ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2017 February 28; 9(6): 293-348

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

A peer-reviewed, online, open-access journal of hepatology

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2014-2017

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World Journal of Hepatology

Contents

Three issues per month Volume 9 Number 6 February 28, 2017

World Journal of Hepatology
Volume 9 Number 6 February 28, 2017

Contents

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
36 Issues/Year (8th, 18th, and 28th of each month)

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Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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PUBLISHER
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8226 Regency Drive,
Pleasanton, CA 94588, USA
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http://www.wjgnet.com

PUBLICATION DATE
February 28, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 February 28; 9(6): 293-299

DOI: 10.4254/wjh.v9.i6.293 ISSN 1948-5182 (online)

EDITORIAL

Hepatorenal syndrome: Update on diagnosis and therapy

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Author contributions: Acevedo JG wrote the manuscript; Cramp ME critically reviewed the manuscript.

Conflict-of-interest statement: Acevedo JG and Cramp ME declare no conflict of interest related to this publication.

Manuscript source: Invited manuscript

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Received: September 29, 2016
Peer-review started: October 2, 2016
First decision: October 20, 2016
Revised: December 30, 2016
Accepted: February 8, 2017
Article in press: February 13, 2017
Published online: February 28, 2017

Abstract

Hepatorenal syndrome (HRS) is a manifestation of extreme circulatory dysfunction and entails high morbidity and mortality. A new definition has been recently recommended by the International Club of Ascites, according to which HRS diagnosis relies in serum creatinine changes instead that on a fixed high value. Moreover, new data on urinary biomarkers has been recently published. In this sense, the use of urinary neutrophil gelatinase-associated lipocalin seems useful to identify patients with acute tubular necrosis and should be employed in the diagnostic algorithm. Treatment with terlipressin and albumin is the current standard of care. Recent data show that terlipressin in intravenous continuous infusion is better tolerated than intravenous boluses and has the same efficacy. Terlipressin is effective in reversing HRS in only 40%-50% of patients. Serum bilirubin and creatinine levels along with the increase in blood pressure and the presence of systemic inflammatory response syndrome have been identified as predictors of response. Clearly, there is a need for further research in novel treatments. Other treatments have been assessed such as noradrenaline, dopamine, transjugular intrahepatic portosystemic shunt, renal and liver replacement therapy, *etc*. Among all of them, liver transplant is the only curative option and should be considered in all patients. HRS can be prevented with volume expansion with albumin during spontaneous bacterial peritonitis and after post large volume paracentesis, and with antibiotic prophylaxis in patients with advanced cirrhosis and low proteins in the ascitic fluid. This manuscript reviews the recent advances in the diagnosis and management of this life-threatening condition.

Key words: Hepatorenal syndrome; Acute-on-chronic liver failure; Liver cirrhosis; Terlipressin; Acute kidney injury

Core tip: Hepatorenal syndrome (HRS) is a life-threatening complication present in very advanced liver cirrhosis. This manuscript addresses many recent advances in this field, including the recent change in the definition of HRS according to acute kidney injury

criteria, the potential consequences of the adoption of this new definition, and the use of biomarkers to help in the diagnostic algorithm. Moreover, it reviews the recent advances in treatment of HRS such as the use of continuous infusion of terlipressin instead of bolus and the low efficacy of midodrine plus octreotide. Potential areas of research are identified as well.

Acevedo JG, Cramp ME. Hepatorenal syndrome: Update on diagnosis and therapy. *World J Hepatol* 2017; 9(6): 293-299 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i6/293.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i6.293

INTRODUCTION

Hepatorenal syndrome (HRS) is a manifestation of extreme circulatory dysfunction. It develops in the setting of advance stage in cirrhosis and carries an ominous prognosis.

HRS is diagnosed clinically. Its definition has been updated recently in accordance with the acute kidney injury (AKI) criteria.

Current standard of care involves the use of vasoconstrictor therapy (*i.e.*, terlipressin) and volume expansion with albumin. Treatment is effective in only 40%-50% of cases and it recurs in up to 50% of those cases responding to treatment. Liver transplant (LT) should be considered in all patients without contraindications for it.

Areas of research would be aimed at improving the accuracy of diagnosis of HRS, identifying predictors of non-response, and testing novel treatments.

PATHOPHYSIOLOGY

HRS is caused by extreme circulatory dysfunction. Hepatocytes and stellate cells in a cirrhotic liver produce numerous local acting vasodilators such as nitric oxide, cannabinoids, *etc*. These vasodilators act locally on the splanchnic circulation producing splanchnic arterial vasodilation. Splanchnic circulation represents an important part of the circulation of the body. Thus, splanchnic vasodilation produces a decrease in mean arterial pressure (MAP), which in turn triggers the activation of the sympathetic nervous system, leading to high levels of circulating noradrenaline, which along with an increase in cardiac output are the early mechanisms compensating circulatory dysfunction during this early stage and keep MAP stable[1].

