was heterogeneous as shown in Figure 2B.

Hepatic steatosis and CVR

The correlation between CAP and CVR showed that 28.9% of the patients with S3 had a high CVR. Although most of our patients were found in S3, the severity of the CAP did not show a significant relationship with the severity of the CVR (P = 0.254). Table 2 shows the correlation between CVR and CAP. In logistic regression analysis, the presence of fibrosis P = 0.007 [95% confidence interval (CI): 0.157–35.376], DM P = 0.000 (95%CI: 0.791–43.555) and hypertension P = 0.035 (95%CI: 0.085–5.228) were independently and significantly associated with the CVR, but not with the presence of steatosis P = 0.220 (95%CI: 0.144–22.921).

DISCUSSION

MAFLD is currently the most common chronic liver disease worldwide, present in 25% to 30% of the population. Although the severity of the disease criteria has not been established, it is described that the

Table 2 Correlation between hepatic steatosis and cardiovascular risk by the Framingham system (*n* = 69)

	Steatosis (CAP), *n* (%)				
	S0, *n* = 13	S1, *n* = 7	S2, *n* = 3	S3, *n* = 46	*P* value
Framingham					
Mild (*n* = 18)	3 (4.3)	2 (2.9)	0	13 (18.8)	0.254
Moderate (*n* = 24)	5 (7.2)	5 (7.2)	1 (1.4)	13 (18.8)	
High (*n* =27)	5 (7.2)	0	2 (2.9)	20 (28.9)	

Degree of steatosis due to controlled attenuation parameter: S0 < 5% of hepatic fatty tissue, S1 between 5% to 33%, S2 between 34% to 66% and S3 > 64%. Cap: controlled attenuation parameter.

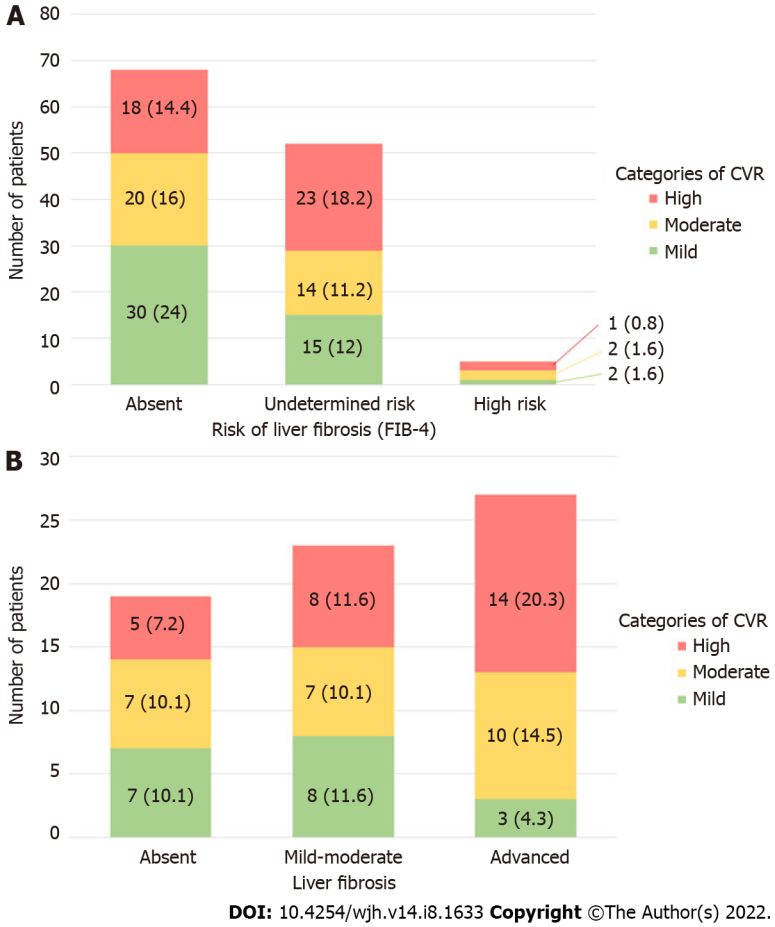

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Figure 2 Distribution of cardiovascular risk. A: Distribution of cardiovascular risk (CVR) according to the Framingham system in patients with metabolic-dysfunction-associated fatty liver disease and risk of liver fibrosis according to fibrosis-4 (FIB-4) (*n* = 125); B: Distribution of CVR estimated by the Framingham system in patients with metabolic-dysfunction-associated fatty liver disease according to hepatic fibrosis estimation with transient elastography (*n* = 69), *n* (%). Degrees of fibrosis by Kpa according to METAVIR: Absent: F0; Mild–moderate: F1 and F2; Advanced: F3 and F4. CVR: Cardiovascular risk; FIB-4: Fibrosis-4.

presence of fibrosis is the most important prognostic marker of mortality, independent of the severity of the fatty infiltration[16,17]. Our study, carried out in a Mexican population, showed that one out of every three patients with MAFLD had a high CVR, and the higher the fibrosis the greater the CVR. We show novel results as it is one of the first studies to use the new definition of MAFLD in association with CVR[18].

The change in diagnostic criteria from NAFLD to MAFLD was made recently. Therefore, in Mexico we have few prevalence studies with the new definition. The study carried out by Bellentani *et al*[19] in 585 healthy volunteers published as a summary showed a prevalence of MAFLD of 42.1; high if we compare it with the prevalence of NAFLD estimated worldwide. In our cohort, we found that 21.4% of the population over 40 years of age had MAFLD, like the prevalence of NAFLD reported in the adult population of various western countries. Although the prevalence of DM in patients with NAFLD is

50% to 70%, in our population, it was lower (22.4%). We found a high prevalence of overweight and obesity (97.6%), like that observed in other populations where between 80% and 90% has been reported. We consider that the differences may be because previously conducted studies consider the NAFLD criteria[20,21].

Liver biopsy is the gold standard in the evaluation of fibrosis, but it carries the risk of complications coupled with high cost. For this reason, the use of noninvasive systems such as serum markers, TE or magnetic resonance imaging is recommended. TE with FibroScan® is the most widely used and validated elastography worldwide with good sensitivity and specificity to diagnose F4 stage (both 92%). The FIB-4 index has been shown to be superior to other noninvasive markers in the identification of advanced fibrosis in patients with MAFLD; therefore, it is the marker of choice in the two-step algorithms[22]. Shah *et al*[23] compared the diagnostic performance of noninvasive fibrosis markers and concluded that the FIB-4 index is better at identifying advanced fibrosis in patients with MAFLD. The diagnostic performance of the markers used in our study showed similar results, with a higher correlation of advanced fibrosis by FIB-4 with TE.

The main cause of death in patients with MAFLD is cardiovascular disease, and the secondary causes are related to the liver. Cardiovascular diseases most frequently observed in patients with MAFLD are left ventricular dysfunction, atherosclerotic disease, disturbances in the cardiac conduction system, and cerebral ischemic events. These observations establish the close relationship between the severity of liver disease and the risk of fatal and nonfatal cardiovascular events[24]. Despite current evidence, in daily clinical practice, MAFLD is considered a benign entity. For this reason, our study focused on the identification of patients with MAFLD and a high risk of cardiovascular events and its relationship with hepatic fibrosis markers. Our purpose was to recommend early identification mechanisms to prevent complications and decrease mortality.

Fatty liver is associated with increased CVR regardless of the presence of DM, dyslipidemia, and hypertension. Therefore, early identification is important to reduce cardiovascular mortality[25]. Our results show that the majority of the population with MAFLD has mild CVR according to the Framingham system. However, more patients with higher CVR were identified at indeterminate risk of fibrosis due to FIB-4 (18.2%). Our results showed that most of the patients with high CVR have advanced fibrosis (F3–F4). In addition to this, these patients had a higher frequency of DM and hypertension. These results reflect that the higher the CVR, the greater the risk of liver fibrosis, which allows the early identification of patients with compensated advanced liver disease.

Cardiovascular disease and arteriosclerosis are the result of endothelial damage, dyslipidemia, and oxidative stress reported more frequently in patients with MAFLD. However, as reported in the literature, the severity of hepatic fatty infiltration did not demonstrate a relationship with CVR[5,26].

Various studies have reported that the FIB-4 index ≥ 2.67 is independently associated with coronary atherosclerosis and cardiovascular events; therefore, with an increase in CVR[27,28]. In our study, results similar to those published in previous clinical trials were observed, exhibiting a correlation between FIB-4 and the CVR systems compared to NAFLD and APRI ($P < 0.05$). Another limitation was that it was not a nationally representative population, as it only included volunteers from the State of Veracruz.

It is important to mention and recognize that our study had limitations that must be considered. One of them was that the prevalence of MAFLD was calculated in volunteers older than 40 years and in different clinical trials the population older than 18 years was included; therefore, the prevalence could be underestimated in our cohort. Finally, it is recognized that the gold standard for evaluating fibrosis and steatosis in patients with MAFLD is liver biopsy. However, due to the risks of this procedure, we performed the evaluation of fibrosis and steatosis with only biochemical markers and transient elastography.

CONCLUSION

One of every three patients with MAFLD had a high CVR and the greater severity of fibrosis correlated with a greater CVR. According to our results, the early identification of CVR in patients with MAFLD will allow establishment of preventive actions and timely treatment to reduce the risk of mortality in this population.

ARTICLE HIGHLIGHTS

Research background

The investigation was carried out in an open population without a diagnosis of metabolic-dysfunction-associated fatty liver disease (MAFLD).

Research motivation
To identify patients with MAFLD and establish their cardiovascular risk (CVR).

Research objectives
The objective is to evaluate the relationship between fibrosis and steatosis measured by transition elastography in patients with MAFLD and CVR scores in Mexican patients.

Research methods
Identification of patients with MAFLD in the open population. Subsequently, determination of the risk of fibrosis by noninvasive methods. Finally, CVR was determined by the Framingham risk scale and was related to the presence of fibrosis and steatosis.

Research results
21.4% of the study population met MAFLD criteria. The severity of CVR was related to the presence of fibrosis, but not with the severity of steatosis.

Research conclusions
Patients with MAFLD and liver fibrosis have a higher CVR compared to patients without fibrosis, regardless of the severity of steatosis.

Research perspectives
Prospective research is required to determine the best CVR score in patients with MAFLD.

FOOTNOTES

Author contributions: Salgado Alvarez GA, Pinto Galvez SM, Garcia Mora U and Cano Contreras AD contributed to the first writing of the manuscript, methodology, analysis of results; Durán Rosas C, Priego-Parra BA and Triana Romero A contributed to review and editing; Amieva Balmori M, Roesch Dietlen F, Martinez Vazquez SE and Mendez Guerrero IO contributed to the conception and development of research; Chi-Cervera LA, Bernal Reyes R, Martinez Roriguez LA, Icaza Chavez ME and Remes Troche JM contributed to the conception and development of research, project management, review and editing.

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Informed consent statement: Patients signed and provided the informed consent to this study.

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ORIGINAL ARTICLE

Observational Study

Prevalence of sarcopenia using different methods in patients with non-alcoholic fatty liver disease

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Abstract

BACKGROUND

Sarcopenia is a clinical condition associated with several liver diseases and it includes non-alcoholic fatty liver disease (NAFLD) in its broad spectrum as steatosis, steatohepatitis and fibrosis. However, the criteria to define sarcopenia are diverse, and even those established in consensus have been discussed regarding their performance in making an accurate diagnosis.

AIM

To evaluate the prevalence of sarcopenia, using different methods, in patients with NAFLD, and its association with clinical-anthropometric parameters.

METHODS

This was an observational study of outpatients with NAFLD. Sarcopenia was defined by the European Working Group Consensus on Sarcopenia in Older People of 2010 (EWGSOP1) and 2018 (EWGSOP2). The skeletal muscle index was used to estimate muscle mass, handgrip strength was assessed using the dynamometer and physical performance by walking a distance of four meters at usual walking speed. The non-invasive fibrosis scores, fibrosis-4 (FIB-4) index and Aspartate aminotransferase to platelet ratio index (APRI), were used to assess the absence and presence of fibrosis.

RESULTS

Fifty-seven individuals with NAFLD were evaluated, the mean age (SD) was 52.7 (11.3) years and 75.4% were female. Fibrosis assessed by FIB-4 and APRI was observed in 3.7% and 16.6% of patients with NAFLD, respectively. The diagnosis of sarcopenia was identified only by EWGSOP1 in 3.5% of NAFLD patients, and the prevalence of probable/pre-sarcopenia was higher using the EWGSOP2 consensus at 26.3%, when compared to 1.8% with EWGSOP1. Sarcopenia defined by EWGSOP1, was associated with grade I steatosis, but without overweight ($P < 0.05$). An association between sarcopenia and fibrosis was not observed ($P > 0.05$). EWGSOP2 showed a greater number of patients with probable sarcopenia, and who were overweight (12 (80.0%)), with a higher degree of steatosis [11 (73.3%) and presence of fibrosis (1 (6.7%), FIB-4 and 3 (20.0%), APRI] compared to EWGSOP1 [1 (100%), 0 (0.0%), 0 (0.0%), FIB-4 and 0 (0.0%), APRI, respectively].

CONCLUSION

The present study showed that sarcopenia in NAFLD was not predominant in patients without fibrosis, by both diagnostic methods. In addition, the prevalence of probable sarcopenia also depends on the method applied.

Key Words: Non-alcoholic fatty liver disease; Sarcopenia; Muscle strength; Physical performance; Liver fibrosis

Core Tip: In Non-Alcoholic Fatty Liver Disease (NAFLD), sarcopenia has been associated with the presence and severity of the disease. However, the diagnostic criteria for sarcopenia are still under evaluation and undergoing constant changes. In patients with NAFLD, sarcopenia was not common, but a higher prevalence of probable sarcopenia was observed by the most current European Working Group Consensus on Sarcopenia in Older People, 2018. This increased sensitivity to the possible early stage of sarcopenia may be an opportunity for accurate and early interventions in this population, preventing the development of sarcopenia and the worse evolution of NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered the most prevalent liver disease and affects approximately 25% to 30% of the world population[1]. Among obese and/or diabetic individuals, the prevalence is even higher, reaching around 75%-90% in these populations[2]. The increased prevalence in these groups may be justified by the association of NAFLD with several metabolic disorders, including insulin resistance, inflammation and altered lipid metabolism[3].

Sarcopenia, defined as the progressive loss of muscle mass, strength and muscle function, shares some pathophysiological mechanisms with NAFLD such as insulin resistance, which is the main link between these two diseases[3-6].

Skeletal muscle is an active endocrine organ responsible for insulin-mediated glucose elimination. Thus, muscle depletion can lead to a reduction in the primary target tissue of insulin action with consequent resistance to it[7,8].

Sarcopenia was considered a diagnostic code muscle disease, in which low muscle strength is the main determinant for triggering diagnostic investigation, surpassing low muscle mass, prioritized by the first publication of the European Working Group on Sarcopenia in Older Persons (EWGSOP)[9,10]. The primary difference between the two consensuses (EWGSOP1 2010 and EWGSOP2 2018) is in the triggering criteria for diagnostic investigation, defined as "pre-sarcopenia".

Although experts in the field accept the use of EWGSOP2, the effect on the identification of sarcopenia has raised concern. To date, all consensuses have agreed on two crucial components in defining sarcopenia, the involvement of both structural damage (low muscle mass) and impaired function (low muscle strength). However, the cut point reductions suggested in EWGSOP2 seem to have lower sensitivity[11,12].

The present study aimed to evaluate the prevalence of sarcopenia using different diagnostic methods in patients with NAFLD, and its association with the severity of this disease.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted at the Nonalcoholic Steatohepatitis Outpatient Clinic (NASH) - Federal University of Bahia, Brazil.

A consecutive and voluntary sample of patients including both sexes aged over 18 years with NAFLD was selected from January 2019 to December 2021. The criteria for NAFLD included the presence of hepatic steatosis on abdominal ultrasound; negative history of ethanol intake (< 140 g of ethanol per week); exclusion of other liver diseases such as hepatitis B and C virus infection; hemochromatosis, and autoimmune hepatitis. Patients with hypothyroidism, pregnant and lactating women, those with hepatomegaly or splenomegaly, ascites, abdominal tumors and recent abdominal surgeries, or any physical limitation that compromised the anthropometric assessment were excluded.

Abdominal ultrasound

All patients underwent ultrasound of the upper abdomen by a single evaluator to measure intrahepatic fat, in a specialized clinic, using the Xario 100 Canon Medical Systems device®.

Non-invasive fibrosis scores

Non-invasive fibrosis scores were used to assess the absence and presence of fibrosis. The fibrosis-4 index (FIB-4), considered the absence of fibrosis to be FIB-4 < 1.30, indeterminate FIB-4 1.30–2.67 and fibrosis FIB-4 > 2.67[13,14]. For the Aspartate aminotransferase to platelet ratio index (APRI), absence of fibrosis was APRI ≤ 0.50, indeterminate APRI > 0.5 - < 1.49 and fibrosis APRI > 1.50[15].

Anthropometric assessment

Anthropometric measurements were performed in duplicate by a trained and standardized team. Body weight (kg) and height (cm) were measured with light clothes and without shoes, using a digital scale with a resolution of 100 g and a stadiometer with 0.5 cm[16]. The body mass index (BMI) was obtained using the formula weight (kg)/height^2 (m^2)[17] and for better interpretation of the data, overweight and non-overweight categorizations were used. To this end, adult individuals were considered overweight when the BMI ≥ 25 kg/m^2 and in the elderly, when BMI ≥ 28 kg/m^2[17,18].

Sarcopenia assessment

As diagnostic criteria, the 2010 European Consensus was used (EWGSOP1)[9], which recommends muscle mass, muscle strength and physical performance for the diagnosis, and the 2018 European Consensus (EWGSOP2)[10] that uses muscle strength and muscle quantity/quality, in that order, maintaining physical performance only as a way of categorizing the severity of the disease (Table 1).

Muscle mass

Muscle mass was evaluated by calculating skeletal muscle mass (SMM), using the prediction equation proposed by Janssen *et al*[19], where height is measured in cm, resistance in ohms, male = 1, female = 0, and age is measured in years.