As the disease progresses and splanchnic vasodilation gets worse other vasoconstrictor systems get activated such as the renin-angiotensin-aldosterone system and vasopressin release[1].

Aldosterone enhances retention of sodium and water by the kidneys leading to development of ascites. Vasopressin enhances retention of free water conducting to hyponatremia. The splanchnic vascular bed is refractory to the action of all these vasoconstrictor systems which on the contrary act effectively on other vascular beds such as the femoral and brachial vessels (producing cramps), in vessels in the brain (potentially playing a role in encephalopathy) and in the renal arteries (leading to HRS)[1,2]. In this sense, mean renal artery resistive index increases gradually from patients with cirrhosis but no ascites, in those with ascites, refractory ascites and HRS[3,4].

Therefore, HRS is a functional disease characterised by marked vasoconstriction of the renal arteries secondary to the effect of hyper-activation of different vasoconstrictor systems aimed at compensating the systemic vasodilation caused by the initial splanchnic vasodilation. HRS always develops in the setting of advance circulatory dysfunction and it is always accompanied by ascites and usually by hyponatremia[1].

HRS can develop in the setting of infection, mainly after spontaneous bacterial peritonitis (SBP), as a consequence of a worsening degree of circulatory dysfunction caused by sepsis. Volume expansion with albumin prevents effectively development of HRS in patients with SBP[5].

HRS can also develop in the setting of circulatory dysfunction after large volume paracentesis (LVP). This complication is prevented by replacing albumin after LVP[6].

DIAGNOSIS OF HRS ACCORDING TO THE NEW DEFINITION OF AKI

Classically, acute renal failure in cirrhosis was defined as an increase in serum creatinine (sCr) levels of $\geqslant$ 50% from baseline to a final level above 1.5 mg/dL (133 μmol/L), and classical definition of HRS type-1 was doubling sCr levels over 2.5 mg/dL or 220 μmol/L within 2 wk. Serum creatinine overestimates renal function in cirrhotic patients due to a number of factors: Creatinine production in patients with cirrhosis is reduced due to muscle wasting, there is an increased secretion of creatinine in the renal tubules, sCr may be diluted due to an increased volume of distribution, and finally, high bilirubin levels may interfere with the assays to measure accurately its level. Recently, the International Club of Ascites (ICA) has adopted the concept of AKI which was developed originally to be used in general critically-ill patients. AKI is defined as the increase of at least 0.3 mg/dL (26 μmol/L) and/or $\geqslant$ 50% from baseline, within 48 h[7].

Diagnostic criteria of HRS according to ICA-AKI criteria are the following[7]: (1) diagnosis of cirrhosis and ascites; (2) diagnosis of AKI according to ICA-AKI criteria; (3) no response after 2 consecutive days of diuretic withdrawal and plasma volume expansion with albumin (1 g/kg of body weight); (4) absence of shock; (5) no current or recent use of nephrotoxic drugs (non-steroidal anti-inflammatory drugs, aminoglycosides, iodinated contrast media, *etc.*); and (6) no macroscopic signs of structural kidney injury, defined as absence of proteinuria (> 500 mg/d), absence of microhematuria (> 50 red blood

cells per high power field) and normal findings on renal ultrasound.

The main change produced by adopting the new definition of HRS is the removal of a rigid very high cut-off value of sCr (2.5 mg/dL or 220 μmol/L) to start pharmacologic treatment. In this way, treatment can be administered early and potentially better efficacy could be achieved.

However, these clinical criteria do not allow differentiation between HRS and parenchymal renal disease, which is extremely important because vasoconstrictors will not be effective and could even worsen the renal dysfunction. Thus, there is a wide interest in developing urinary biomarkers to help in the differential diagnosis of HRS.

URINARY BIOMARKERS IN AKI

Currently, numerous biomarkers have been assessed in the setting of AKI and liver cirrhosis including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18, liver-type fatty acid binding protein (L-FABP), kidney injury molecule-1, toll-like receptor 4, π-glutathione S-transferase and α-glutathione S-transferase[8]. Among all of them, current data show that NGAL is the most useful marker. NGAL detects patients with acute tubular necrosis (ATN). On the contrary, NGAL is not helpful to differentiate between pre-renal azotemia and HRS. NGAL urinary levels are much higher in patients with ATN compared to patients with other causes of AKI. Urinary levels of NGAL in ATN were 417 μg/L, compared with levels at 30 μg/L in pre-renal azotemia, 82 μg/L in chronic kidney disease and 76 μg/L in HRS, $P < 0.001$[9,10]. Thus, incorporating NGAL into the clinical decision algorithm would be of benefit to rule out structural kidney injury and detecting a group of patients in whom treatment with vasoconstrictors wouldn't be effective and only would produce potentially serious side effects[11].