The resistance value was obtained through bioelectrical impedance, using the tetrapolar Biodynamics®, model 450. The technique and previous procedures were performed according to Kyle *et al*[20]. From the SMM, the equation skeletal muscle index (SMI) calculated the SMI = MM/height^2[19].

Low muscle mass was defined according to the cutoff points predicted by the EWGSOP1[9] (women < 6.42 kg/m^2, men < 8.87 kg/m^2) and EWGSOP2[10] (women < 5.5 kg/m^2, men < 7 kg/m^2).

Muscle strength

Muscle strength was assessed by the maximal handgrip strength test, with a portable handheld dynamometer SAEHAN Spring Hand Dynamometer (Smedley-Type SH5002), and a 0-100 kg/force grading scale (kg/f).

Two measurements were taken on each hand, alternately, with 1 min of rest between them. The average between each pair of measurements was obtained and the highest average obtained was considered for analysis. For EWGSOP1, low muscle strength is defined as < 30 kgf for men and < 20 kgf for women[9] and for EWGSOP2, < 27 kgf for men and < 16 kgf for women[10].

Physical performance

Usual gait speed was measured in meters per second (m/s). The patient walked four meters in a straight and flat environment, at their usual walking speed. The test was repeated twice, and the shortest time spent was used for analysis. Individuals with gait speed < 0.8 m/s were evaluated with reduced gait

Table 1 Classification of the diagnosis of sarcopenia according to the European Working Group on Sarcopenia in Older People, 2010 and 2018

Diagnosis	EWGSOP1	EWGSOP2
No sarcopenia	MM + MS + PP adequate	MQ + MS + P adequate
Pre-sarcopenia/Probablesarcopenia	MM insufficient	MS insufficient
Sarcopenia	MM + (MS or PP)insufficient	MS + MQ insufficient
Severe sarcopenia	MM + MS + PP insufficient	MS + MQ + P insufficient

EWGSOP1: European Working Group of Sarcopenia in Older People, 2010; EWGSOP2: European Working Group of Sarcopenia in Older People, 2018. MM: Muscle mass; MS: Muscle strength; PP: Physical performance; MQ: Muscle quantity; P: Performance.

speed or poor physical performance[9,10].

Statistical analysis

For tabulation and analysis of the data, the statistical program Statistical Package for the Social Sciences® (SPSS) version 20.0 was used. The results of categorical variables were expressed as absolute and relative frequency and continuous variables were expressed as mean and standard deviation. Pearson's chi-square test was used to compare qualitative variables. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Characteristics of the population studied

The study included fifty-seven patients with NAFLD. A total of 75.4% were female, and the ages ranged between 26 and 73 years, mean (SD) of 52.7 (11.3) years. 84.2% of patients were overweight, 63.1% had grade II and III steatosis. Hepatic fibrosis by FIB-4 was observed in 3.7% of the NAFLD patients and in 16.6% by APRI.

Prevalence of EWGSOP1 vs EWGSOP2 Sarcopenia

The diagnosis of sarcopenia in these NAFLD patients was identified only by EWGSOP1, in 3.5% patients. The prevalence of probable/pre-sarcopenia was higher when using the EWGSOP2 consensus when compared to EWGSOP1, 26.3% *vs* 1.8% of patients with NAFLD.

When evaluated separately by the items that define sarcopenia, most patients had preserved muscle mass and physical performance, both by EWGSOP1 and EWGSOP2. However, it is observed that the number of people with preserved muscle strength was higher by the EWGSOP2 (71.9%), when compared to those evaluated by the EWGSOP1 (47.4%) (Table 2).

Prevalence of sarcopenia according to BMI, degrees of steatosis and fibrosis

Patients with NAFLD diagnosed with sarcopenia by EWGSOP1 presented grade I steatosis and were not overweight ($P = 0.027$ and $P = 0.003$, respectively). However, no association was observed between sarcopenia and fibrosis, either by FIB-4 or APRI ($P > 0.05$) (Table 3).

By EWGSOP2, no association was observed between probable-sarcopenia and degree of steatosis or probable-sarcopenia and excess weight ($P > 0.05$). However, using this method, a greater number of patients with probable sarcopenia and who were overweight, with a higher degree of steatosis and presence of fibrosis were observed (Table 3).

DISCUSSION

This sample of NAFLD patients was composed mostly of overweight adult women, who had the highest degrees of hepatic steatosis, but without fibrosis. The diagnosis of sarcopenia was identified by EWGSOP1 criteria, and the EWGSOP2 algorithm identified probable sarcopenia or pre-sarcopenia major. According to the EWGSOP2, we observed more cases of NAFLD with probable sarcopenia, who were overweight, had a higher degree of steatosis and the presence of fibrosis.

Due to the wide variety of methods available, the identification of sarcopenia varies, and consequently discrepancies are observed between the prevalence of sarcopenia when applying the EWGSOP1 and EWGSOP2 criteria[21]. A systematic review, which compared the prevalence of sarcopenia in the geriatric population based on these two consensuses found 6.2% to 35.3% had sarcopenia by EWGSOP1

Table 2 Variables for the definition of sarcopenia by the European Working Group of Sarcopenia in Older People, 2010 and 2018

Variable	EWGSOP1	EWGSOP2
Muscle mass, *n* (%)		
Adequate	54 (94.7)	57 (100)
Low	3 (5.3)	0 (0.0)
Muscle strength, *n* (%)		
Adequate	27 (47.4)	41 (71.9)
Low	30 (52.6)	16 (28.1)
Physical performance, *n* (%)		
Adequate	48 (84.2)	41 (71.9)
Low	9 (15.8)	16 (28.1)

EWGSOP1: European Working Group of Sarcopenia in Older People, 2010; EWGSOP2: European Working Group of Sarcopenia in Older People, 2018.

Table 3 Association of sarcopenia with the classification of body mass index, degrees of steatosis and presence of fibrosis (Pearson's chi-square test)

Variable	EWGSOP1				EWGSOP2		
	No Sarcopenia, *n* = 54	Pre-sarcopenia, *n* = 1	Sarcopenia, *n* = 2	*P* value	No sarcopenia, *n* = 42	Probable sarcopenia, *n* = 15	*P* value
BMI, *n* (%)							
No excess weight	7 (13.0)	0 (0.0)	2 (100.0)	0.003	6 (14.3)	3 (20.0)	0.685
Overweight	47 (87.0)	1 (100.0)	0 (0.0)		36 (85.7)	12 (80.0)	
Degree of steatosis, *n* (%)							
Grade I	18 (33.3)	1 (100.0)	2 (100.0)	0.027	17 (40.5)	4 (26.7)	0.534
Grade II-III	36 (66.7)	0 (0.0)	0 (0.0)		25 (59.5)	11 (73.3)	
FIB-4[1]							
No fibrosis	49 (96.1)	1 (100.0)	2 (100.0)	0.740	38 (97.4)	14 (93.3)	0.484
Fibrosis	2 (3.9)	0 (0.0)	0 (0.0)		1 (2.6)	1 (6.7)	
APRI[1]							
No fibrosis	42 (82.4)	1 (100.0)	2 (100.0)	0.448	33 (84.6)	12 (80.0)	0.688
Fibrosis	9 (17.6)	0 (0.0)	0 (0.0)		6 (15.4)	3 (20.0)	

[1]*n* = 54, considering three patients who did not have biochemical tests.

EWGSOP1: European Working Group of Sarcopenia in Older People, 2010; EWGSOP2: European Working Group of Sarcopenia in Older People, 2018; BMI: Body mass index; FIB-4: Fibrosis-4 index; APRI: Aspartate aminotransferase to platelet ratio index.

and 3.2% to 26.3% by EWGSOP2[22]. Some studies have shown that EWGSOP1 seems to have great sensitivity in identifying individuals at higher risk for health outcomes[12,21,23]. However, the sensitivity assessment of both methods has been, in its entirety, applied in geriatric populations associated with other diseases.

Sarcopenia has been associated with an increased incidence and risk of NAFLD[24,25]. Despite the divergence of methods used to identify sarcopenia in several studies, and considering the specific characteristics of each population, the literature shows a positive association between sarcopenia and NAFLD, with a significant prevalence in this population[24,26,27].

The association of steatosis with fibrosis in patients with sarcopenia was evaluated by scores FIB-4 and APRI[26-28]. The currently available scores, including FIB-4 and APRI, have some limitations in the diagnosis of fibrosis. There is also difficulty in defining a cutoff point capable of differentiating between the absence of fibrosis or the presence of advanced fibrosis in NAFLD. In general, predictive fibrosis scores have a good Negative Predictive Value to exclude advanced fibrosis with low Positive Predictive

Value. Therefore, these scores can be safely used for basal risk stratification to exclude advanced and nonexistent fibrosis. There is an interval considered undetermined or gray area. In these cases, other methods such as liver biopsy may be necessary for the diagnosis of fibrosis[14,29].

Different methods have been used for the diagnosis of sarcopenia in these studies, among them, the ratio between appendicular skeletal muscle mass and BMI with different cutoff points, and fibrosis identified through liver biopsy and/or non-invasive markers.

The association between sarcopenia and NAFLD still requires further evaluation, considering the standardization and identification of the best diagnostic method for both sarcopenia and hepatic fibrosis.

Considering that sarcopenia is often not noticeable in the preliminary stages, detecting probable sarcopenia is important so that appropriate intervention can be established early[30,31]. In our population, EWGSOP2 better identified cases of probable sarcopenia when compared to EWGSOP1. In addition to the difference in cutoff points, the criteria used in the initial screening are different between the consensuses (muscle mass *vs* muscle strength). In a longitudinal analysis performed in the geriatric population, a higher prevalence of low muscle strength was observed[32].

The cohort study by Xia *et al*[33] found that hand pressure strength is inversely associated with the incidence of NAFLD. This finding had been demonstrated in other studies, which also pointed to a probable relationship between NAFLD and muscle strength[34,35]. Patients with NAFLD seem to be more likely to have low muscle strength when compared to controls, and a higher prevalence of NAFLD was identified in those with low muscle strength. Thus, the evaluation of muscle strength, prioritized by the EWGSOP2, in patients with NAFLD may be a more valuable parameter to identify the early stages of sarcopenia when compared to the analysis of muscle mass[33].

These study results suggest that prioritization of muscle strength by EWGSOP2 may allow greater identification of early cases of sarcopenia in individuals with NAFLD. The measurement of muscle strength is easy to perform and of low cost, which is a positive factor for clinical applicability when compared with the measurement of muscle mass. From a clinical viewpoint, early detection of cases is essential, considering that it is better to prevent skeletal muscle depletion than to try to restore it once it has progressed[36,37].

This seems to be one the first studies to investigate the impact of using the two most frequently used consensuses for the detection of sarcopenia in patients with NAFLD. Although the cross-sectional design limits the possibility of inferring causality, and the small sample size restricts the extrapolation of results to the entire NAFLD population, our results suggest a new clinical approach to sarcopenia in patients with NAFLD.

CONCLUSION

In conclusion, the prevalence of sarcopenia varies depending on the sensitivity of the method applied. In addition, due to the pathophysiological association of sarcopenia with NAFLD, it is important to identify the best method for early detection of loss of muscle function in this population.

It is also possible to identify viable strategies to screen for sarcopenia in clinical practice, using muscle strength as a primary diagnostic indicator, as determined by the EWGSOP2. Thus, this method makes it easier to identify probable sarcopenia in the initial screening at an outpatient level, and consequently the early detection of cases of sarcopenia in this population.

ARTICLE HIGHLIGHTS

Research background

Sarcopenia is a clinical condition possibly associated with Non-Alcoholic Fatty Liver Disease (NAFLD) as they share common pathophysiological mechanisms, such as insulin resistance. The diagnostic criteria available in the literature to define sarcopenia are diverse, and even those established in consensus have been questioned in relation to their diagnostic accuracy.

Research motivation

Previous studies demonstrated an association between sarcopenia and NAFLD. However, the assessment of sarcopenia is performed by various diagnostic methods, which implies discrepant prevalence. The search for the best method led to the two most used consensuses in the scientific community for the diagnosis of sarcopenia in the population and that were not previously investigated in patients with NAFLD.

Research objectives

To evaluate the prevalence of sarcopenia, using different methods, in patients with NAFLD, and its association with the severity of this disease.

Research methods

Sarcopenia was defined by the European Working Group Consensus on Sarcopenia in Older People of 2010 (EWGSOP1) and 2018 (EWGSOP2). Abdominal ultrasound was used to diagnose hepatic steatosis. The non-invasive fibrosis scores, FIB-4 and APRI, were used to assess the absence and presence of fibrosis.

Research results

The diagnosis of sarcopenia was identified only by EWGSOP1, and the EWGSOP2 algorithm identified probable sarcopenia or pre-sarcopenia. Sarcopenia, defined by EWGSOP1, was associated with grade I steatosis, but without excess weight ($P < 0.05$). EWGSOP2 showed a greater number of patients with probable sarcopenia, overweight, with a greater degree of steatosis and presence of fibrosis compared to EWGSOP1.

Research conclusions

Sarcopenia in NAFLD was not predominant in patients without fibrosis, by both consensuses. In addition, the prevalence of probable sarcopenia, a promising early indicator of sarcopenia, was higher by the EWGSOP2 method.

Research perspectives

Validation of muscle strength measurement in the early identification of sarcopenia is essential in NAFLD patients.

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FOOTNOTES

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Observational Study

Metabolic-associated fatty liver disease is associated with low muscle mass and strength in patients with chronic hepatitis B

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Abstract

BACKGROUND

Although the prognostic relevance of sarcopenia has been increasingly recognised in the context of liver disease, there is a paucity of data evaluating body composition in patients with chronic hepatitis B (CHB). Beyond virus-related factors, nutritional and metabolic aspects can be associated with skeletal muscle abnormalities in these patients and should not be disregarded.

AIM

To evaluate the association between components of sarcopenia and demographic, clinical, lifestyle, nutritional, and biochemical variables in CHB patients.

METHODS

Dual-energy X-ray absorptiometry (DXA) was used to assess muscle mass by quantifying appendicular lean mass (ALM) adjusted for body mass index (ALM_{BMI}). Muscle function was evaluated by hand grip strength (HGS) and the timed up and go test. Metabolic-associated fatty liver disease (MAFLD) was defined according to the criteria proposed by an international expert panel. A body shape index and the International Physical Activity Questionnaire were used to assess central obesity and physical activity level, respectively.

RESULTS

This cross-sectional study included 105 CHB outpatients followed at the tertiary care ambulatory centre (mean age, 48.5 ± 12.0 years; 58.1% males; 76.2% without cirrhosis; 23.8% with compensated cirrhosis). The DXA-derived fat mass percentage was inversely correlated with the ALM_{BMI} (r = - 0.87) and HGS (r = - 0.63). In the multivariable analysis, MAFLD, sedentarism and central obesity were positively and independently associated with low ALM_{BMI}. MAFLD and central obesity were independently associated with low HGS.

CONCLUSION

MAFLD and central obesity were associated with low muscle mass and strength in patients with chronic hepatitis B, independent of the liver disease stage.

Key Words: Chronic hepatitis B; Appendicular lean mass; Muscle strength; Metabolic associated fatty liver disease; Central obesity; Physical performance

Core Tip: Recently, the clinical significance of sarcopenia in hepatic disease has been increasingly recognised. In patients with chronic hepatitis B, metabolic-associated fatty liver disease and central obesity were associated with low muscle mass and strength. Metabolic and skeletal muscle abnormality appraisal should be encouraged among individuals chronically infected with hepatitis B virus.

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INTRODUCTION

Globally, approximately 462 million adults are underweight, whereas 1.9 billion are either overweight or obese[1,2]. In this scenario, according to the World Health Organization definition, the double burden of malnutrition is "characterized by the coexistence of undernutrition along with overweight, obesity or diet-related noncommunicable diseases, within individuals, households and populations, and across the life-course"[1,2]. Translating this definition into the hepatic disease context, several investigations have demonstrated that malnutrition and overweight can simultaneously be present in a patient[3-8]. Malnutrition contributes to the development of skeletal muscle abnormalities[3,4]. The loss of skeletal muscle mass, function and performance is considered primary when it is associated with ageing itself, *i.e.*, primary sarcopenia; however, it can also be related to chronic diseases, *i.e.*, secondary sarcopenia[9, 10]. Furthermore, abnormalities in muscle mass and function may coexist with obesity, resulting in sarcopenic obesity, which is associated with liver-related complications and adverse outcomes[5,6]. The interaction between skeletal muscle abnormalities and metabolic factors such as obesity, insulin

resistance and metabolic syndrome play a key role in the progression of liver fibrosis[5-8].

In real-world settings, researchers have identified an overlap between two or more factors associated with the progression of fibrosis in a substantial number of patients with cirrhosis[11-15]. Although in patients with chronic hepatitis B (CHB), long-term antiviral therapy is effective in discontinuing viral replication and reducing the development of cirrhosis and/or hepatocellular carcinoma (HCC), subgroups of patients are still prone to fibrosis progression, even achieving virological sustained response with potent nucleos(t)ide analogue therapy[16-18]. This evidence sheds light on putative risk factors for fibrosis advancement other than hepatitis B virus (HBV)-related factors. Among these factors, host and environmental factors should be highlighted, such as nutritional and metabolic characteristics.

With respect to nutritional status, in a previous study including individuals chronically infected with HBV or hepatitis C virus (HCV), sarcopenia was identified in 7.1%, 11.8%, and 21.9% of noncirrhotic, compensated cirrhotic (Child-Turcotte-Pugh A), and decompensated cirrhotic (Child-Turcotte-Pugh B/C) patients, respectively[19]. More recently, Han and colleagues examined the influence of sarcopenia on liver fibrosis among 506 patients with CHB[7]. Sarcopenia was significantly associated with liver disease severity, especially among HBV-positive subgroups with obesity, insulin resistance, metabolic syndrome and liver steatosis[7]. Although secondary sarcopenia is a well-known predictor of liver fibrosis in patients with nonalcoholic fatty liver disease (NAFLD), the interaction between sarcopenia and CHB is poorly understood. On the other hand, in line with the increasing prevalence of NAFLD, the coexistence of HBV infection and fatty liver disease has frequently been identified worldwide[8,20].