CURRENT TREATMENT (STANDARD OF CARE)

Once patients with AKI have received volume expansion with albumin (1 g per kilogram) with no response achieved in the following 48 h, and criteria of HRS are fulfilled, then treatment with terlipressin is recommended. Expansion with albumin should be continued at the dose of 20-40 g daily.

Response to treatment should be assessed regularly and terlipressin should be titrated gradually up to a maximum dose of 12 mg per day. Terlipressin should be used for a maximum of 14 d and stopped in case of lack of response[7].

Response is defined as a reduction of at least 25% from baseline sCr level, that is from sCr level before treatment with terlipressin was started[7].

Response is achieved in around 40%-50% of patients. The rate of recurrence of HRS is 30%. A definitive treatment of the circulatory dysfunction and the underlying liver cirrhosis with liver transplantation should be considered in all cases with no contraindications. Otherwise, the persistent advanced circulatory dysfunction makes HRS recur frequently and predispose the patient to other major decompensations[12]. This is the rationale supporting prioritization of patients with HRS on the waiting list for LT in some centres. Terlipressin and albumin is not a definitive treatment but should be considered as a bridge to a definitive treatment, *i.e.*, LT.

Two randomized studies showed that HRS reversal rate when terlipressin plus albumin was employed was higher compared to the reversal achieved employing albumin alone. Martín-Llahí *et al*[13] reported a much higher rate of improvement in renal function in patients treated with terlipressin and albumin compared to those patients treated only with albumin (43.5% *vs* 8.7%, $P = 0.017$). This result may be influenced by the fact that patients who did not tolerate terlipressin were excluded from the analysis. Sanyal *et al*[14] also showed that HRS reversal was achieved more frequently in those patients treated with terlipressin and albumin compared with those treated only with albumin (33.9% *vs* 12.5%, $P = 0.008$). Any of these studies showed difference in survival at 3-mo and 6-mo. A large randomized trial has been published recently and it showed a higher rate of HRS reversal in those patients receiving terlipressin (23.7% *vs* 15.2%, $P = 0.13$). This difference did not reach statistical significance, probably due to the fact that one third of patients received fewer than three days of treatment, which could affect the effectiveness of the treatment. When the analyses were done stratifying patients by the degree of reduction in serum creatinine level, data showed that a decrease in sCr level, even if not reaching a complete reversal, has a positive impact on survival[15].

Traditionally, terlipressin has been used in bolus 0.5-1.0 mg every 4-6 h. Recent data show that continuous infusion of terlipressin has the same efficacy compared with bolus administration and it is better tolerated presenting fewer side effects (35.29% *vs* 62.16%, $P < 0.025$). Probably, side effects were lower because the total effective daily dose required was lower in the infusion groups compared to the bolus group (2.23 ± 0.65 mg/d *vs* 3.51 ± 1.77 mg/d, $P < 0.05$)[16].

Therefore, we recommend employing terlipressin at 2 mg per day in continuous infusion (diluted in 250 mL of Dextrose 5%) along with albumin (20-40 g per day). Response should be assessed every 48 h. If response is not achieved in 48 h, then terlipressin dose should be increased in a stepwise manner (increase in 2 mg per day).

These patients need careful observation, including review of ischaemic side effects on acral parts, ischaemic heart events, bowel ischaemia (diarrhoea). They can also develop hyponatremia and arrhythmias.

PREDICTORS OF RESPONSE TO TERLIPRESSIN AND ALBUMIN

There are only few published studies assessing pre-

dictors of response to treatment in HRS. These studies show there is a close relationship between effectiveness of treatment and capacity to improve systemic hemodynamics. Patients in whom terlipressin did not increase the MAP in at least 5 mmHg at day 3 of treatment had a lower rate of response. Effectiveness of treatment is also related with degree of liver dysfunction. Those patients who did not increase MAP at day 3 and who also had high baseline bilirubin levels ⩾ 171 μmol/L (10 mg/dL) had a poor response rate, of only 9%[17]. Another study showed that baseline creatinine levels predicted HRS reversal, suggesting that early intervention would be more effective[18]. A recent retrospective study showed that those patients with systemic inflammatory response syndrome (SIRS) had a much higher response rate to terlipressin (42.9% *vs* 6.7%, $P = 0.018$), while terlipressin did not show more efficacy than placebo when employed in patients without SIRS (15.9% *vs* 18.8%, P = NS)[19].

A recent abstract showed that no response to treatment was associated with higher urinary NGAL levels (728.8 μg/L *vs* 182.9 μg/L, $P = 0.02$), probably related to the presence of acute tubular necrosis in those patients[20].

In summary, the following markers to predict response to treatment (terlipressin) have been identified: Low baseline creatinine and bilirubin levels, increase in blood pressure, presence of SIRS and low urinary NGAL.

OTHER TREATMENTS

LT

Patients with HRS type-1 with no contraindications for a LT should be invariably worked up and place in the LT waiting list because LT is the only definitive treatment for HRS. LT reverses liver dysfunction and portal hypertension. Patients with HRS have worse survival expectancy than other patients with cirrhosis for any given value of MELD score, which suggests HRS is a factor of poor prognosis independently from MELD score[21,22]. Furthermore, there is evidence that structural injury to the renal tubules occur early in the course of HRS-1 and the longer the patient is awaiting the transplant and suffering from HRS the higher the risk of not recovering their renal function or even requiring a renal transplant after LT[23]. In this sense, experts recommend to prioritize these patients by using pre-treatment levels of creatinine or considering the pharmacological treatment of HRS as haemodialysis when calculating MELD score[24]. Currently, there is no general consensus about prioritization of patients with HRS awaiting a LT. Some centres prioritize these patients and some others don't. The major challenge LT programmes face is the shortage of donors and consequently optimization in the allocation of the few organs available becomes extremely necessary. Thus, we suggest that those patients with recurrent episodes of HRS-1, hence at high risk of developing refractory HRS, are at high risk of dropping out of the LT waiting list or at risk of not recovering their renal function after LT, and therefore will get most benefit from early transplantation.

Midodrine and octreotide

Combination of midodrine and octreotide (MID/OCT) plus albumin is widely used in countries where terlipressin is not available. A recent randomized trial showed a much lower response rate in patients treated with MID/OCT compared to patients treated with terlipressin (4.8% *vs* 55.6%, $P < 0.01$). Three-month survival rate, after exclusion of patients who received rescue treatment, was also lower in the MID/OCT group (29% *vs* 56%, $P = 0.06$)[25]. These data show midodrine in combination with octreotide is not an effective treatment for HRS.

Noradrenaline

A recent randomized study comparing noradrenaline with terlipressin showed HRS reversal is achieved in 43.4%, similar to the reversal rate achieved with terlipressin (39.1%). Survival at 15 d of therapy was similar in the noradrenaline and terlipressin group (39.1% *vs* 47.8%, $P = 0.461$)[26]. A recent meta-analysis analysed 4 studies including 152 patients and suggested that treatment with noradrenaline is as effective as terlipressin in reversing HRS when used along with albumin[27]. Therefore, noradrenaline is an effective therapy for HRS. Noradrenaline main drawback is that its use generally requires an intensive care unit setting.

Dopamine

Low-dose dopamine increases renal blood flow but shows no effect on glomerular filtration rate or on the outcome in HRS. In a recent study, dopamine didn't show reduction of creatinine levels after 5 d of treatment[28,29]. It is not considered an appropriate treatment for HRS.

Transjugular intrahepatic portosystemic shunt

HRS type-1 usually occurs in the setting of advanced liver dysfunction and transjugular intrahepatic portosystemic shunt (TIPS) is usually contraindicated on this basis. There are few small trials showing improvement on renal function and deactivation of vasoconstrictor system, *i.e.*, reduction in levels of renin, aldosterone and noradrenaline after TIPS insertion[30,31]. However, data is very limited to recommend its use in clinical practice.

Renal and liver replacement therapy

Haemodialysis is employed in those patients awaiting LT whose renal function failed to respond to medical treatment and at the same time bring the extra points required for prioritization.

Liver support with molecular adsorbent recirculating system (MARS) has been tested in small cohorts of patients who did not respond to vasoconstrictors and had advanced liver dysfunction, which usually precludes TIPS insertion. One trial showed the reduction in creatinine and bilirubin levels was higher in the MARS group compared with the continuous haemodialysis group[32]. Another study showed no significant changes in systemic

haemodynamics and glomerular filtration rates following MARS treatment[33]. Treatments employed at this stage should be restricted to patients awaiting a definitive treatment (*i.e.*, LT). It would be controversial to employ such invasive treatments in patients with contraindication for LT, and thus with no option for a definitive treatment.

Serelaxin

Serelaxin is a recombinant form of the human peptide hormone relaxin-2, increases renal perfusion in healthy human volunteers. Its properties have been explored in a pilot study on compensated cirrhotic patients and it showed increase renal blood flow by 65.4% from baseline with no effect on systemic blood pressure[34]. Data on this hormone is still scarce.

PREVENTION

HRS can be prevented in different clinical scenarios. The first one is in the setting of SBP. The deleterious effect on circulatory dysfunction produced by SBP can be prevented by volume expansion with albumin. The pioneer study of the Barcelona group showed that those patients receiving albumin prevented development of renal failure (10% *vs* 33%, $P = 0.002$) and reduced short-term mortality (mortality at 3-mo, 22% *vs* 41%, $P = 0.03$)[9]. There are still no convincing data to recommend plasmatic expansion with albumin in patients with other types of infections different from SBP. One trial showed a tendency to develop renal failure less frequently in those patients without renal failure at baseline and receiving expansion with albumin (3% *vs* 10%, P = NS)[35].