Recently, an international expert panel outlined metabolic-associated fatty liver disease (MAFLD) as hepatic steatosis in the presence of overweight, diabetes, and/or a combination of other metabolic disorders[21]. In contrast to the previous criteria for the diagnosis of NAFLD, the diagnosis of MAFLD is based on the degree of metabolic derangement and does not require the exclusion of other aetiologies of hepatic disease[21]. The role of superimposed MAFLD in CHB progression is still unclear. Despite the risks and consequences associated with low muscle mass in subjects chronically infected with HBV, there is a paucity of data evaluating body composition in this population. Thus, the aim of this study was to investigate the association between components of sarcopenia and demographic, clinical, lifestyle, nutritional, and biochemical variables in patients chronically infected with HBV.

MATERIALS AND METHODS

This was a cross-sectional study comprising 105 consecutive outpatients who were aged > 18 years with confirmed CHB diagnosis attending the Viral Hepatitis Outpatient Clinic, University Hospital, Belo Horizonte, Brazil, between 2017 and 2020. Each patient met the inclusion criteria of the study for CHB as confirmed by the presence of specific HBV seromarkers and HBV-DNA.

The Viral Hepatitis Outpatient Clinic is an outpatient care ambulatory of a metropolitan tertiary teaching hospital that admits patients for the treatment of chronic viral hepatitis. All participants signed the informed consent form. The study was designed and conducted following the Declaration of Helsinki and was approved by the Ethics Committee of Federal University of Minas Gerais/UFMG (ETIC 0404.0.203.000 - 10; CAAE, 07761212.2.0000.5149).

Study population

All patients were screened for other hepatic diseases. The following patients were excluded from the study: those aged < 18 years; women who were pregnant or breastfeeding; those with hepatic encephalopathy, HBV/HCV or HBV/human immunodeficiency virus (HIV) coinfection; patients who had causes of liver disease other than HBV infection and advanced diseases such as chronic kidney disease, heart failure, chronic pulmonary disease, and neoplasia, including HCC. Patients were also excluded if they were using drugs known to be associated with fatty liver disease.

Since fluid overload interferes with body composition assessment, the Child-Pugh-Turcotte score was assessed for each patient, and those with a Child-Pugh-Turcotte score > 7 points and/or decompensated cirrhosis were not included in the study[22,23]. The diagnosis of cirrhosis was based on standard clinical, biochemical, radiological, and histological parameters[15]. Each patient underwent a detailed physical examination, particularly for the presence of bilateral lower extremity oedema and ascites. Additionally, all included patients had serum albumin levels ≥ 3.5 g/dL and the absence of ascites confirmed by abdominal ultrasound.

Laboratory parameters

Blood samples were obtained from each patient after 12 h of overnight fasting for HBV diagnosis and biochemical and haematological evaluation. Fasting blood glucose levels, glycated haemoglobin, total cholesterol and fractions, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase, alkaline phosphatase, albumin, total bilirubin, prothrombin activity, complete blood count test and creatinine were evaluated by routine laboratory methods.

Diagnosis and classification of chronic hepatitis B virus infection

CHB infection was classified as recommended by the EASL Clinical Practice Guidelines[24]. HBeAg-positive or HBeAg-negative chronically infected patients who presented HBV DNA > 2000 IU/mL, ALT > 2 × upper limits of normal and/or at least moderate liver necroinflammation or fibrosis during clinical follow-up were categorised as having CHB and underwent antiviral treatment[24]. All of them had undetectable HBV-DNA viral loads. Patients who were treatment-naïve with intrinsically low HBV viral load met the HBV chronic infection classification[24].

HBV status and HBV-DNA viral load were evaluated by chemiluminescence immunoassay (Ortho-Clinical Diagnostics™ VITROS™, Cumberland County, NJ) and a commercial test (Abbott Real Time HBV Viral Load, Lake Bluff, IL), respectively, according to the manufacturers' instructions.

Clinical comorbidities and metabolic derangement evaluation

Hypertension, diabetes mellitus, dyslipidaemia and metabolic syndrome were defined in accordance with international guidelines[25-28]. Hepatic steatosis was diagnosed as the presence of fatty liver determined by ultrasound and/or histological assessment. The diagnosis of liver steatosis on ultrasound was based on increased hepatic echogenicity, hepatic attenuation of the ultrasound beam and hepatorenal index[29-31]. In addition, the hepatic steatosis index (HSI), a quantitative method for the evaluation of fatty liver disease validated for patients with HBV, was calculated according to the following formula: 8 × (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes mellitus)[32,33]. MAFLD was defined according to the International Expert Consensus Statement[21].

Liver histological assessment

The METAVIR score was used to assess the severity of fibrosis and the degree of liver inflammation/activity[34]. The grading and staging of fatty liver were defined using criteria proposed by Brunt *et al*[35] for histological lesions.

Lifestyle assessment

A current/past history of alcohol use was investigated as part of the lifestyle evaluation. Risky alcohol consumption was defined as a consumption of more than 20 g and 30 g of alcohol daily for women and men, respectively, for more than five years[36].

Participant habitual physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) short version validated for the Brazilian population[37]. Physical activity was dichotomised into normal [moderate-to-high categorical scale of IPAQ ≥ 600 metabolic equivalent of task (MET)-min/wk] or low (< 600 MET-min/wk). A trained person administered the questionnaires.

Anthropometry assessment and nutritional status

A nutritionist carried out all nutritional evaluations (C.M.L.S.). Weight and height were measured with a mechanical platform-type Filizola® (Filizola, São Paulo, Brazil). Light indoor clothing could be worn, excluding sweaters, belts, and shoes. We used Quetelet's formula to calculate BMI as a ratio between weight in kilograms and height in metres squared (kg/m^2), and for elderly subjects, we used the Lipschitz classification[38,39].

Waist circumference (WC) was measured in the horizontal plane midway between the lower rib edge and the upper iliac crest in the standing position with a nonstretchable tape (cm). Central obesity was diagnosed as waist circumference > 102 cm in males and > 88 cm in females[28].

"A body shape index" (ABSI), an indirect measure of central obesity, was calculated as WC/(BMI$^{2/3}$ × height$^{1/2}$) and expressed in m$^{11/6}$.kg$^{-2/3}$[40-42]. The original ABSI values were < 0.1 and were multiplied by 1,000 to derive numbers on the order of magnitude of WC[42]. The fourth sex-specific quartile was used as the cut-off point to categorise the patients into the following groups: "higher ABSI" (> 82.4 for men and > 83.2 for women) and "nonhigher ABSI".

Malnutrition was evaluated by using subjective global assessment (SGA). Patients were classified as follows: Nourished (SGA A), suspected to be malnourished or moderately malnourished (SGA B), and severely malnourished (SGA C)[43].

Evaluation of body composition

Whole-body dual-energy X-ray absorptiometry (DXA) exams were performed according to the procedures recommended by the manufacturer on a Discovery W densitometer (Hologic, Inc., Bedford, MA), software version 3.3.0. All procedures were carried out by blinded assessors and interpreted by the same operator (O. B. M.). The analysis included whole-body DXA measurements as fat mass (FM) and appendicular lean mass (ALM) or appendicular lean soft tissue (ALST), which is the sum of the lean mass of the arms and legs (kg)[44]. ALM was adjusted for BMI (ALM_{BMI}), and patients in the first sex-specific quintile (< 0.767 for men and < 0.501 for women) were considered to have low ALM_{BMI}. The criteria were adapted from the Foundation for the National Institutes of Health Biomarkers Consortium Sarcopenia Project consensus (FNIH Consensus)[10].

High DXA-derived FM was considered greater than 27.0% for men and 38.0% for women[45].

Handgrip strength assessment

Handgrip strength, used to evaluate muscle strength, was measured with the hand-held dynamometer JAMAR® (Asimow Engineering Co., Los Angeles, CA). Subjects were seated with their elbows flexed at 90° and supported at the time of the measurement[46]. During handgrip strength measurement, we asked the patient to grip the dynamometer with maximum strength and hold the grip for 3 s. We collected three measurements from each hand in an alternating manner, and the maximum strength was defined as the greatest of the six measurements[46]. Handgrip strength was considered low when it was < 30 kg for males and < 20 kg for females (1.0 SD below the mean of a reference Brazilian population) [47,48].

Timed up-and-go test

The timed up-and-go test (TUG) measures the time it takes an individual to stand up from an armchair, walk a distance of three metres, turn, walk back to the chair and sit down again[49]. Patients in the fourth age- and sex-specific quartile were considered to have low physical performance according to the TUG values [for both men and women (age in years), 20-29 years, 9 s; 30-39 years, 10 s; 40-39 years, 11 s; 50-59 years, 12 s. For men 60-80 years, 14 s and for women 60-80 years, 18 s] modified from Furlanetto *et al*[50].

To improve the accuracy of the results, biochemical evaluation, abdominal ultrasound, liver biopsy, DXA, interview as well as lifestyle evaluation, anthropometric assessment and nutritional status were obtained from each patient at the time of her or his inclusion in the study.

Statistical analysis

Data were analysed with IBM SPSS (IBM Corp., Armonk, NY), statistical software package version 26.0. Descriptive statistics were used to provide information regarding the demographic, clinical, metabolic, lifestyle, nutritional, and biochemical data. The Shapiro-Wilk test was used to evaluate whether the data were normally distributed. For the comparison of percentages, the asymptotic Pearson's χ^2 test was used. The Mann-Whitney U test or Kruskal-Wallis test was used for comparing the medians, and Student's t test or ANOVA was used for comparing the means.

The strength of the associations between, FM and ALM_{BMI} and FM and HGS was analysed by Spearman's correlation. The correlation coefficient was interpreted as follows: 0.00-0.30 negligible, 0.30-0.50 Low, 0.50-0.70 moderate, 070-0.90 high and 0.90-1.00 very high[51].

Multiple logistic regression models were used to appraise the factors independently associated with the components of sarcopenia, low $ALMB_{MI}$, low HGS and low physical performance (dependent variables, categorised as 0, absent or 1, present). We selected the following independent variables: demographics (age and sex); anthropometrics (ABSI); stage of liver disease (with compensated cirrhosis and without cirrhosis); metabolic derangement (MAFLD); sedentary lifestyle (IPAQ < 600 MET-min/wk); and prescribed medications (polypharmacy). Associations were evaluated by univariate analysis, and all variables with *P* values < 0.20 were included in the full models of logistic regression. Odds ratios and 95% confidence intervals were used as estimates of the risk. The Hosmer-Lemeshow test was used to assess the adequacy of the models.

To avoid the effect of collinearity, muscle abnormalities, low ALM_{BMI} and low HGS were not included in the same logistic regression models.

The level of significance was set at *P* values ≤ 0.05.

RESULTS

Characteristics of the study population

The baseline characteristics of the patients are summarised in Table 1. The mean age of the patients was 48.5 ± 12.0 years, and 58.1% were men. At clinical follow-up, 61 (58.1%) and 44 (41.9%) patients met the criteria of CHB and HBV chronic infection, respectively[24]. Those categorised as CHB underwent antiviral treatment for at least 12 mo and had undetectable viral loads (Table 1). Out of 105 patients, 94 (89.5%) were diagnosed as HBeAg-negative, and 25/105 (23.8%) had compensated cirrhosis, which was more frequent in men than in women.

With respect to the nutritional data, ALM_{BMI} (0.882 ± 0.147 *vs* 0.589 ± 0.097; *P* < 0.001) and HGS (43.5 ± 11.0 *vs* 24.9 ± 4.8; *P* < 0.001) were significantly higher in men than in women. Women had a significantly higher mean BMI (27.4 ± 4.6 *vs* 25.5 ± 4.1 kg/m^2; *P* = 0.02) and mean FM (40.9 ± 5.2 *vs* 26.7 ± 6.2; *P* < 0.001) than men. Most patients (98.1%) were well nourished according to the SGA [SGA = 103 and SGB = 2 (1.9%)]. No differences were observed in mean or median of waist circumference (90.3 ± 12.2 *vs* 89.9 ± 12.2 cm; *P* = 0.87), ABSI [80.0 (77.0; 82.4) *vs* 78.9 (75.7; 83.2) ($m^{11/6}$. $kg^{-2/3}$); *P* = 0.37] or the timed up and go test [10.0 (8.9; 11.7) *vs* 10.0 (8.1; 10.9) (sec); *P* = 0.42] between men and women, respectively.

Table 1 Main characteristics of the patients with chronic hepatitis B according to sex (*n* = 105)

Variables	Total (*n* = 105)	Male (*n* = 61)	Female (*n* = 44)	*P* value
Demographic				
Age (yr)[1]	48.5 ± 12.0	48.9 ± 12.9	48.0 ± 10.7	0.69
HBV infection				
HBeAg negative *n* (%)	94 (89.5)	52 (85.2)	42 (95.5)	0.12
HBeAg positive *n* (%)	11 (10.5)	9 (14.8)	2 (4.5)	
HBV-DNA log10 (IU)/mL[2]	3.23 (2.59; 4.33)	3.66 (2.75; 5.12)	2.97 (2.53; 3.70)	0.05
Phases of HBV infection[3]				
HBeAg-positive or -negative HBV chronic infection *n* (%)	44 (41.9)	19 (31.2)	25 (56.8)	0.008
HBeAg-positive or -negative chronic hepatitis B *n* (%)	61 (58.1)	42 (68.8)	19 (43.2)	
Time of HBV diagnosis (years)[2]	13.0 (5.0; 19.0)	19.5 (15.0; 24.0)	8.0 (4.0; 15.0)	0.17
Antiviral therapy				
Entecavir *n* (%)	35 (33.3)	29 (47.6)	6 (13.6)	0.009
Tenofovir disoproxil fumarate *n* (%)	26 (24.8)	13 (21.3)	13 (29.6)	
Time of antiviral treatment (months)[2]	36.0 (12.0; 60.0)	36.0 (12.0; 60.0)	39.0 (12.0; 49.5)	0.58
Stage of liver disease				
Without cirrhosis *n* (%)	80 (76.2)	37 (60.7)	43 (97.7)	< 0.001
Compensated cirrhosis *n* (%)	25 (23.8)	24 (39.3)	1 (2.3)	
Child-Pugh-Turcotte score (A5/A6)	19/6	18/6	1/0	
Biochemical parameters[2]				
Serum albumin, g/dL	4.4 (4.1; 4.6)	4.5 (4.2; 4.7)	4.2 (4.1; 4.5)	0.02
Clinical and metabolic abnormalities *n* (%)				
Blood hypertension	34 (32.4)	20 (32.8)	14 (31.8)	0.92
Diabetes mellitus	11 (10.5)	9 (14.8)	2 (4.5)	0.12
Dyslipidaemia	19 (18.1)	11 (18.0)	8 (18.2)	0.98
Overweight/obesity[4]	60 (57.1)	31 (50.8)	29 (65.9)	0.12
Metabolic syndrome[5]	19 (18.1)	10 (16.4)	9 (20.5)	0.59
Hepatic steatosis	40 (38.1)	27 (44.3)	13 (29.6)	0.13
Metabolic associated fatty liver disease[6]	29 (27.6)	18 (29.5)	11 (25.0)	0.61
Polypharmacy[7]	10 (9.5)	6 (9.8)	4 (9.1)	0.9
Lifestyle data *n* (%)				
Low IPAQ (<600 met-min/week)	65 (61.9)	38 (62.3)	27 (61.4)	0.92
Current alcohol consumption[8]	7 (6.7)	4 (6.6)	3 (6.8)	1
Risk drinking consumption[9]	3 (2.9)	2 (3.3)	1 (2.3)	1

[1]mean ± SD.
[2]Median [(interquartile range), 25th - 75th percentile].
[3]According to guidance The European Association for the Study of the Liver[24].
[4]Body mass index (BMI) > 25 (< 60 yr) and BMI > 27 (> 60 yr).
[5]The International Diabetes Federation worldwide definition of the metabolic syndrome[28].
[6]Metabolic associated fatty liver disease according to an international expert consensus statement[21].
[7]Defined as regular use of at least five medications.
[8]The median [(interquartile range), 25th - 75th percentile] current alcohol intake per day was 7.9 g (2.7-19.0) and 5.0 g (0.6-10.5) for men and women, respectively.

[9]> 30 g per day for men and > 20 g per day for women.

n: Number of subjects; HBV: Hepatitis B virus; HBeAg: Hepatitis B e-antigen; ALT: Alanine aminotransferase; γ-GT: γ-glutamyltranspeptidase; IPAQ: International Physical Activity Questionnaire (normal: ≥ 600 METs-min/wk). The asymptotic Pearson's χ^2 test was used to compare categorical variables. The *t* test and Mann-Whitney U test were used for comparison of normal and nonnormal continuous variables: Respectively.

Overweight/obesity (57.1%) was the most frequent clinical and metabolic abnormality, followed by hepatic steatosis (38.1%), blood hypertension (32.4%), dyslipidaemia (18.1%), metabolic syndrome (18.1%) and diabetes mellitus (10.5%). As an elevated prevalence of clinical and metabolic abnormalities was identified, the patients were categorised into non-MAFLD and MAFLD groups. MAFLD was diagnosed in 29 of 105 patients with CHB. Among these patients, 14 (48.2%) had overweight or obesity; 20.7% had overweight/obesity, hypertension and diabetes mellitus; 10.3% had overweight/obesity, hypertension and dyslipidaemia; 6.9% had overweight/obesity, hypertension, diabetes mellitus and dyslipidaemia; 6.9% had hypertension and diabetes mellitus; 3.5% had hypertension and dyslipidaemia; and 3.5% had dyslipidaemia.

Concerning hepatic steatosis assessment, all patients underwent liver ultrasound evaluation, and liver biopsy was available in 41 patients (39%). In the MAFLD group, hepatic steatosis was diagnosed by ultrasound in 17/29 (58.6%) patients and by both histological analysis and ultrasound in 12 (41.4%) patients. The HIS [median (interquartile range, 25th - 75th percentile)] was significantly higher in the MAFLD group [42.5 (37.6-44.8)] than in the non-MAFLD group [34.8 (30.9-40.4); $P < 0.001$].