HRS can be prevented after LVP, albumin at a dose of 6-8 g per litre of ascites removed is the dose most commonly used to prevent worsening of circulatory dysfunction, and thus minimize the impact on electrolytes, creatinine and renin levels. Volume expansion with albumin also improves survival after LVP and it is recommended by international societies[36,37].

HRS can also be prevented with primary antibiotic prophylaxis of SBP. Fernández *et al*[38] showed in a cohort of patients with advanced cirrhosis that SBP primary prophylaxis reduced development of HRS (28% *vs* 41%, $P = 0.02$) and mortality at 3 mo (94% *vs* 62%, $P = 0.003$), this effect is probably related to the effect of Norfloxacin in reducing the levels of bacterial products within the gut and hence reducing bacterial translocation.

AREAS FOR FUTURE RESEARCH

Definition of HRS is continuously changing and it is based on clinical grounds, relying on serum creatinine levels, which has many limitations as marker of renal function. Research focused on new biomarkers, such as urinary NGAL, to make the diagnostic algorithm of HRS more accurate is clearly needed and fortunately, interest in this field is increasing.

Moreover, identifying patients with low probability of responding to treatment is of major importance in order to start early alternative treatments and potentially prioritize these patients on the LT waiting list.

Finally, research looking for novel treatments besides intravenous terlipressin and expansion with albumin is also needed.

CONCLUSION

HRS is a major decompensation in advanced liver cirrhosis. It entails a high short-term mortality rate. Current definition is based on clinical grounds and has been recently modified adopting AKI definition. Recent data on urinary NGAL show it is useful to differentiate acute tubular necrosis and should be incorporated in the diagnostic algorithm of HRS. Terlipressin and noradrenaline are the only effective treatment currently available and reversal rate is only 40%-50% of cases. Data on predictors of response to treatment suggest that treatment should be started as early as possible. In this sense, ICA new definition of HRS allows an early diagnosis. New treatments should be tested for this life-threatening condition. Finally, LT is the only curative treatment and should be always considered.

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P- Reviewer: Ding MX, Sato T, Thomopoulos KC
S- Editor: Song XX **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 February 28; 9(6): 300-309

DOI: 10.4254/wjh.v9.i6.300 ISSN 1948-5182 (online)

MINIREVIEWS

Clinical features, histology, and histogenesis of combined hepatocellular-cholangiocarcinoma

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Conflict-of-interest statement: All the authors of this study have nothing to declare.

Manuscript source: Invited manuscript

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Received: September 11, 2016
Peer-review started: September 14, 2016
First decision: October 20, 2016
Revised: November 18, 2016
Accepted: January 2, 2017
Article in press: January 3, 2017
Published online: February 28, 2017

Abstract

Combined hepatocellular-cholangiocarcinoma (CHC) is a rare tumor with poor prognosis, with incidence ranging from 1.0%-4.7% of all primary hepatic tumors. This entity will be soon renamed as hepato-cholangiocarcinoma. The known risk factors for hepatocellular carcinoma (HCC) have been implicated for CHC including viral hepatitis and cirrhosis. It is difficult to diagnose this tumor pre-operatively. The predominant histologic component within the tumor largely determines the predominant radiographic features making it a difficult distinction. Heterogeneous and overlapping imaging features of HCC and cholangiocarcinoma should raise the suspicion for CHC and multiple core biopsies (from different areas of tumor) are recommended before administering treatment. Serum tumor markers CA19-9 and alpha-fetoprotein can aid in the diagnosis, but it remains a challenging diagnosis prior to resection. There is sufficient data to support bipotent hepatic progenitor cells as the cell of origin for CHC. The current World Health Organization classification categorizes two main types of CHC based on histo-morphological features: Classical type and CHC with stem cell features. Liver transplant is one of the available treatment modalities with other management options including transarterial chemoembolization, radiofrequency ablation, and percutaneous ethanol injection. We present a review paper on CHC highlighting the risk factors, origin, histological classification and therapeutic modalities.

Key words: Combined hepatocellular-cholangiocellular carcinoma; Hepatocellular carcinoma; Cholangiocellular carcinoma; Hepatic progenitor cell(s); Histogenesis; Classification

Core tip: Combined hepatocellular-cholangiocarcinoma is a rare tumor with ambiguous data in literature in

relation to its clinical features, histogenesis, pathological classification and prognosis. The goal of our study was to review the literature and highlight the new updates on this entity.

Gera S, Ettel M, Acosta-Gonzalez G, Xu R. Clinical features, histology, and histogenesis of combined hepatocellular-cholangiocarcinoma. *World J Hepatol* 2017; 9(6): 300-309 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i6/300.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i6.300

INTRODUCTION

Combined hepatocellular-cholangiocarcinoma (CHC) is a rare tumor, with variation reported from 1.0%-4.7% of all primary hepatic tumors in series of patients undergoing hepatic resection[1-6], although accurate incidence is not known. CHC has been known with several nomenclatures in the literature including mixed hepatocellular carcinoma-cholangiocarcinoma (HCC-CC), hybrid HCC-CC or combined liver and bile duct carcinoma[7]. Some of the more common risk factors for CHC mentioned in the literature are hepatitis B virus (HBV) infection, male predominance, cirrhosis and hepatitis C virus (HCV) infection[8-12]. Molecular evidence supports that the hepatic progenitor cell (HPC) is the cell of origin for CHC. On histology, it is divided into two main subtypes-classical type and subtypes with stem cell features (further discussed in histology section)[13]. Separate HCC and CC in the same liver does not classify as CHC. We present a review of current understanding of clinical features, histogenesis and histology of the combined hepatocellular-cholangiocarcinoma that will be soon renamed as hepato-cholangiocarcinoma.

CLINICAL FEATURES AND RISK FACTORS

Owing to the rarity of CHC, the majority of the series describing clinical features and prognostic factors consist of retrospective studies from single institutions encompassing small patient populations with little statistical power. This is further compounded by inconsistent histologic inclusion criteria and definition of CHC across the studies, with many series including collision tumors and separate nodules of HCC and CC as CHC. Thus, it is not surprising that identification of clinical features and prognostic factors are not consistent or reproducible among the different studies. A clear profile of the patient demographic afflicted by this rare primary hepatic malignancy has remained vague and is highly dependent on geographic region.

Etiology and risk factors for these tumors may be common or differ in different regions in eastern and western series. This reflects variability in the prevalence of infectious agents such as hepatitis viruses and liver flukes, as well as the lifestyle and nutritional differences. Multiple studies have highlighted risk factors such as male gender, cirrhosis, hepatitis infection, family history of liver cancer, heavy alcohol consumption and diabetes mellitus[4,8-11,14-16]. The high male: Female ratio and prevalence of HBV in CHC patients in Asian countries are generally more similar to HCC compared to CC[15,16]. On the other hand, western studies have shown a less pronounced male predominance of CHC, paralleling with relatively low prevalence of HBV (15%-16.6%) and high prevalence of HCV[1,4,12,17]. Taken together, these findings suggest that geographical characteristics heavily influence clinical profiles of patients with CHC. This demonstrates that CHC is associated with overlapping clinical features of both HCC and CC. Some studies have reported that CHC has poor prognosis and more aggressive behavior in comparison to HCC and CC[18-20], which some authors attribute to increased lymph node involvement[8].

IMAGING CHARACTERISTICS AND PRE-OPERATIVE DIAGNOSIS

Historically, CHC has been an elusive and difficult pre-operative diagnosis. This is due to its heterogeneous imaging characteristics with overlapping features of both HCC and CC. The predominant histologic component within the tumor largely determines the predominant radiographic features. This is also true in tissue specimens, as sampling error (*e.g.*, sampling only the area of HCC or CC within a CHC) may also lead to an erroneous pre-operative diagnosis. Thus, the majority of CHC cases in the literature were initially misdiagnosed as either HCC or CC and the proper diagnosis was only reached in the surgical resection specimens. Correct pre-operative diagnosis is important, especially distinction from HCC, as it may determine different management strategy. In the United States the vast majority of HCCs are diagnosed based on characteristic radiological features alone without pathologic confirmation. HCC in selected patients is an indication for liver transplant, with excellent outcomes equivalent to non-neoplastic entities and 5-year survival > 70%[21]. Taking into account the scarcity of grafts available for transplantation and the poor prognosis associated with CHC, differentiation from HCC becomes paramount.

The characteristic features of HCC on contrast enhanced CT and MRI are arterial phase diffuse enhancement, portal venous washout, and an enhanced pseudocapsule on delayed imaging. The hallmark radiological findings of CC are arterial peripheral rim enhancement with progressive fibrous stroma central enhancement, dilation of the biliary system, and retraction of the capsule[22]. CHC may show all of these radiographic characteristics to varying degrees, making distinction from HCC and particularly from CC very challenging. Some authors have suggested that the presence of heterogeneous or overlapping imaging features should prompt an extended tissue biopsy from different appearing tumor areas to aid in this diagnostic

conundrum and mitigate sampling bias[22]. Another clue that should raise suspicion for CHC pre-operatively is discordant tumor markers. Generally, elevated alpha-fetoprotein (AFP) levels are associated with HCC, while elevated CA 19-9 levels are associated with CC. If a tumor shows characteristic imaging features of HCC, but is associated with elevated CA 19-9 levels, or if a tumor has characteristic CC imaging features and is associated with elevated AFP levels, or if both serum markers are elevated, biopsy for pathologic confirmation should be strongly considered.

HISTOGENESIS

The concept of cancer stem cells may explain the origin and progression of different kinds of cancers, and CHC is no exception. Although histogenesis of the CHC has been a topic of debate, three types of tumor origins have been hypothesized: (1) collision tumors; (2) de-differentiation or re-differentiation of a primary HCC into a biliary phenotype or vice versa; (3) derivation from bipotent HPC[5,23]. The first theory, collision tumor consisting of separate populations of HCC and CC occurring in the same liver without intimate relationship, does not qualify as CHC. De-differentiation or redifferentiation of HCC or CC into the other component is controversial, as some studies have shown differences in the clinical features, histology and molecular genetics of CHC and HCC/CC while others have supported this theory owing to existing similarities between CHC and HCC as well as CHC and CC.