Clinical characteristics of patients with or without muscle abnormalities

Out of 105 participants, 8 (7.6%) had low ALM_{BMI} and HGS combined, and 5 (4.8%) had low ALM_{BMI}, HGS and physical performance combined.

Patients with low ALM_{BMI} were older, had a higher prevalence of general or central obesity, high DXA-derived FM, low HGS, compensated cirrhosis, clinical and metabolic disorders, sedentary lifestyle and risky alcohol consumption (Supplementary Table 1). General or central obesity, high FM, low ALM_{BMI}, polypharmacy, and clinical and metabolic abnormalities were more frequent in patients with low HGS than in those without low muscle strength (Supplementary Table 2). There were no significant differences between low ALM_{BMI} (Figure 1A) and low HGS (Figure 1B) within different age range groups.

Polypharmacy tended to be more frequent in CHB patients with low physical performance (19.4%) than in those without abnormal functional performance (5.4%, $P = 0.06$) (Supplementary Table 3). Angiotensin-converting inhibitor, angiotensin-receptor blockers, amlodipine, amitriptyline, atenolol, carvedilol, diltiazem, entecavir, furosemide, hydrochlorothiazide, indapamide, insulin, metformin, omeprazole, propranolol, spironolactone, statin and tenofovir disoproxil fumarate were the medications used by the patients. None of the individuals taking statins had myalgia, muscle weakness or increased creatine phosphokinase.

Neither ALM_{BMI} nor muscle function was associated with antiviral therapy use. All patients with coexisting low ALM_{BMI}, HGS and physical performance had MAFLD, central obesity, sedentary lifestyle and high FM (Supplementary Table 4).

Correlation between DXA-derived fat mass percentage and muscle abnormalities

DXA-derived FM was inversely correlated with ALM_{BMI} ($r = -0.87$; $P < 0.001$) (Figure 2A) and HGS ($r = -0.63$; $P < 0.001$) (Figure 2B).

Liver necroinflammatory activity and muscle abnormalities

Neither ALM_{BMI} nor muscle function was associated with abnormal aminotransferase levels.

Factors associated with low appendicular lean mass adjusted for body mass index

In the univariate analysis, age, high ABSI, compensated cirrhosis, MAFLD and low IPAQ (< 600 MET-min/wk) were included (Table 2). High ABSI, MAFLD and sedentary lifestyle remained positively and independently associated with low ALM_{BMI} in the multivariable analysis (Table 2). In patients with hepatic steatosis, HSI was inversely correlated with ALM_{BMI} ($r = -0.50$; $P < 0.001$) (Figure 3A), and HSI was higher in patients with low ALM_{BMI} than in those without (Figure 3B).

Factors associated with low handgrip strength adjusted for body mass index

High ABSI, compensated cirrhosis, MAFLD and polypharmacy were included in the univariate analysis (Table 2). High ABSI and MAFLD remained positively and independently associated with low HGS in the univariate analysis (Table 2).

Factors associated with low physical performance

Age and polypharmacy were included in the univariate analysis (Table 2). Polypharmacy remained positively and independently associated with low physical performance in the multivariable analysis.

Table 2 Univariate and multivariable analyses of variables associated with skeletal muscle abnormalities and function in 105 patients with chronic hepatitis B

Variables	Univariate analysis					Multivariable analysis		
	Low ALM_{BMI}							
	Present *n* = 22	Absent *n* = 83	OR	95%CI	*P* value	OR	95%CI	*P* value
Male/Female *n* (%)	13 (59.1)/9 (40.9)	48 (57.8)/35 (42.2)	1.05	0.41-2.73	0.92	-	-	-
Age > 50 yr	13 (59.1)	33 (39.8)	2.19	0.84-5.70	0.1	1.03	0.98-1.08	0.2
High ABSI ($m^{11/6}.kg^{-2/3}$)	10 (45.5)	16 (19.3)	3.49	1.29-9.50	0.01	3.53	1.18-10.60	0.03
Compensated cirrhosis	9 (40.9)	16 (19.3)	2.9	1.06-7.96	0.03	1.64	0.51-5.27	0.41
MAFLD	11 (50.0)	18 (21.7)	3.61	1.35-9.68	0.008	3.81	1.30-11.19	0.02
Low IPAQ (< 600 met-min/wk)	19 (86.4)	46 (55.4)	5.09	1.40-18.55	0.01	3.13	1.17-8.32	0.02
Polypharmacy[1]	4 (18.2)	6 (7.2)	2.85	0.73-11.17	0.21	-	-	-
Variables	**Low HGS**							
	Present *n* = 22	**Absent *n* = 83**	**OR**	**95%CI**	***P* value**	**OR**	**95%CI**	***P* value**
Male/Female *n* (%)	12 (54.5)/10 (45.5)	49 (59.0)/34 (41.0)	0.83	0.32-2.14	0.7	-	-	-
Age > 50 years	11 (50.0)	35 (42.2)	1.37	0.58-3.51	0.51	-	-	-
High ABSI ($m^{11/6}.kg^{-2/3}$)	10 (45.5)	16 (19.3)	3.49	1.29-9.50	0.01	3.54	1.26-9.89	0.02
Compensated cirrhosis	8 (36.4)	17 (20.5)	2.22	0.80-6.15	0.12	1.45	0.46-4.55	0.53
MAFLD	10 (45.5)	19 (22.9)	2.81	1.05-7.50	0.04	2.85	1.02-7.91	0.04
Low IPAQ (< 600 met-min/wk)	13 (59.1)	52 (62.7)	0.86	0.33-2.25	0.76	-	-	-
Polypharmacy[1]	5 (22.7)	5 (6.0)	4.59	1.19-17.63	0.02	3.13	0.74-13.22	0.12
Variables	**Low physical performance**							
	Present *n* = 31	**Absent *n* = 74**	**OR**	**95%CI**	***P* value**	**OR**	**95%CI**	***P* value**
Male/Female *n* (%)	17 (54.8)/14 (45.2)	44 (59.5)/30 (40.5)	0.83	0.36-1.93	0.66	-	-	-
Age > 50 years	21 (67.7)	61 (82.4)	0.45	0.17-1.17	0.1	0.69	0.46-1.05	0.08
High ABSI ($m^{11/6}.kg^{-2/3}$)	7 (22.6)	19 (25.7)	0.84	0.32-2.27	0.74	-	-	-
Compensated cirrhosis	6 (19.4)	19 (25.7)	0.69	0.25-1.96	0.49	-	-	-
MAFLD	9 (29.0)	20 (27.0)	1.1	0.44-2.80	0.83	-	-	-
Low IPAQ (< 600 met-min/wk)	21 (67.7)	44 (59.5)	1.43	0.59-3.47	0.43	-	-	-
Polypharmacy[1]	6 (19.4)	4 (5.4)	4.2	1.09-16.13	0.06	5.69	1.38-23.44	0.02

[1]Defined as regular use of at least five medications.

n: Number of patients; ALM_{BMI}: Low appendicular lean mass adjusted by body mass index; ABSI: A Body Shape Index; MAFLD: (Metabolic associated fatty liver disease) according to an international expert consensus statement [21]; IPAQ: International Physical Activity Questionnaire (normal: ≥ 600 METs-min/wk); HGS: Hand grip strength.

DISCUSSION

Muscle abnormalities have been identified in 13.0% to 40.0% of patients with liver cirrhosis, and recent reports have recognised their clinical significance[4-8]. However, there are limited data evaluating the loss of muscle quantity and quality in patients with CHB[7].

To the best of our knowledge, this is the first study to demonstrate that MAFLD and central obesity are associated with muscle abnormalities in the setting of CHB. Patients chronically infected with HBV with MAFLD had a 3.8-fold increased risk of muscle wasting compared to those without MAFLD. We also found that patients with central obesity had a threefold increased risk of muscle abnormalities in comparison with patients without central obesity.

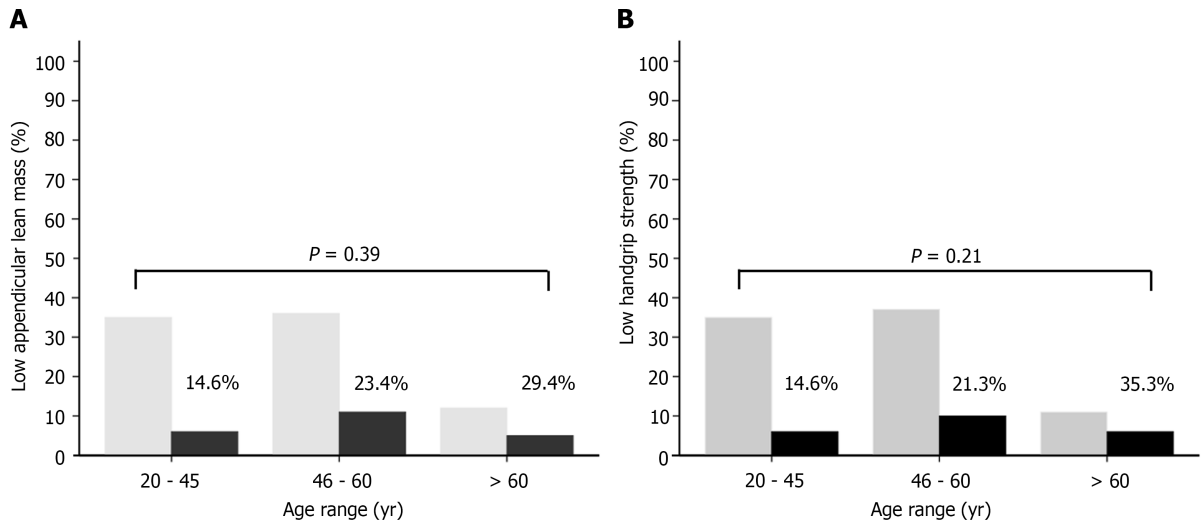

Figure 1 Mean percentage of patients chronically infected with hepatitis B virus. A: With low appendicular lean mass adjusted for body mass index (BMI); B: Low handgrip strength adjusted by BMI according to age range (Student's *t*-test, $P \leq 0.05$).

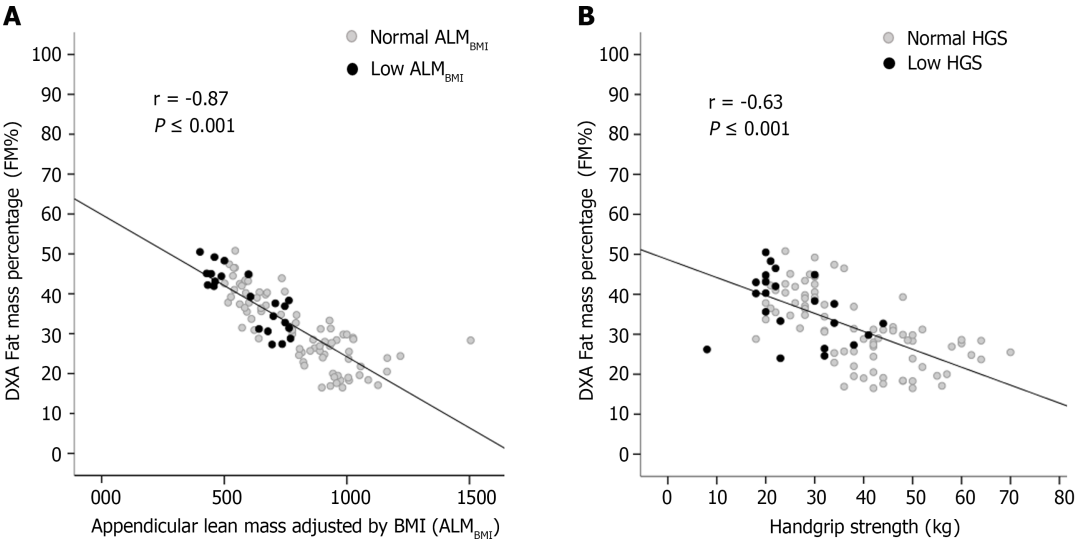

Figure 2 Correlation between Dual-energy X-ray absorptiometry-derived fat mass percentage and muscle abnormalities. A: Correlations between fat mass percentage and appendicular lean mass adjusted for body mass index; B: Correlations between fat mass percentage and handgrip strength in patients chronically infected with hepatitis B.

In the current study, all CHB patients had quiescent virological activity. Although the long-term risk factors for cirrhosis and HCC, such as elevated levels of ALT and high HBV viral load, were not verified in our patients, 27.6% of them fulfilled the MAFLD criteria[14,21,52]. Thus, the presence of overweight, obesity or diet-related noncommunicable diseases should not be disregarded.

Concerning patients with cirrhosis, recent studies have shown that the presence of both obesity and muscle abnormalities was associated with higher rates of mortality than either condition alone[5,6]. Myosteatosis, the infiltration of fat in skeletal muscle, has been associated with worse survival in cirrhotic patients compared to those with normal body composition[53]. In individuals with NAFLD, the presence of low muscle volume and high muscle fat has been associated with poor functional performance and metabolic comorbidities[53].

Conversely, investigations exploring muscle composition abnormalities in patients with CHB are scarce. Our findings are similar to those of a previous study reporting that the frequency of obesity was higher in CHB patients with muscle abnormalities than in those without this condition[7]. When the authors categorised the participants according to metabolic factors, a strong association between muscle abnormalities and advanced fibrosis was identified in patients with obesity, insulin resistance, metabolic syndrome and hepatic steatosis[7]. These data suggest that mechanisms associated with muscle abnormalities identified in patients with NAFLD/NASH could be found in patients chronically infected with HBV[8,53].

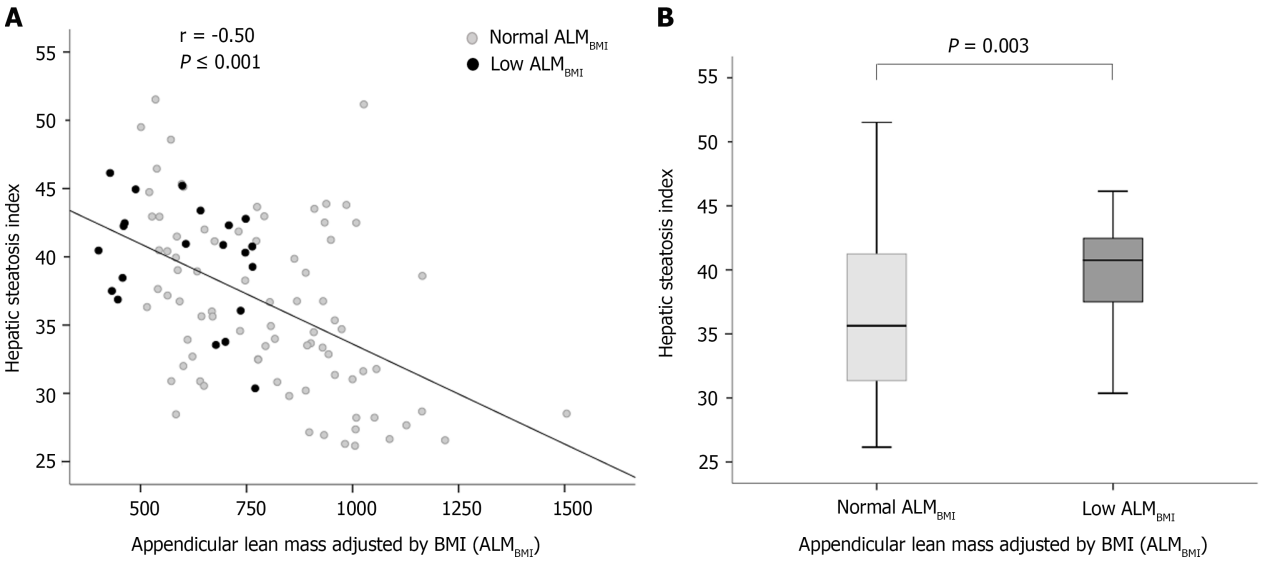

Figure 3 Factors associated with low appendicular lean mass adjusted for body mass index. A: Correlation between hepatic steatosis index and appendicular lean mass adjusted by body mass index in patients chronically infected with hepatitis B; B: Box plots representing the hepatic steatosis index. The upper and lower limits of the boxes represent the 75th and 25th percentiles, respectively; the horizontal bar across the box indicates the median, and the ends of the vertical lines indicate the minimum and maximum data values (P = 0.003).

Regarding fatty liver disease, we must bear in mind the complexity of mechanisms implicated in skeletal muscle damage. Lee and colleagues identified that up to 12.0% of patients diagnosed with NAFLD had sarcopenia independent of obesity and insulin resistance, and approximately 30.0% of sarcopenic individuals without metabolic syndrome and obesity had NAFLD[54,55]. These results point to a bidirectional muscle-liver axis as a possible pathophysiological contributor to either nonhepatic- or hepatic-related complications. The mechanisms involved in muscle-liver crosstalk include insulin resistance, increased inflammation, myokines secreted by skeletal muscles, myostatin, adiponectin, vitamin D deficiency and physical inactivity[8]. Therefore, based on these facts, it remains of utmost importance to detect additional risk predictors, other than those related to HBV, for adverse liver and nonliver outcomes.

Given the relevance of metabolic derangement in the liver disease course, an expert panel proposed a new definition for metabolic dysfunction in the presence of liver disease, renaming NAFLD as MAFLD, which, unlike NAFLD, does not require the exclusion of other hepatic diseases[21]. Large longitudinal cohort investigations demonstrated that superimposed MAFLD, NAFLD and nonalcoholic steatohepatitis (NASH) in adults with CHB were associated with advanced fibrosis, necroinflammatory activity, liver-related complications and all-cause mortality[11-14].

Nevertheless, the impact of coexisting hepatic steatosis on HBV-related disease progression remains complex and controversial[20]. A recent investigation reported that although coexisting fatty liver was observed in approximately 34.0% of CHB patients receiving HBV antiviral therapy, hepatic steatosis was associated with a low risk of HCC[56].

In our study, low physical performance was associated with polypharmacy. Recently, Venter and colleagues[57] observed a significantly greater weight gain in patients with HIV treated with dolutegravir plus two prodrugs of tenofovir (tenofovir disoproxil fumarate and tenofovir alafenamide fumarate), especially in combination with TAF, than in participants who were treated with the standard-care regimen. Translating this evidence into the CHB context, it is important to mention that prodrugs of tenofovir have been extensively used worldwide as first-line options and long-term therapy for patients with chronic HBV infection[24]. The effects of nucleos(t)ide analogues on body composition in HBV-infected individuals have scarcely been investigated. However, previous studies have demonstrated fat body increases and mitochondrial alterations with long-term antiviral treatment[58,59].

Limitations of our study were the inclusion of patients attending a referral centre, which may have made them not be representative of all patients with CHB, and the cross-sectional nature of our investigation that precluded the possibility of recognising any cause-effect relationship between adverse skeletal muscle status and the coexisting fatty liver in patients with CHB. In addition, a detail of DXA-derived measurements is their restraint in discerning any level of intramuscular fat infiltration. Furthermore, the inclusion of a control group of patients with MAFLD but without CHB should be assessed in a sequential investigation.

Although skeletal muscle abnormalities have been highly important in the course of chronic liver disease, there is no universal consensus to define and diagnose this condition in this population. Especially in patients with CHB, there is a need to endorse the definitions and cut-off values for

assessing muscle mass (quantity/quality) and function. Regarding issues related to coexisting liver steatosis and myosteatosis[8,56], body composition assessment could shed light on the interplay among muscle, adipose tissue and liver in patients with CHB[7,8,53].

CONCLUSION

In conclusion, MAFLD and central obesity were associated with muscle abnormalities in patients with CHB, independent of the stage of liver disease. These findings point to crosstalk between metabolic factors and skeletal muscle abnormalities in CHB. Both clinicians and researchers should emphasise the importance of holistic and integrated management of patients infected with HBV. The coexistence of CHB, muscle abnormalities, obesity, and metabolic dysregulation may be involved in the pathophysiology of nonhepatic- and hepatic-related outcomes. Thus, metabolic and skeletal muscle abnormality assessments should be encouraged among HBV-chronically infected individuals.

ARTICLE HIGHLIGHTS

Research background

Recently, the clinical significance of sarcopenia in hepatic disease has been increasingly recognised. However, in chronic hepatitis B patients, the factors linked to skeletal muscle abnormalities have scarcely been investigated. Among them, host and environmental factors, such as nutritional and metabolic characteristics, should be evaluated.

Research motivation

Sarcopenia was identified in 7.1%, 11.8%, and 21.9% of noncirrhotic, compensated cirrhotic (Child-Turcotte-Pugh A), and decompensated cirrhotic (Child-Turcotte-Pugh B/C) patients, respectively. More recently, Han and colleagues observed that sarcopenia was significantly associated with liver disease severity, especially among hepatitis B virus (HBV)-positive subgroups with obesity, insulin resistance, metabolic syndrome and liver steatosis.

Research objectives

To investigate the association between components of sarcopenia and demographic, clinical, lifestyle, nutritional, and biochemical variables in HBV-chronically infected patients.

Research methods

Dual-energy X-ray absorptiometry (DXA) was used to assess muscle mass by quantifying appendicular lean mass (ALM) adjusted for body mass index (ALM_{BMI}). Muscle function was evaluated by hand grip strength (HGS) and the timed up and go test. Metabolic-associated fatty liver disease (MAFLD) was defined according to the criteria proposed by an international expert panel. A Body Shape Index and the International Physical Activity Questionnaire were used to assess central obesity and physical activity level, respectively.

Research results

This cross-sectional study included 105 chronic hepatitis B (CHB) outpatients followed at the tertiary care ambulatory centre (mean age, 48.5 ± 12.0 years; 58.1% males; 76.2% without cirrhosis; 23.8% with compensated cirrhosis). The DXA-derived fat mass percentage was inversely correlated with the ALM_{BMI} (r = - 0.87) and HGS (r = - 0.63). In the multivariable analysis, MAFLD, sedentarism and central obesity were positively and independently associated with low ALM_{BMI}. Central obesity was independently associated with low HGS. MAFLD and central obesity were independently associated with low HGS.

Research conclusions

Among patients with CHB, metabolic-associated fatty liver disease (MAFLD) and central obesity were associated with low muscle mass and strength. Metabolic and skeletal muscle abnormality appraisal should be encouraged among HBV-chronically infected individuals.

Research perspectives

Further large-scale case-control studies are needed to evaluate the role of MAFLD in HBV-chronically infected patients, including individuals with MAFLD but without CHB.

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FOOTNOTES

Author contributions: All authors have made substantial contributions; Santos CML, Rocha GA, Silva LD, and Bering T designed the research (project conception, development of overall research plan, and study oversight); Santos CML, Malheiro OB, Brito MD, Castro PASV, Vries TP, Viana NL, Coelho MPP, and Cambraia RD conducted the research (data collection); Malheiro OB, Teixeira R, and Gonzalez MC provided essential materials for the research; Santos CML, Malheiro OB, Brito MD, Castro PASV, Vries TP, Viana NL, Coelho MPP, Cambraia RD, and Silva LD analysed the data or performed the statistical analysis; Santos CML, Rocha GA, and Silva LD wrote the paper; and Santos CML, Rocha GA, and Silva LD had primary responsibility for the final content; all authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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

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ORIGINAL ARTICLE

Randomized Controlled Trial

Effect of probiotics on hemodynamic changes and complications associated with cirrhosis: A pilot randomized controlled trial

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Abstract

BACKGROUND

Bacterial translocation exacerbates the hyperdynamic circulation observed in cirrhosis and contributes to a more severe disease course. Probiotics may reduce bacterial translocation and may therefore be useful to redress the circulatory imbalance.

AIM

To investigate the effect of probiotics on hemodynamic parameters, systemic inflammation, and complications of cirrhosis in this randomized placebo-controlled trial.

METHODS

This single-blind randomized placebo-controlled study included 40 patients with Child-Pugh class B and C cirrhosis; 24 patients received probiotics (*Saccharomyces boulardii*) for 3 mo, and 16 patients received a placebo over the same period. Liver function and the systemic hemodynamic status were evaluated pre- and post-intervention. Echocardiography and simultaneous blood pressure and heart rate monitoring were performed to evaluate systemic hemodynamic indicators. Cardiac output and systemic vascular resistance were calculated.

RESULTS

Following a 3-mo course of probiotics in comparison to the control group, we

observed amelioration of hyperdynamic circulation [a decrease in cardiac output (P = 0.026) and an increase in systemic vascular resistance (P = 0.026)] and systemic inflammation [a decrease in serum C-reactive protein levels (P = 0.044)], with improved liver function [an increase in serum albumin (P = 0.001) and a decrease in the value of Child-Pugh score (P = 0.001)] as well as a reduction in the severity of ascites (P = 0.022), hepatic encephalopathy (P = 0.048), and cholestasis [a decrease in serum alkaline phosphatase (P = 0.016) and serum gamma-glutamyl transpeptidase (P = 0.039) activity] and an increase in platelet counts (P < 0.001) and serum sodium level (P = 0.048).

CONCLUSION

Probiotic administration was associated with amelioration of hyperdynamic circulation and the associated complications of cirrhosis.

Key Words: Gut; Gut-liver axis; Microbiota; Hemodynamics; Heart; Gut-heart axis; *Saccharomyces boulardii*; Portal hypertension

Core Tip: Bacterial translocation exacerbates the hyperdynamic circulation observed in cirrhosis and contributes to a more severe disease course. Probiotics may reduce bacterial translocation and may therefore be useful to redress the circulatory imbalance. The aim of the study was to investigate the effect of probiotics on hemodynamic parameters, systemic inflammation, and complications of cirrhosis in this randomized placebo-controlled trial. Following a 3-mo course of probiotics, we observed amelioration of hyperdynamic circulation and systemic inflammation, improvement liver function, regression of ascites and hepatic encephalopathy, and an increase in serum sodium level.

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INTRODUCTION

Cirrhosis, which represents the culmination of chronic liver disease[1], is characterized by changes in liver morphology, reduced liver function, and the onset of portal hypertension. However, in addition to the liver, the intestine and its microbiota are affected by the pathophysiological derangements in cirrhosis. Cirrhosis is known to be associated with disturbances in the composition of the gut microbiota (gut dysbiosis[2-16]), expansion of the microbiota of the small intestine (small intestine bacterial overgrowth[17]), and increased permeability of the intestinal barrier[18], all of which result in bacterial translocation, which refers to the entry of bacteria and their components from the intestinal contents through the intestinal wall into the lymph, blood, and body tissues[19,20]. Bacterial translocation leads to systemic inflammation, which precipitates hemodynamic alterations [hyperdynamic circulation indicated by increased cardiac output and decreased systemic vascular resistance (SVR)] that contribute to liver decompensation[21-25]. This bidirectional association between the gut along with its microbiota and the liver is referred to as the gut-liver axis[26] or the gut-heart-liver axis[25]. Studies have shown that certain drugs that affect this axis can redress the hemodynamic imbalance and improve the clinical course in patients with cirrhosis. Among these drugs, probiotics are live microorganisms, which when administered in adequate amounts confer several health benefits on the host[27]. Although evidence-based research supports the role of probiotics in cases of hepatic encephalopathy, their effects on other symptoms and manifestations of cirrhosis remain unclear[28]. A non-controlled study reported that a 6-wk course of probiotics reduced the cardiac output and heart rate and increased the SVR and serum sodium levels in the study population[29].

Saccharomyces boulardii (*S. boulardii*), a probiotic yeast, has shown significant effectiveness for the treatment or prevention of diarrhea, inflammatory bowel disease, irritable bowel syndrome, *Helicobacter pylori* infection, and dyslipidemia, among other such conditions[30,31]. *S. boulardii* produces pleiotropic effects; it reestablishes the gut microbiome after dysbiosis[32], strengthens the intestinal immune barrier [33], improves the trophic function of gut microbiota[34], restores the impaired gut barrier, and protects against bacterial translocation[35] in experimental models and in patients with gut diseases. *S. boulardii*

administration in an experimental mouse model of cirrhosis led to correction of gut dysbiosis, decreased intestinal permeability, as well as reduced severity of liver inflammation and fibrosis[36]. However, the role of this probiotic is not known in humans with cirrhosis.

In this randomized placebo-controlled trial, we investigated the effect of probiotic administration (*S. boulardii*) on hemodynamic parameters, systemic inflammation, and complications of cirrhosis.

MATERIALS AND METHODS

Patients

In this single-blind randomized placebo-controlled trial, 198 consecutive patients with cirrhosis who underwent health check-ups at the Department of Hepatology's Clinic for Internal Diseases, Gastroenterology, and Hepatology at Sechenov University were screened for inclusion. The study procedures were explained to potential participants, and written informed consent was obtained before enrollment. The study was approved by the Ethics Committee of Sechenov University and was registered at clinicaltrials.gov (NCT05231772). The research protocol can be accessed from this website.

Inclusion criteria were as follows: (1) Diagnosis of cirrhosis based on histopathological, or clinical, biochemical, and ultrasonographic findings; (2) Child-Pugh class B or C cirrhosis; (3) Age between 18 years and 70 years; and (4) Signed informed consent. Exclusion criteria were as follows: (1) Administration of lactulose, lactitol, or other prebiotics, probiotics, antibiotics, or metformin during the 6 wk preceding study commencement; (2) Alcohol consumption 6 wk preceding study commencement; or (3) Diagnosis of inflammatory bowel disease, cancer, or any other serious disease.

There are no data to calculate the required sample size.

Of the 198 patients initially screened for inclusion, 40 met the inclusion criteria and were enrolled in the study (Figure 1). Patients included in the study were randomized into the test and control arms (ratio 1.5:1). The Excel function RANDBETWEEN (1:5) was used as a random number generator; for numbers 1 to 3, patients were assigned to the test arm and for numbers 4 or 5, patients were assigned to the placebo group. Patients who prematurely discontinued ingestion of the experimental probiotic/placebo or were administered antibacterial drugs, other probiotics, or prebiotics during the follow-up period were excluded from the study.

Intervention and controls

Patients in the test arm received *S. boulardii* at a dose of 250 mg twice a day for 3 mo and those in the control group received a placebo preparation at the same dose over the same period. Patients were not aware whether they were administered a placebo or the experimental drug. Additionally, all patients received standard of care treatment for cirrhosis. Drugs administered did not significantly differ between patient groups (Table 1). Patients were re-evaluated 3 mo after initiation of *S. boulardii* or placebo treatment.

Outcomes

All patients underwent a standard medical check-up for evaluation of cirrhosis and for measurement of indicators of systemic hemodynamics before and 3 mo after initiation of *S. boulardii* or placebo treatment (the first and second visit, respectively). There were no additional visits or examinations between these two time points. The outcomes included changes in cardiac output, SVR, the extent of systemic inflammation (represented by serum C-reactive protein levels), severity of ascites and hepatic encephalopathy, serum levels of liver biomarkers, and Child-Pugh scale scores.

Echocardiography was performed at rest based on the guidelines of the American Society of Echocardiography[37-40]. The systolic and diastolic blood pressure and heart rate were measured using an automatic oscillometric sphygmomanometer (A and D, Japan) simultaneously with measurement of the stroke volume. Table 2 shows the hemodynamic parameters calculated in this study[37-42].

The degree of ascites was determined based on the International Ascites Club scale as follows: 0 = No ascites; 1 = Minimal ascites (measurable only with instrumental methods); 2 = Clinically significant ascites (determined on physical examination); and 3 = Gross ascites[43].

The degree of hepatic encephalopathy was determined based on the following scale: 0 = No hepatic encephalopathy; 1 = Minimal hepatic encephalopathy; and 2 = Overt hepatic encephalopathy[44].

Statistical analysis

Statistical analysis was performed with STATISTICA 10 (StatSoft Inc., Tulsa, OK, United States) software. The data were presented as medians interquartile ranges]. Differences between continuous variables were assessed with the Mann-Whitney test because many variables were not distributed normally. Fisher's exact test was used to assess the differences between categorical variables. P values ≤ 0.05 were considered as statistically significant. We performed per-protocol analysis.

Table 1 Main indicators and drugs used of enrolled patients at inclusion by arms

	The test arm, *n* = 24	The control arm, *n* = 16	*P* value
Age, yr	48.5 (42.5-59.0)	53.5 (44.5-59.0)	0.730
Body mass index, kg/m^2	25.4 (22.3-27.9)	26.5 (24.3-28.7)	0.553
Male/female	8/16	9/7	0.134
Etiology of cirrhosis: Alcohol	12 (50.0)	8 (50.0)	> 0.050
Metabolically associated fatty liver disease	2 (8.3)	0	
HBV	2 (8.3)	0	
HCV	3 (12.5)	2 (12.5)	
Mixed	3 (12.5)	3 (18.8)	
Cryptogenic	2 (8.3)	3 (18.8)	
Child–Pugh score	9 (8-10)	9 (8-10)	0.730
Child–Pugh class, B/C	17/7	12/4	0.533
End-diastolic volume of the left ventricle, mL	109 (84-116)	100 (90-143)	0.689
Ejection fraction of the left ventricle, %	61.5 (59.4-62.8)	59.8 (57.5-62.0)	0.263
Stroke volume, mL	67 (52-72)	58 (55-82)	0.689
Heart rate, bpm	71 (69-75)	70 (69-74)	0.709
Cardiac output, L/min	4.8 (3.6-5.2)	4.4 (4.0-5.8)	0.473
Mean blood pressure, mmHg	84.7 (80.3-89.7)	87.3 (79.8-93.7)	0.499
Systemic vascular resistance, dyn · s · cm^{-5}	1442 (1243-1874)	1470 (1255-1744)	0.945
Mean pulmonary artery pressure, mmHg	23.4 (22.1-25.8)	23.4 (20.9-26.4)	0.749
Esophageal varices (Grade 1), *n* (%)	8 (33.3)	6 (37.5)	0.338
Esophageal varices (Grade 2-3), *n* (%)	16 (66.7)	7 (43.8)	
Minimal hepatic encephalopathy, *n* (%)	18 (75.0)	10 (62.5)	0.484
Overt hepatic encephalopathy, *n* (%)	2 (8.3)	2 (12.5)	
Hepatic encephalopathy, *n* (%)	20 (83.3)	12 (75.0)	0.399
Ascites, *n* (%)	22 (91.7)	12 (75.0)	0.160
Minimal ascites, *n* (%)	15 (62.5)	6 (37.5)	0.249
Clinically significant ascites, *n* (%)	7 (29.2)	6 (37.5)	
Red blood cells as 10^{12} cell/L	3.7 (3.3-4.3)	3.6 (3.1-4.1)	0.689
White blood cells as 10^9 cell/L	4.3 (3.2-5.6)	3.8 (2.6-7.2)	0.553
Platelets as 10^9 cell/ L	94 (69-107)	94 (48-104)	0.669
Serum total protein, g/L	73 (63-77)	70 (61-77)	0.649
Serum albumin, g/L	33 (31-36)	33 (29-37)	0.967
Serum total bilirubin, μmol/L	37 (27-64)	55 (29-67)	0.499
International normalized ratio	1.48 (1.39-1.68)	1.58 (1.31-1.73)	0.978
Serum cholesterol, mmol/L	4.1 (3.1-5.5)	3.5 (2.6-4.6)	0.230
Serum triglyceride, mmol/L	1.1 (0.7-1.4)	1.0 (0.7-1.4)	0.626
Serum creatinine, mg/dL	76 (65-88)	78 (70-105)	0.448
Serum sodium, mmol/L	140 (139-141)	141 (140-142)	0.235
Serum potassium, mmol/L	4.3 (4.0-4.8)	4.4 (4.1-4.6)	0.804
Serum glucose, mmol/L	4.7 (4.2-5.7)	4.8 (4.6-5.4)	0.464
Serum iron, μmol/L	13.9 (8.1-20.6)	11.3 (7.3-22.0)	0.761

Alanine aminotransferase, U/L	31 (20-46)	26 (20-51)	0.782
Aspartate aminotransferase, U/L	54 (34-72)	52 (38-79)	0.945
Gamma glutamyl transferase, U/L	82 (28-299)	84 (49-118)	0.934
Alkaline phosphatase, U/L	268 (221-395)	214 (173-274)	0.098
Cholinesterase, U/L	3650 (2861-3961)	3803 (2778-4215)	0.827
C-reactive protein, mg/L	9 (6-15)	7 (2-20)	0.347
Beta blockers, *n* (%)	17 (70.8)	11 (68.8)	0.580
Spironolactone, *n* (%)	22 (91.7)	14 (87.5)	0.529
Loop diuretics, *n* (%)	11 (45.8)	7 (43.8)	0.578
Ademetionine, *n* (%)	15 (62.5)	9 (56.3)	0.472
Entecavir or tenofovir, *n* (%)	2 (8.3)	0	0.354

HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Table 2 Calculations of hemodynamic parameters

Parameter	Calculation
End-diastolic and end-systolic volume of the left ventricle	Modified Simpson's disk method
Ejection fraction of the left ventricle	[(End-diastolic volume) – (end-systolic volume)]/(end-diastolic volume)
Stroke volume	(Doppler velocity time integral) × (cross-sectional aorta area)[41]
Mean arterial pressure	[(systolic blood pressure)+2 × (diastolic blood pressure)]/3
Cardiac output	(Stroke volume) × (heart rate)
Systemic vascular resistance	(Mean arterial pressure)/(cardiac output)
Systolic pulmonary artery pressure	(Right atrium pressure estimated from diameter of inferior vena cava and respiratory changes) + 4 × (the peak velocity of the tricuspid valve regurgitant jet)2[39,40]
Mean pulmonary artery pressure	0.61 × (systolic pulmonary artery pressure) + 2 mmHg[42]

RESULTS

The study included 40 patients [24 (test group) and 16 (control group)] (Figure 1). No significant differences were observed between the groups at the time of study inclusion (Table 1). All included patients completed the study. None of the patients were hospitalized between the visits.