The bipotent HPC is a stem cell that differentiates into both hepatocytes and bile duct epithelial cells, and is suspected to be the cancer stem cell responsible for CHC growth[5,23-26]. Theise *et al*[23] described four primary liver cancers with three different components including hepatocellular, cholangiocellular and a third component of small or undifferentiated cells (oval-like cells) with high N/C ratio, scant basophilic cytoplasm and nuclear pleomorphism. Oval cells (described in animal models) or intermediate cells (in humans) or stem cells can be found in regenerative nodules. These cells are located in canals of Hering[23,27] and can differentiate bidirectionally into hepatocytes and cholangiocytes, also called as bipotent progenitor cells[5,23,24]. There is morphological and immunohistochemical similarity between these oval-cell like progenitors and hepatoblasts (both positive for CK19 and Hep-Par1).

HPC and stem cell markers which have been studied in relation to CHC include CD133, CD90, CD44, epithelial cell adhesion molecule (EpCAM), nuclear cell adhesion molecule (NCAM/CD56), OV6, CD13, c-kit, YAP1, SALL4 and Delta-like 1 homolog (DLK-1)[5,28-33]. Kim *et al*[24] demonstrated that c-kit-positive HPCs have a potential to differentiate into both hepatocytes and cholangiocytes and are neoplastic counterparts of HPCs.

Evidence including identification of HPC-like cells merging with HCC and CC components, as well as shared expression of HPC markers in the different components, supports these cells as the origin for CHC[23,25]. Furthermore, inoculation of cells from a CHC cell line positive for the HPC marker EpCAM has been associated with development of CHC in mice[26]. HPC activation in non-tumor liver in CHC cases has been linked with recurrence and poor prognosis in CHC[34]. Microdissection has shown that both components share a single clonal background, which is consistent with the shared origin of both components deriving from HPCs[35].

The role of the HPC is reflected in the current World Health Organization (WHO) classification, which is subdivided into CHC, classical type and three subtypes of CHC with stem cell features[13]. In making this classification, the authors noted that it was uncertain whether biological differences existed between these subtypes, and determination of subtype relied mainly on histological and immunohistochemical features. HPC marker expression has been shown to varying degree in all stem cell subtypes and to a lesser degree in the classical subtype, while prominent in transitional areas of CHC[24,29-31]. Ikeda *et al*[30] showed that the stem cell markers DLK-1 and NCAM/CD56 were expressed most frequently in CHC with stem cell features, and were most frequently expressed in typical and cholangiocellular subtypes. Akiba *et al*[29] showed that stem cell markers CD133 and EpCAM were more often expressed in CHC with stem cell features compared to those with classical features. They further showed that among CHC subtypes with stem cell features, cholangiocellular subtype more often expressed CD133 and EpCAM in comparison to intermediate subtype (their study did not include sufficient cases of typical subtype for statistical analysis)[29]. Komuta *et al*[36] supported origin of cholangiolocellular carcinoma from HPCs that was initially a subtype of cholangiocarcinoma.

Molecular studies have shown that CHC shares some traits with HCC and others with CC, confirming its status as a distinct entity. Gene profiling of CC, HCC and CHC by microarray shows increased differential expression in CC *vs* HCC as compared to CC *vs* CHC, reflecting this concept[37]. Analysis of copy number changes in CC and HCC components of CHC showed concordance in the overall trend of gain or loss for several target genes although magnitude of copy number change differed. The copy number gains in the CC component were likely to be paired with a similar but not identical copy number gain in the HCC component of the tumor, with the same holding true for copy number losses. The specific genes most often amplified in this study were MYC, ADAMTSL4, TM4SF1 and CUL4A, which are each associated with HCC although CUL4A has also been associated with CC[38]. Similarly, comparative genomic hybridization showed specific chromosomal gains and losses similar to those of HCC[39]. This study also showed high prevalence of chromosomal imbalances similar to those seen in CC. Similarly, a high level of chromosomal instability, in addition to recurrent loss of heterozygosity at 3p and 14q is also noted[40].