After a 3-mo course of the probiotic in comparison to the control group, we observed evidence of amelioration of hyperdynamic circulation (a decrease in cardiac output and end-diastolic volume and an increase in SVR) and systemic inflammation (a decrease in serum C-reactive protein levels), improved liver function (an increase in serum albumin and cholinesterase levels and a decrease in the value of Child-Pugh score), regression of ascites and hepatic encephalopathy, increased serum sodium levels, as well as a reduction in the severity of cholestasis (a decrease in serum alkaline phosphatase and serum gamma-glutamyl transpeptidase activity), and hypersplenism (an increase in platelet count). However, in contrast to patients in the test group, those in the control group showed an increase in mean blood pressure. No significant changes were observed in the levels of other variables, including in the grade of esophageal varices and the international normalized ratio (Table 3).

In the test arm, an improvement in liver function (a decrease in the value of Child-Pugh score: -2 [-3-(-1)] *vs* -0.5 [-1-0]; $P = 0.042$) and a decrease in the degree of ascites (-1[-1-(-1)] *vs* 0 [0-0]; $P = 0.015$) was observed only in those patients ($n = 18$) who had a decrease in cardiac output after the course of the probiotic.

No patient developed acute-on-chronic liver failure and bleeding esophageal varices during the study, and no patient died during the study period.

Only 1 patient in the test arm developed self-limited itching as an adverse effect.

Table 3 Changes of the main indicators of the included patients after treatment

	The test arm (*n* = 24)	The control arm (*n* = 16)	*P* value
Body mass index, kg/m^2	-0.2 (-0.9-1.5)	0.0 (0.0-0.5)	0.281
Child–Pugh score	-1.5 [-3.0-(-0.5)]	0 (-0.5-1.5)	0.001
Child–Pugh class (from B or C to A), *n* (%)	8 (33.3)	1 (6.3)	0.048
End-diastolic volume of the left ventricle, mL	-13 [-17-(-10)]	0 (-3-12)	< 0.001
Ejection fraction of the left ventricle, %	0.5 (-0.7-0.3)	0.0 (0.0-0.3)	0.590
Stroke volume, mL	-7 [-12-(-4)]	0 (-3-8)	< 0.001
Heart rate, bpm	2 (-4-8)	-2 (-7-7)	0.234
Cardiac output, L/min	-0.5 [-1.0-(-0.1)]	0.3 (-0.3-0.9)	0.026
Mean blood pressure, mmHg	1.8 (-1.7-7.5)	3.8 (-3.3-15.3)	0.847
Systemic vascular resistance, dyn·s·cm^{-5}	237 (39-358)	30 (-200-227)	0.026
Mean pulmonary artery pressure, mmHg	0.0 (-2.1-0.6)	1.2 (0.0-1.8)	0.043
Esophageal varices, a decrease in grade, *n* (%)	2 (4.3)	0	0.354
Esophageal varices, an increase in grade, *n* (%)	0	0	-
Regression hepatic encephalopathy, *n* (%)	8 (33.3)	1 (6.3)	0,048
Ascites, a decrease in degree, *n* (%)	15 (62.4)	4 (25.0)	0.022
Ascites, an increase in degree, *n* (%)	1 (4.2)	3 (18.8)	0.167
Red blood cells as 10^{12} cell/L	0.2 (-0.1-0.5)	0.2 (-0.1-0.4)	0.730
White blood cells as 10^9 cell/L	0.1 (-1.2-0.6)	-0.1 (-2.1-0.7)	0.782
Platelets as 10^9 cell/ L	11 (4-32)	-6 (-14-1)	< 0.001
Serum total protein, g/L	-0.2 (-4.2-8.9)	-1.4 (-3.5-5.6)	0.793
Serum albumin, g/L	3.0 (0.6-7.7)	-3.1 (-5.1-2.4)	0.001
Serum total bilirubin, μmol/L	-5.1 [-23.9-(-0.9)]	-3.7 (-10.0-17.2)	0.181
International normalized ratio	0.0 (-0.1-0.1)	0.0 (-1.0-0.1)	0.544
Serum cholesterol, mmol/L	0.2 (-0.4-1.2)	0.5 (-1.0-2.0)	0.571
Serum triglyceride, mmol/L	0.1 (-0.2-0.2)	0.1 (-0.6-0.4)	0.836
Serum creatinine, mg/dL	2.4 (-9.1-8.1)	-3.5 (-11.2-4.2)	0.264
Serum sodium, mmol/L	1.5 (0.5-3.0)	-1 (-3-2)	0.048
Serum potassium, mmol/L	0.0 (-0.4-1.2)	0.1 (-0.2-0.3)	0.523
Serum glucose, mmol/L	0.4 (-0.1-0.7)	0.0 (-0.9-0.8)	0.423
Alanine aminotransferase, U/L	-1 (-15-6)	2 (-15-8)	0.740
Aspartate aminotransferase, U/L	-9 (-32-1)	-2 (-26-20)	0.116
Gamma glutamyl transferase, U/L	-8 (-171-11)	6 (-15-23)	0.039
Alkaline phosphatase, U/L	-40 (-95-3)	26 (-57-91)	0.016
Cholinesterase, U/L	257 (28-734)	-155 [-239-(-68)]	0.016
C-reactive protein, mg/L	-3.0 [-4.5-(-0.5)]	1.0 (-2.0-4.5)	0.044

DISCUSSION

This is the first randomized controlled study that investigated the effect of probiotics on hemodynamic disturbances in patients with cirrhosis. Our results concur with those reported by a previous uncontrolled study[29], which showed that these drugs reduce cardiac output and increase SVR. In our study, the reduced cardiac output was attributable to a decrease in the end-diastolic volume, which may indicate a reduction in the effective circulating blood volume.

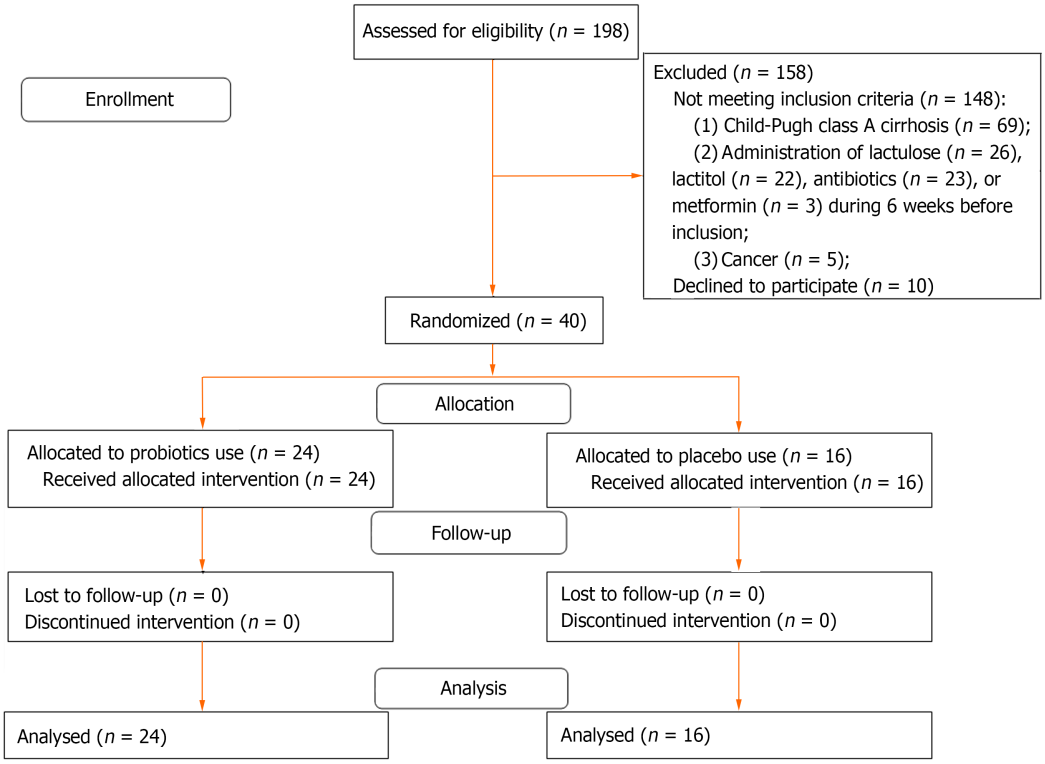

Figure 1 CONSORT 2010 flow diagram.

The use of the probiotic also increased serum albumin and sodium levels and decreased in the degree of ascites. We assume that the probiotics inhibit bacterial translocation and thereby ameliorate hyperdynamic circulation, with a consequent reduction in the degree of ascites, which corrects hypoalbuminemia and hyponatremia. Unfortunately, we could not evaluate the indicators of intrahepatic hemodynamics (for example, the hepatic venous pressure gradient, among other variables).

Interestingly, this study highlights that probiotics reduced serum levels of biomarkers of cholestasis (alkaline phosphatase and gamma-glutamyl transpeptidase). This is the first study to report these findings; further studies are warranted to investigate the mechanisms underlying these changes. We observed probiotic-induced reduction in the severity of hepatic encephalopathy, which is consistent with the results reported by previous research[28]. In our study, probiotic ingestion did not affect the degree of esophageal varices and prothrombin levels (indicated by the international normalized ratio); our results were consistent with those reported by a previous study[28].

Probiotic use was associated with a significant improvement in liver function in our study; 33.3% of patients in the probiotic arm and only 6.3% of patients in the control arm showed Child-Pugh class A cirrhosis after a course of probiotic or placebo administration. We observed no significant probiotic-induced adverse effects during the study. Overall, our study showed that probiotic administration may be a useful therapeutic strategy for correction of gut-heart-liver axis disturbances.

This is the first randomized placebo-controlled trial that confirms the role of probiotics in amelioration of hemodynamic disorders in cirrhosis, together with improvement in levels of several liver biomarkers, which serves as a strength of our study.

A limitation of our study is the fact that biomarkers of bacterial translocation and intestinal permeability, biomarkers of systemic inflammation in addition to C-reactive protein, as well as indicators of intrahepatic hemodynamics were not evaluated. Further research is warranted to overcome this challenge.

CONCLUSION

Probiotic administration was associated with amelioration of hyperdynamic circulation and the associated complications of cirrhosis.

ARTICLE HIGHLIGHTS

Research background

Bacterial translocation exacerbates the hyperdynamic circulation observed in cirrhosis and contributes to a more severe disease course.

Research motivation

Probiotics may reduce bacterial translocation and may therefore be useful to redress the circulatory imbalance.

Research objectives

To investigate the effect of probiotics on hemodynamic parameters, systemic inflammation, and complications of cirrhosis in this randomized placebo-controlled trial.

Research methods

This single-blind randomized placebo-controlled study included patients with Child-Pugh class B and C cirrhosis that received probiotics (*Saccharomyces boulardii*) or a placebo for 3 mo. Liver function and the systemic hemodynamic status were evaluated pre- and post-intervention. Echocardiography and simultaneous blood pressure and heart rate monitoring were performed to evaluate systemic hemodynamic indicators. Cardiac output and systemic vascular resistance were calculated.

Research results

Following a 3-mo course of probiotics in comparison to the control group, we observed amelioration of hyperdynamic circulation and systemic inflammation with improved liver function, reduction in the severity of ascites, hepatic encephalopathy, and cholestasis, and an increase in platelet counts.

Research conclusions

Probiotic administration was associated with amelioration of hyperdynamic circulation and the associated complications of cirrhosis.

Research perspectives

To study the changes in the levels of the biomarkers of bacterial translocation, intestinal permeability, and in indicators of intrahepatic hemodynamics after the use of the probiotic in decompensated cirrhosis.

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FOOTNOTES

Author contributions: Ivashkin V provided the research idea; Ivashkin V, Efremova I, and Roman Maslennikov R designed the study; Efremova I and Maslennikov R wrote the draft; Maslennikov R was the guarantor; All authors edited the draft and contributed to the research and data analysis.

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Clinical trial registration statement: The study was registered at https://clinicaltrials.gov (NCT05231772).

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CASE REPORT

Secondary sclerosing cholangitis after critical COVID-19: Three case reports

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Abstract

BACKGROUND

The global coronavirus disease 2019 (COVID-19) pandemic has caused more than 5 million deaths. Multiorganic involvement is well described, including liver disease. In patients with critical COVID-19, a new entity called "post-COVID-19 cholangiopathy" has been described.

CASE SUMMARY

Here, we present three patients with severe COVID-19 that subsequently developed persistent cholestasis and chronic liver disease. All three patients required intensive care unit admission, mechanical ventilation, vasopressor support, and broad spectrum antibiotics due to secondary infections. Liver transplant protocol was started for two of the three patients.

CONCLUSION

Severe COVID-19 infection should be considered a potential risk factor for chronic liver disease and liver transplantation.

Key Words: SARS-CoV-2; Persistent cholestasis; Liver chemistry; Hypoxic cholangiopathy; Case report

Core Tip: Coronavirus disease 2019 (COVID-19) multiorganic involvement is well described, including liver disease. In patients with critical COVID-19 requiring invasive mechanical ventilation and management in the intensive care unit, a new entity called "post-COVID-19 cholangiopathy" has been described. It is characterized by persistent cholestasis and chronic liver disease. Therefore, severe COVID-19 infection should be considered a potential risk factor for chronic liver disease probably requiring liver transplantation.

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INTRODUCTION

The global coronavirus disease 2019 (COVID-19) pandemic has caused more than 5100000 deaths worldwide, and as it grows, the knowledge of the disease as well as the discovery of new complications increases. Up to 30% of patients with COVID-19 present with abnormal liver chemistry during the course of the disease[1]; this can occur due to the expression of angiotensin-converting enzyme II in cholangiocytes, a shared mechanism responsible for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry into the cell. While most patients with COVID-19 develop mild and transient elevation of aminotransferases, in patients with critical disease requiring invasive mechanical ventilation and management in the intensive care unit (ICU), a new entity called "post-COVID-19 cholangiopathy" has been described, with only few cases reported to date[2].

Here, we present three patients with severe COVID-19, who subsequently developed persistent cholestasis and chronic liver disease.

CASE PRESENTATION

Chief complaints

Case 1: A 45-year-old male presented to the emergency department of our hospital complaining of malaise, cough, fever, and progressive dyspnea.

Case 2: A 52-year-old male presented to the emergency department of our hospital with severe dyspnea and a positive real-time PCR (RT-PCR) SARS-CoV-2 test.

Case 3: A 46-year-old woman presented to the emergency department of our hospital complaining of malaise, headache, cough, fever, and progressive dyspnea.

History of present illness

Case 1: Patient´s symptoms started 10 d before hospital admission, with dyspnea at rest as the main complaint at admission.

Case 2: Patient's symptoms started 7 d before his admission, and included malaise, cough, fever, and progressive dyspnea. Two days before admission, the patient presented with nausea, emesis, non-inflammatory diarrhea, and dyspnea at rest.

Case 3: Patient´s symptoms started 13 d before admission, including cough, malaise and headache. During this time, a positive RT-PCR SARS-CoV-2 test was obtained and she received symptomatic treatment with acetaminophen. Forty-eight hours before admission, she presented with persistent fever and resting dyspnea.

History of past illness

Case 1: Patient's history was relevant for longstanding type 2 diabetes mellitus, systemic arterial hypertension, and chronic kidney disease KDIGO III. No history of hepatic disease was reported.

Case 2: Patient's history was relevant for chronic kidney disease on hemodialysis, type 2 diabetes, and hypertension. No history of hepatic disease was reported.

Case 3: Patient's history was relevant for history of chronic kidney disease on hemodialysis, type 2 diabetes mellitus, and hypertension. No history of hepatic disease was reported.

Personal and family history

No SARS-CoV-2 vaccine was available at the time of presentation. Family history was unremarkable in all three patients.

Physical examination

Case 1: Physical examination was relevant for oxygen saturation (SpO2) measured by pulse oximeter of 52%, tachypnea, respiratory distress, and crackles on chest auscultation.

Case 2: Physical examination was relevant for SpO2 measured by pulse oximeter of 50%, tachypnea, temperature of 37.8 °C, respiratory distress, and crackles on chest auscultation. Bilateral lower extremity edema was present.

Case 3: Physical examination was relevant for SpO2 measured by pulse oximeter of 80%, tachypnea, and crackles on chest auscultation.

Laboratory examinations

Case 1: At admission, blood tests showed lymphopenia, D-dimer 1093 ng/mL, ferritin 1436 ng/mL, creatinine 8.8 mg/dL and normal liver chemistry. SARS-CoV-2 infection was subsequently confirmed by RT-PCR, and the patient required invasive mechanical ventilation due to respiratory failure type 1 [partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2) ratio of 80].

Case 2: At admission, blood tests showed lymphopenia and elevated inflammatory blood markers. Liver chemistry was normal.

Case 3: Initially, her liver chemistry was normal and elevated inflammatory blood markers were reported. After 72 h of admission, she developed severe hypoxemia (PaO2/FiO2 ratio of 91) requiring mechanical ventilation and admission to the ICU.

Imaging examinations

Chest computed tomography (CT) was performed in all cases, which showed peripheral, bilateral ground glass opacities consistent with severe pulmonary involvement (> 50%) secondary to SARS-CoV-2 infection.

Further evolution and diagnostic work-up

Case 1: During hospitalization after 33 d of stay in the ICU, the patient required sedation with midazolam, fentanyl, and ketamine, high positive end-expiratory pressure (up to 20 cm H_2O) and use of norepinephrine (maximum dose of 0.45 μg/kg/min). In addition, he was treated with meropenem, vancomycin, ceftriaxone, and co-trimoxazole due to blood and tracheal aspirate cultures yielding *Enterobacter cloacae, Stenotrophomonas maltophilia,* and *Klebsiella pneumoniae*. Finally, the patient developed gastrointestinal bleeding caused by duodenal ulcers and required hemodialysis for acute renal failure and metabolic acidosis.

Interestingly, during his stay in the ICU, liver chemistry showed a cholestatic pattern (R factor of 0.7) with an isolated and persistent increase in alkaline phosphatase (ALP) levels. The initial diagnostic workup, included abdominal ultrasound and CT, which did not show bile duct dilatation. The patient eventually improved his clinical conditions, including liver chemistry showing a decrease in ALP levels, extubation on the 35th day, and discharged 42 d after his initial presentation at the endoscopy.

Six weeks after discharge, he developed jaundice, pruritus, and sleep disturbances. New biochemical parameters reported a total bilirubin (TB) 5.8 mg/dL, direct bilirubin (DB) 3.4 mg/dL, and ALP 1328 U/L. Interestingly, hypercholesterolemia developed in the patient, with peak levels reaching 1920 mg/dL (normal < 200 mg/dL). A contrast-enhanced CT scan showed intrahepatic bile duct dilatation and a common bile duct diameter of 8 mm with biliary sludge. An endoscopic retrograde cholangiography (ERCP) was performed and cholangiography confirmed dilation of intrahepatic and extrahepatic bile ducts, a sphincterotomy and balloon sphincteroplasty were also performed, obtaining a bile duct stone, bile duct casts and dark bile, ultimately a biliary plastic stent was placed (Figure 1A). Despite this, there was no improvement in liver chemistry, showing a persistent elevation of ALP levels (> 15 × upper limit of normal); therefore, magnetic resonance cholangiography was performed, showing multiple areas of stenosis in the distal intrahepatic bile ducts (Figure 1B). Differential diagnosis of liver chemistry abnormalities included autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis (SSC), immunoglobulin G4-related disease, viral hepatitis, and drug-induced liver injury (DILI), all of which were ruled out by negative specific antibodies, Ig, and liver biopsy. Percutaneous liver biopsy showed findings consistent with intracanalicular cholestasis, portal inflammation, ductular reaction, and moderate portal fibrosis (Figure 2).

Case 2: He required invasive mechanical ventilation with intermittent prone positioning due to respiratory failure type 1 (PaO2/FiO2 ratio of 73). On day 28 of ICU stay, the patient required hemodialysis, red blood cell transfusions, high positive end-expiratory pressure (up to 20 cm H_2O), and

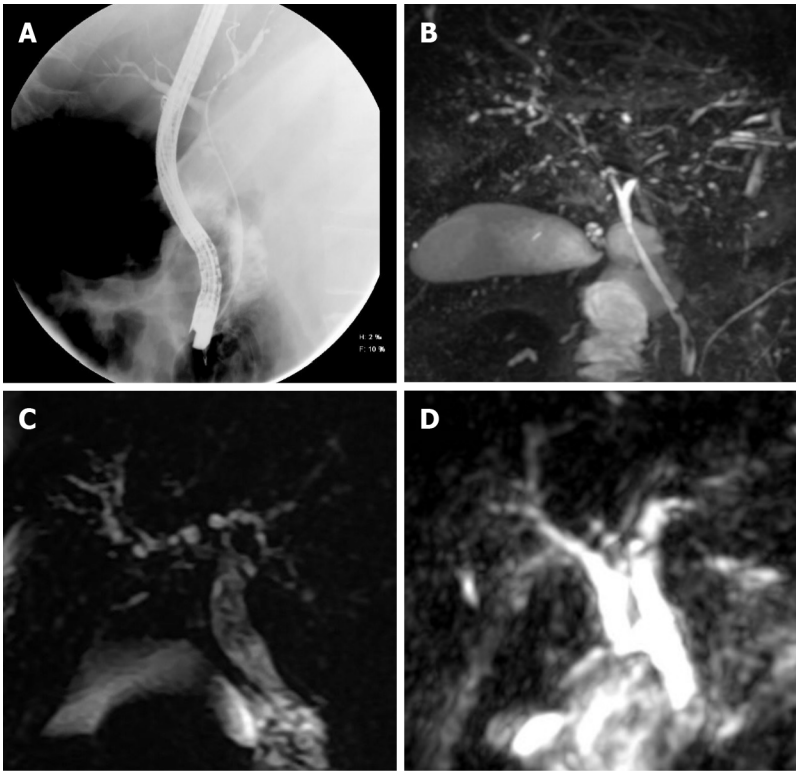

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Figure 1 Sclerosing cholangitis imaging findings. A: Cholangiogram showed dilatation of the common bile duct and its intrahepatic branches in the first case; B: Magnetic resonance imaging (MRI) demonstrated multiple short stenosis of the intrahepatic bile ducts; C and D: In the second and third case, the MRI showed multiple stenosis of the intrahepatic bile duct.

use of norepinephrine (maximum dose of 0.5 μg/kg/min). He received treatment with meropenem, vancomycin, moxifloxacin, co-trimoxazole and voriconazole due to *Streptococcus pneumoniae* and *Staphylococcus aureus* bacteremia (endocarditis was ruled out); ventilator-associated pneumonia due to *S. maltophilia*, *E. cloacae*, and *Aspergillus fumigatus*. Subsequently, liver chemistry showed a cholestatic pattern (R factor < 2) with a persistent increase in ALP and gamma-glutamyl transferase (GGT) levels. Initial diagnostic workup with abdominal ultrasound was negative.

The patient improved his general condition and was discharged 2 mo after admission. During follow-up, he presented with jaundice; liver chemistry reported TB 9.47 mg/dL, DB 5.62 mg/dL, and ALP of 1695 U/L. Viral hepatitis panel and autoimmune cholestatic disease-specific antibodies were negative. Magnetic resonance cholangiography was performed, showing multiple areas of short stenosis with a pattern of SSC (Figure 1C). ERCP was performed in which filling defects of the main bile duct were identified in cholangiography; after sphincterotomy, bile sludge and biliary casts were obtained. Despite the ERCP, there was no improvement in liver function test, showing persistent elevation of ALP levels and TB 22.7 mg/dL.

Case 3: During her 20-d ICU stay, the patient required hemodialysis, high positive end-expiratory pressure (up to 20 cm H_2O), and use of norepinephrine (maximum dose of 0.13 μg/kg/min). She developed ventilator-associated pneumonia due to *Pseudomonas aeruginosa* and received treatment with imipenem, piperacillin/tazobactam, and moxifloxacin.

During her stay, she presented with progressive cholestasis (R factor of < 2) reaching TB up to 17.32 mg/dL, DB 11.59 mg/dL, GGT 211 U/L, and ALP 705 U/L. Abdominal CT scan showed intrahepatic and extrahepatic biliary dilation without evident cause of obstruction. Viral hepatitis panel and autoimmune cholestatic disease-specific antibodies were negative. Magnetic resonance cholangiography was performed, showing intrahepatic and extrahepatic bile ducts with irregular morphology, without evidence of obstruction and periportal edema (Figure 1D).

FINAL DIAGNOSIS

With these findings, including clinical course, ruling out other alternative diagnoses and a close and temporal relationship with SARS-CoV-2 infection, a diagnosis of secondary SSC due to severe COVID-19 was made.

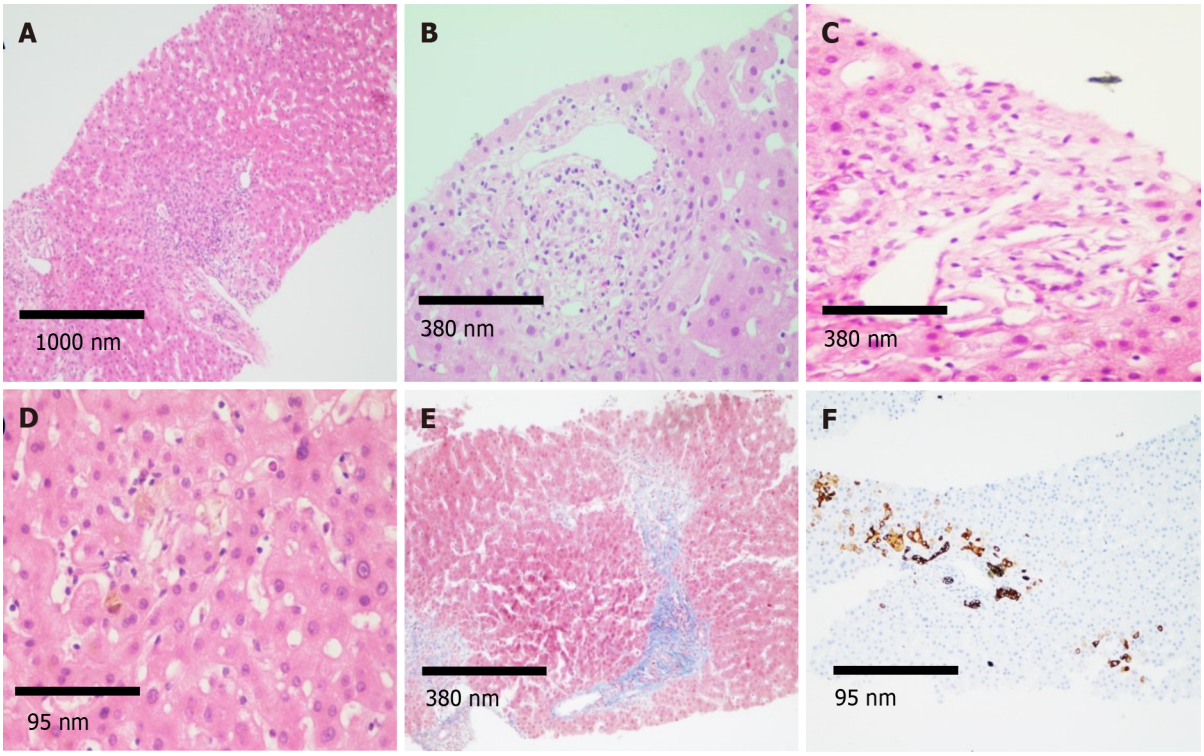

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Figure 2 Histological findings. A: Histological sections of the liver (magnification 4 ×) stained with hematoxylin and eosin (H&E) showing mixed inflammatory infiltrate in portal spaces; B and C: H&E (magnification 10 ×) showing regenerative changes and swelling of cholangiocytes, as well as presence of inflammatory infiltrate in the portal space vein and hepatic artery; D and E: Intracanalicular and cytoplasmic cholestasis is observed predominantly in space 3, fibrosis in portal and periportal space (magnification 40 × and 10 ×, respectively); F: Immunohistochemistry for cytokeratin 7 (CK7) demonstrating CK7 metaplasia in hepatocytes and ductular reaction (magnification 40 ×).

TREATMENT

Case 1: Treatment with ursodeoxycholic acid, cholestyramine, and sertraline was started, showing no clinical improvement on liver chemistry at 8 wk, with persistent elevation of ALP, TB, and GGT.

Case 2: Treatment with ursodeoxycholic acid was started, showing no clinical improvement on liver chemistry.

Case 3: Treatment with ursodeoxycholic acid was started, with persistent elevation of ALP, TB, and GGT.

OUTCOME AND FOLLOW-UP

Case 1: Currently, the patient remains under follow-up without cholestasis improvement and is being evaluated for liver transplantation at our center.

Case 2: A vibration-controlled transient elastography was performed 6 mo after severe COVID-19 admission showing a median of 20.2 KPa (interquartile range/med 17%; FibroScan Echosens™, M probe). Currently, the patient is under palliative care due to Fournier's gangrene and penile necrosis associated sepsis. Liver transplantation protocol was stopped.

Case 3: The clinical evolution of the patient was protracted, and 1 mo after admission, she presented with cardiorespiratory arrest that was not reversible after advanced cardiopulmonary resuscitation maneuvers.

DISCUSSION

SCC is a chronic cholestatic disease, derived from multiple insults to the biliary tract including chronic obstruction, infectious disease, autoimmune, and ischemic cholangiopathy. Similar to primary SSC, its

manifestations include chronic cholestasis, radiologic evidence of stenosis and dilations of the biliary tract, and the potential to progress to liver cirrhosis.

In 2001, Scheppach *et al*[3] reported a series of 3 patients admitted to the ICU due to extrahepatic infections without preexisting biliary or hepatic disease. During their stay, all three developed progressive persistent cholestasis with radiologic (magnetic resonance imaging [MRI] and ERCP) evidence of biliary dilation and stenosis without mechanical obstruction, and eventually progression to liver cirrhosis. In recent years, many centers worldwide have reported SSC in a growing number of patients who have recovered from critical illnesses.

The key element in the pathophysiology of SCC in critically ill patients (SSC-CIP) seems to be ischemia. Severe hypotension, mechanical ventilation, hypoxemia, red blood cell transfusion, and the use of vasopressors all cause significant hemodynamic changes, which directly damage the epithelium of the intrahepatic biliary tract, whose only source of arterial blood supply comes from the peribiliary vascular plexus, this favors the formation of strictures and biliary casts from necrosis tissue and collagen, which also causes mechanical obstruction. These patients present with persistent cholestasis, 7-9 d after the beginning of the condition that led them to the ICU, followed by hyperbilirubinemia; usually with normal or mildly elevated aspartate aminotransferase to alanine aminotransferase ratio; cholestasis persists even after the critical illness has resolved. Filling defects from biliary casts, stenosis and dilations of the intrahepatic biliary tract can be found in imaging studies (MRI and ERCP). Histopathology is highly unspecific, with only 30% of biopsies showing cholestasis associated morphologic changes with different degrees of liver fibrosis[4].

In 2020 with the emergence of COVID-19, many patients were admitted to the ICU, requiring prolonged mechanical ventilation and use of vasopressors due to shock and severe hypoxemia; which are factors associated with ischemic injury to the biliary tract. Since then, some centers have reported cases of progressive and persistent cholestasis in COVID-19, 16 patients (Table 1) with abnormal findings on MRI or ERCP (beading of intrahepatic ducts, bile duct wall thickening with enhancement, and peribiliary diffusion high signal) some associated with the use of Ketamine[2,5-10]. Roth *et al*[2] described 3 patients who developed prolonged and severe cholestasis during recovery from severe COVID-19. Clinical, histologic, and imaging features of these 3 patients were similar to those of SSC-CIP with few exceptions; no biliary casts were found during ERCP and biopsies revealed severe cholangiocyte injury and intrahepatic microangiopathy suggesting direct biliary injury from SARS-CoV-2. Only 1 of 3 biopsies was positive for SARS-CoV-2 in immunohistochemistry and *in situ* hybridization.

The 3 cases described here (Table 2), could also represent a confluence between SSC-CIP and direct hepatic injury from COVID-19. Our patients were admitted to the ICU due to severe COVID-19 requiring prolonged mechanical ventilation and vasopressors and developed cholestasis after admission, which was progressive and persisted even after resolution of choledocholithiasis and long after cardiopulmonary recovery. Characteristic imaging changes were found in MRI in our patients such as intrahepatic bile ducts stenosis and histopathologic changes were identical to those reported by Roth *et al*[2], suggesting a direct biliary injury from SARS-CoV-2. We did not perform immunohistochemistry and *in situ* hybridization for SARS-CoV-2 due to lack of availability in our center.

Nevertheless, we must take into consideration that the differential diagnosis of cholestasis in the ICU is broad, and one important diagnosis to consider is DILI. Bile duct injury due to DILI has emerged as a distinct entity, causing persistent cholestasis and cholangiographic changes consistent with SSC. Our patients received antibiotics and ketamine, both associated with bile duct injury due to DILI. However, the Council for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method Score discarded causality in all cases, mostly because other causes of cholestasis could not be ruled out.

Prognosis in patients with SSC-CIP is poor, with a median transplant-free survival of 13-44 mo; significantly lower than other causes of SSC. Transplant-free survival at 1 year is 55% and 14% at 6 years [4]. In patients with COVID-19 cholangiopathy, prognosis is not well known; to our knowledge, there is one reported case of a 47-year-old man with a successful orthotopic liver transplantation post COVID-19 and is doing well with normal liver tests for 7 mo[9].

CONCLUSION

We believe that our diagnosis is consistent with post-COVID-19 cholangiopathy, although elements of the clinical course, histopathology and radiologic findings may be shared with SSC-CIP, severe COVID-19 is the common element in these patients, and seems to be associated with unique histopathologic features not previously observed in SSC-CIP. Further investigation into treatment and prognosis is required, mostly because persistent cholestasis may lead to liver cirrhosis. Therefore, we propose that severe COVID-19 infection should be considered a potential risk factor for chronic liver disease and liver transplantation.

Table 1 Clinical, images and histological characteristics of the patients reported with post-coronavirus disease 2019 cholangiopathy

Ref.	Patients	Underlying conditions	Drugs	ERCP	MR cholangiography	Liver biopsy	Follow-up
Knooihuizen *et al*[5]	Female, 54 yr	Diabetes, hypothyroidism, hypertension, and hyperlipidemia	Hydromorphone, midazolam, propofol and ketamine	No reported	Intrahepatic dilatationwith a beaded appearance and dilated common bile duct with distal narrowing	Biliary ductular reaction with lobular inflammation and one small non-necrotizing lobular granuloma without viral inclusions	Continued improvement
Edwards *et al*[6]	Male, 59 yr	None	Vancomycin and co-trimoxazole	Sclerosing cholangitis in the intrahepatic ducts	Hypointense filling defects within the common bile duct and intrahepatic bile ducts were also dilated and demonstrated some beading	Not reported	Not reported
Mallet *et al* [7]	3 males and 2 females	Hypertension, diabetes, one with KT and one with HBV infection	Ketamine and no other drugs reported	Filling defects in CBD and rarefication of the intrahepatic biliary tract	Sclerosing cholangitis, with strictures and dilatations of intrahepatic bile ducts, peribiliary cysts and multiple biliary casts	Biliary obstructions, cholangiolar proliferation, biliary plugs, portal inflammation with neutrophil infiltrates, extensive biliary fibrosis and cirrhosis	1 died SSC and cirrhosis, 1 died biliary sepsis, 1 pruritus without jaundice and 2 recurrent biliary sepsis
Sanders *et al* [8]	Male, 57 yr	Hypertension and diabetes	No reported	Bile duct stone cast and intrahepatic duct stenosis without dilation	No reported	No reported	No reported
Durazo *et al* [9]	Male, 47 yr	Obesity, OSA, hypertension, and hyperlipidemia	HCQ	Small pigment stone and diffuse intrahepatic biliary strictures	Mild intrahepatic biliary ductal dilatation with multifocal strictures or beading without extrahepatic biliary dilatation	Mononuclear inflammatory infiltration within the wall of the bile duct, bile lake associated with bile duct injury, microarteriopathy with endothelial cell swelling and obliteration of the lumen and obliterative portal venopathy	On day 108, the patient underwent an OLT
Roth *et al*[2]	2 males and 1 female	None	Multiple antibiotics	2 sludge and stone extracted	Beading, with multiple short segmental strictures	Ductal reaction, bile duct paucity, cholangiocyte swelling, cholangiocyte regenerative change, portal tract inflammation, endothelial swelling, focal endophlebitis portal veins, cholestasis hepatocanalicular and fibrosis	No reported
Bütikofer *et al*[10]	3 males and 1 female	Diabetes	Ketamine	No reported	Diffuse irregularities of the bile ducts with dilatations and strictures	Portal edema, mixed portal inflammation and pronounced bile duct damage with ductular reaction as well as lobular bile infarcts and severe hepatocellular, canalicular, focally ductular cholestasis and pericellular fibrosis around portal tracts and central veins	1 cirrhosis Child B, MELD 17, 2 died pulmonary infection and 1 persistently increased ALP

ALP: Alkaline phosphatase; CBD: Common bile duct; ERCP: Endoscopic retrograde cholangiopancreatography; HBV: Hepatitis B virus; HCQ: Hydroxychloroquine; KT: Kidney transplantation; MELD: Model for End-stage Liver Disease; MR: Magnetic resonance; OLT: Orthotopic liver transplantation; OSA: Obstructive sleep apnea; SSC: Sclerosing secondary cholangiopathy.

Table 2 Clinical characteristics

	Patient 1	Patient 2	Patient 3
Demographics			
Age (yr)	45	52	46
Sex	Male	Male	Female
Comorbidities	T2D, HT, CKD KDIGO III	T2D, HT, CKD KDIGO V	T2D, HT, CKD KDIGO V
COVID-19 infection			
ICU admission	Yes	Yes	Yes
Mechanical ventilation	Yes	Yes	Yes
Vasopressor support	Yes	Yes	Yes
Renal replacement therapy	Yes	Yes (on hemodialysis before admission)	Yes (on hemodialysis before admission)
Secondary infections	Ventilator-associated pneumonia due to *Enterobacter cloacae, Stenotrophomonas maltophilia* and *Klebsiella pneumoniae*	*Streptococcus pneumoniae* and *Staphylococcus aureus* bacteremia. Ventilator-associated pneumonia due to *Stenotrophomonas maltophilia, Enterobacter cloacae* and *Aspergillus fumigatus*	Ventilator associated pneumonia due to *Pseudomonas aeruginosa*
Antibiotics	Meropenem, vancomycin, ceftriaxone and co-trimoxazole	Meropenem, vancomycin, moxifloxacin, co-trimoxazole and voriconazole	Imipenem, piperacillin/tazobactam and moxifloxacin
COVID-19 specific therapy	Dexamethasone	Dexamethasone	Dexamethasone
Liver chemistries on admission			
TB (mg/dL)	0.36	0.37	0.47
ALT (U/L)	37	20	11.8
AST (U/L)	33	46	35.9
ALP (U/L)	89	128	91
Peak liver chemistries			
TB (mg/dL)	11.72	22.7	17.32
ALT (U/L)	63	62.7	7.9
AST (U/L)	119	184.1	46.4
ALP (U/L)	2146	2370	705
Last liver chemistries			
TB (mg/dL)	6.41	8.82	
ALT (U/L)	48	9.3	
AST (U/L)	129	52.6	
ALP (U/L)	3250	1870	
Sclerosing cholangitis imaging findings (CT, ERCP, MRI)	Yes	Yes	Yes
Histology	Intracanalicular cholestasis, portal inflammation, ductular reaction and moderate portal fibrosis	None	None
Evidence of liver fibrosis	Yes (histology)	Yes (VCTE)	No
Death	No	No	Yes

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; COVID-19: Coronavirus disease 2019; CKD: Chronic kidney disease; CT: Computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography; HT: Hypertension; ICU: Intensive care unit; MRI: Magnetic resonance imaging; TB: Total bilirubin; T2D: Type 2 diabetes; VCTE: Vibration-controlled transient elastography.

FOOTNOTES

Author contributions: Mayorquín-Aguilar JM, Lara-Reyes A, and Revuelta-Rodríguez LA were the patient´s gastroenterology fellows during their hospitalization; Macías-Rodríguez RU and Flores-García NC were the attending hepatologists; Mayorquin-Aguilar JM, Lara-Reyes A, Macías-Rodríguez RU, and Ruiz-Margain A reviewed the literature and contributed to the manuscript drafting; Jiménez-Ferreira MA was the gastrointestinal pathology fellow in charge of the interpretation and handling of the pathology images.

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CASE REPORT

Hemorrhagic colitis induced by trientine in a 51-year-old patient with Wilson's disease waiting for liver transplantation: A case report

Andreas Schult, Matts Andersson, Jorge Asin-Cayuela, Karl Sigvard Olsson

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Abstract

BACKGROUND

Wilson's disease (WD) is a rare inherited disorder of copper metabolism. Treatment consists of chelating agents, but side effects are common. We describe a patient who developed colitis during trientine treatment leading to decompensation of liver cirrhosis.

CASE SUMMARY

A healthy 51-year-old woman was diagnosed with liver cirrhosis due to decompensation with ascites. Etiologic evaluation raised suspicion of hereditary hemochromatosis because of compound heterozygosity *HFE* p.C282Y/p.H63D, and phlebotomy was started. Re-evaluation showed low ceruloplasmin, increased urinary copper excretion and the presence of Kayser-Fleischer rings. WD was confirmed by genetic analysis. Because of decompensated cirrhosis, she was referred for liver transplant evaluation. Simultaneously, treatment with trientine was initiated. Liver function initially stabilized, and the patient was not accepted for a liver transplant. Shortly after this, she developed severe hemorrhagic colitis, most probably a side effect of trientine. During that episode, she decompensated with hepatic encephalopathy. Because of a second decompensating event, she was

accepted for liver transplantation, and an uneventful transplantation was carried out after clinical improvement of colitis.

CONCLUSION
Despite WD being a rare disorder, it is important to consider because it can present with a plethora of symptoms from childhood to an elderly age. Colitis should be recognized as a serious adverse drug reaction to trientine treatment that can result in decompensated liver disease.

Key Words: Wilson's disease; Colitis; Trientine; Liver transplantation; Adverse effect; Case report

Core Tip: Even if Wilson's disease is a rare disorder, it is important to consider as a cause of liver disease. Treatment with chelating agents is associated with multiple side effects, and colitis should be recognized as a serious adverse drug reaction to trientine. Such a serious adverse event can trigger hepatic decompensation with the need for liver transplantation.

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INTRODUCTION

Wilson's disease (WD) is a rare recessively inherited disorder in which toxic amounts of copper accumulate in the liver and the brain due to a defective excretion to the bile[1]. It is caused by mutations in the *ATP7B* gene, impairing copper excretion into bile. The prevalence of WD is estimated to be between 1 case in 10000 to 30000 live births[2]. WD can manifest with neuropsychiatric symptoms, chronic liver disease or acute liver failure. Treatment usually consists of copper chelating agents, such as penicillamine and trientine or zinc, which reduces enteric copper uptake. Patients with decompensated liver cirrhosis or acute liver failure may require liver transplantation, which corrects the underlying metabolic defect[3].

CASE PRESENTATION

Chief complaints

The present case was a 51-year-old married woman with two children who was employed as a worker at a warehouse. She had never smoked and consumed 1-2 glasses of wine per week. On a routine health check at age 50, the local general practitioner awarded her a star for excellent health. However, shortly afterward she began to feel fatigued and swollen and was diagnosed with ascites at her local hospital.

Physical examination

She was not jaundiced.

Laboratory examinations

The liver function tests showed slightly elevated bilirubin (30 μmol/L, reference 5-25), and albumin was decreased to 20 g/L (reference 36-45). Alkaline phosphatase was within the normal range, alanine aminotransferase was normal, and aspartate aminotransferase was just above the upper limit of normal, resulting in an aspartate aminotransferase/alanine aminotransferase ratio > 2. Because of a prothrombin time/international normalized ratio of 1.9, a liver biopsy was not undertaken.

Viral hepatitis was ruled out by serology, and negative autoantibodies (antinuclear, smooth muscle and antimitochondrial) made autoimmune hepatitis and primary biliary cholangitis unlikely. A negative phosphatidylethanol confirmed the absence of harmful drinking[4]. Transferrin saturation was 52% and ferritin 206 μg/L (reference 13-150), hence hereditary hemochromatosis (HH) was considered. Genetic analysis showed *HFE* p.C282Y/p.H63D compound heterozygosity, and iron removal by phlebotomy was initiated. There was a slight improvement, but after 3 mo, her liver tests were still abnormal, which led to the consideration of other diagnoses.

The suspicion of WD was supported by a low serum ceruloplasmin concentration (0.14 g/L, reference 0.22-0.58) and increased urinary copper excretion (4.8 μmol/24 h, reference 0.15-0.60). Detailed eye examination revealed the presence of Kayser-Fleischer rings. Genetic analysis of *ATP7B*, covering all coding exons +/- 25 flanking intronic bases, showed the presence of two heterozygous pathogenic variants, namely c.3207C>A, p.(His1069Gln) and c.2305A>G, p.(Met769Val) (NM_000053.3). The analysis was carried out on DNA extracted from blood after enrichment with a custom-made next-generation sequencing gene panel that included *ATP7B* (SureSelectQXT, Agilent TechnologiesR), on a MiSeq instrument (IlluminaR). Results were verified by Sanger sequencing. Compound heterozygosity of the two variants was confirmed by genotyping of the patient's parents.

FINAL DIAGNOSIS

She was diagnosed with liver cirrhosis due to decompensation with ascites.

TREATMENT

Upon confirmation of WD, the patient underwent neurological evaluation. Besides slight numbness of legs, especially at night, there were no neurologic symptoms. A complete neurological exam showed a slightly decreased blink rate and somewhat abrupt saccades. There were no signs of dysarthria, gait abnormalities or parkinsonism.

Chelating treatment with trientine 300 mg bid was initiated. Simultaneously, the patient was referred for liver transplant evaluation at Sahlgrenska University Hospital, Gothenburg, due to decompensated liver cirrhosis. At the time of evaluation, she had been on treatment with trientine for 6 wk. She was free from ascites on low-dose diuretics and had no other decompensating events. Her model for end-stage liver disease score was 13 and Child-Pugh class B (8 points). Because of stable disease during ongoing treatment, she was not accepted for liver transplantation.

However, on the day of leaving the university hospital, loose stools appeared. During the following days, her symptoms worsened, and her stools became bloodstained. At her local hospital, a sigmoidoscopy showed hemorrhagic colitis. Biopsies were negative for cytomegalovirus, and stool cultures returned negative. As colitis has been described as a side effect of trientine[5,6], the drug was withdrawn, and treatment with prednisolone 30 mg q.d. was initiated.

Her colitis improved rapidly, but after some days, she became somnolent. There were no clinical signs of gastrointestinal bleeding, spontaneous bacterial peritonitis or other infection. A cranial computed tomography showed normal findings, and electroencephalography was compatible with metabolic encephalopathy. A diagnosis of hepatic encephalopathy West Haven grade 3 was made. The patient improved on treatment with lactulose and rifaximin. Treatment with zinc acetate 25 mg t.i.d. to reduce copper absorption was started. Steroids were tapered within 1 wk. She was again referred for transplant evaluation and subsequently accepted.

OUTCOME AND FOLLOW-UP

After another episode of severe hepatic encephalopathy requiring intubation, liver transplantation with a whole graft from a deceased donor was carried out 3 mo later. Vessel reconstruction consisted of a side-to-side cavo-caval, end-to-end artery and duct-to-duct biliary anastomosis. Immunosuppressive induction therapy was given by 1000 mg methylprednisolone intraoperatively and 20 mg basiliximab before reperfusion and on postoperative day 4. Mycophenolate mofetil 1 g b.i.d. was started before transplantation, and tacrolimus was introduced on postoperative day 4. No steroids were used for maintenance immunosuppression. The clinical course was uneventful, and the patient was discharged to home on postoperative day 10.

During the 1st month, a mild acute T-cell mediated rejection (rejection activity index 3) was treated with oral corticosteroids. Because of cytomegalovirus mismatch (D+/R-), she received prophylaxis for 6 mo with valganciclovir 450 mg q.d. After discontinuation of prophylaxis, she developed cytomegalovirus disease with pancytopenia, and oral treatment with valganciclovir was reinstated. After viral clearance, the further course was uneventful. Protocol liver biopsy after 1 year only showed mild inflammation without sign of rejection or fibrosis. Up to now, 3 years after liver transplantation, there have been no further complications, and the patient is now back to normal active life.

DISCUSSION

This case illustrates two important learning points. The first one is the difficulty to diagnose WD. It can present with both neuropsychiatric as well as acute or chronic liver disease. Because WD is a rare disease, it may not be included in differential diagnosis of liver disease although its prevalence is probably significantly higher than the number of clinically diagnosed cases[7]. A delayed diagnosis is not uncommon, as in another Swedish female observed during family screening of HH[8] in which WD was confirmed by sequencing of *ATP7B* showing homozygosity for the variant c.3207C>A (His1069Gln)[9].

An initial diagnosis of HH was feasible because this is a common disorder in central Sweden[10], and the patient had elevated ferritin. However, compound heterozygosity HFE p.C282Y/p.H63D seldom results in HH-related morbidity[11]. Comorbid factors should always be considered, and WD has previously been reported in the patient's home area[12].

The other point is the awareness of potential side effects of trientine. Although drugs for the treatment of WD were introduced in the 1960s, there is still a lack of high-quality studies. Initial treatment of patients not presenting with acute liver failure usually aims at promoting urinary copper excretion with chelating agents. Penicillamine is a drug with high incidence of adverse reactions such as hypersensitivity, gastrointestinal symptoms, proteinuria and bone marrow depression with rare cases of aplastic anemia.

Trientine is often used as a first-choice treatment because of less side-effects compared to penicillamine[3]. It is, however, not an uncomplicated drug, and besides skin reactions and neurologic worsening, cases of colitis have been described[5,6]. New compounds for the treatment of WD are under development[13,14] and may widen the available armamentarium, offering alternative therapies in case of adverse drug reactions.

Our patient developed severe hemorrhagic colitis due to trientine treatment, which may have triggered decompensation of her liver cirrhosis. Decompensated liver cirrhosis and acute liver failure are indications for liver transplantation in patients with WD. It can only be speculated if liver transplantation could have been avoided if the patient had not developed severe colitis. However, after improvement of colitis with steroid treatment, the patient could undergo liver transplantation with excellent functional status after 3 years of follow-up.

CONCLUSION

Even if WD is a rare disorder, it is important to consider because it can present with a plethora of symptoms from childhood to an elderly age. Colitis should be recognized as a serious adverse drug reaction from trientine treatment that can result in decompensated liver disease.

FOOTNOTES

Author contributions: Schult A wrote the part of the manuscript concerning transplantation, pretransplant work-up and revised the manuscript; Andersson M contributed to pretransplant clinical information; Asin-Cayuela J wrote the part concerning genetic analyses and critically revised the manuscript; Olsson KS drafted the main manuscript and critically revised the manuscript.

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CORRECTION

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Abstract

Addition of authors' affiliation to "Hepatitis B virus detected in paper currencies in a densely populated city of India: A plausible source of horizontal transmission?" *World J Hepatol* 2020 Oct 27; 12(10): 775-791. In this article, one of the affiliations of two authors was not mentioned. Ruchi Supekar, a joint first author and Subhajit Biswas, the corresponding author are affiliated to Academy of Scientific and Innovative Research (AcSIR) Ghaziabad- 201002, India.

Key Words: Addition; Affiliation; Correction

Core Tip: This manuscript is an authors' affiliation addition to "Hepatitis B virus detected in paper currencies in a densely populated city of India: A plausible source of horizontal transmission?" *World J Hepatol* 2020 Oct 27; 12(10): 775-791.

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TO THE EDITOR

Addendum

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FOOTNOTES

Author contributions: Biswas S and Das P conceptualized, designed and executed the present work and wrote the manuscript; Supekar R contributed substantially to the virology experiments and in writing of the manuscript; Chatterjee R, Roy S and Ghosh A took part in collecting the samples for this study and also conducted experiments; Das P and Supekar R contributed equally and are joint first authors; All authors have read and approved the final manuscript.

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