Genome-wide transcriptional analysis of 20 CHC cases

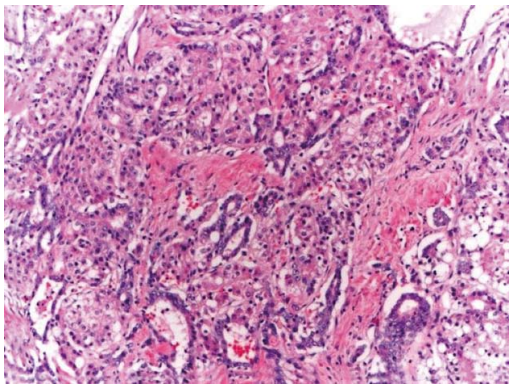

Figure 1 Representative picture classic type combined hepatocellular-cholangiocarcinoma, hematoxylin and eosin, 10 × - intermediate areas with both hepatocytic and chloangiocytic components.

showed that CHC clustered with CC and separately from HCC, with upregulated signaling pathways of TGFβ and Wnt similar to those seen in CC. The TGFβ pathway upregulated in CHC recalled the key role of fibrosis and extracellular matrix remodeling in CC, and the Wnt pathway signature was similar to that seen in biliary ductal morphogenesis. However, CHC also clustered with a subset of poorly differentiated HCC with progenitor cell features, as would be expected given the postulated HPC origin of CHC, while CHC showed repression of the transcription factor HNF4A associated with mature hepatocyte differentiation[41]. Likewise it is also shown that CHCs were clustered with CC by gene expression profiling[42]. A recent whole genome sequencing analysis showed that genome-wide substitution patterns in liver cancers of biliary phenotype (both CC and CHC) overlapped with those of HCC in cases associated with chronic viral hepatitis, while biliary cancers (mostly CC in this study) unrelated to chronic hepatitis differed from HCC[43]. TERT promoter mutations, for example, were common in CHC and in other hepatitis-related cancers. Carcinogenesis arising from HBV acts largely through the HBV X protein that promotes HPC tumorigenesis so it is possible that these tumors may share a similar pathogenesis[44].

HISTOLOGY

Classification

There are multiple classifications for CHC in the literature. Allen and Lisa[7] made the first histological classification for CHC in 1949. They described three subtypes. Type 1 consisted of discrete foci of HCC and CC. Type 2 had contiguous masses with features of both HCC and CC. Type 3 was described as a solitary mass comprising of both components. Goodman *et al*[45] in 1985 proposed another classification, also encompassing three subtypes: Type 1 or collision tumor with separate and colliding areas of HCC and CC in the same liver; type 2 or transitional tumor with transitional areas with intimate intermingling of two components with actual transition of HCC elements to CC elements in the same tumor; and type 3 or mucin producing fibrolamellar tumor. Allen and Lisa[7]'s type 3 and Goodman *et al*[45]'s type 2 has similar features to the current WHO criteria for CHC. The current edition of the WHO classification describes two main types of CHC: Classical type and CHC with stem cell features. The stem-cell features type is further divided into 3 subtypes: Typical subtype, intermediate cell subtype and cholangiolocellular subtype[13]. Representative images of each subtype highlighting histological features and immunohistochemical profile are shown in Figures 1-4.

Histopathology

WHO 2010 classification defines these tumors as histological demonstration of unequivocally differentiated hepatocellular and biliary components in the tumor with intermingling of the two components[13]. Collision tumor, which is a separate entity, consists of HCC and CC occurring in the same liver without intimate relationship and is not categorized as CHC. The definite diagnosis of CHC can be made by histology only along with use of IHC and special stains[5]. The diagnosis of CHC is a challenging diagnosis on core biopsy as it depends on the area sampled[46]. Hepatocytic differentiation is defined by bile production, Mallory-Denk bodies, alpha-1 antitrypsin globules and trabecular arrangement of tumor cells. The cholangiocarcinoma component is appreciated by mucin production, prominent desmoplastic stroma and glandular structures. Combined fibrolamellar HCC in combination with cholangiocellular component has also been described in the literature[45,47].

In the CHC classic type (Figure 1), both the hepatocytic and cholangiocarcinoma components are present and can vary from well to poorly differentiated[13] with intermediate areas demonstrating features of both the components. Hepatocytic component is usually represented by thickened trabeculae composed of polygonal cells with abundant granular eosinophilic cytoplasm and scant stroma while cholangiocarcinoma component has gland formation with low cuboidal/columnar cells and dense fibrotic stroma. HCC with pseudoglandular pattern and expression of CK7/CK19 is not classified as CHC[5].

The first subtype of CHC with stem cell features, the typical subtype (Figure 2A and B), is characterized by peripheral small cells with hyperchromatic nuclei and a high nuclear to cytoplasmic ratio with nests of mature appearing hepatocytes in the center. The intermediate cell subtype (Figure 3A and B) consists of tumor cells with intermediate features between hepatocytes and cholangiocytes. Kim *et al*[24] described these tumor cells as small and oval shaped with hyperchromatic nuclei and scant cytoplasm arranged in either trabeculae, solid nests or strands, present within a desmoplastic stroma. Well-formed glands are not seen but ill-formed gland like structures may be present. These tumors were initially termed as intermediate carcinomas (hepatocyte-cholangiocyte) as these had features that were intermediate between HCC and CC[13,24]. The cholangiolocellular subtype (Figure 4A and B) is characterized

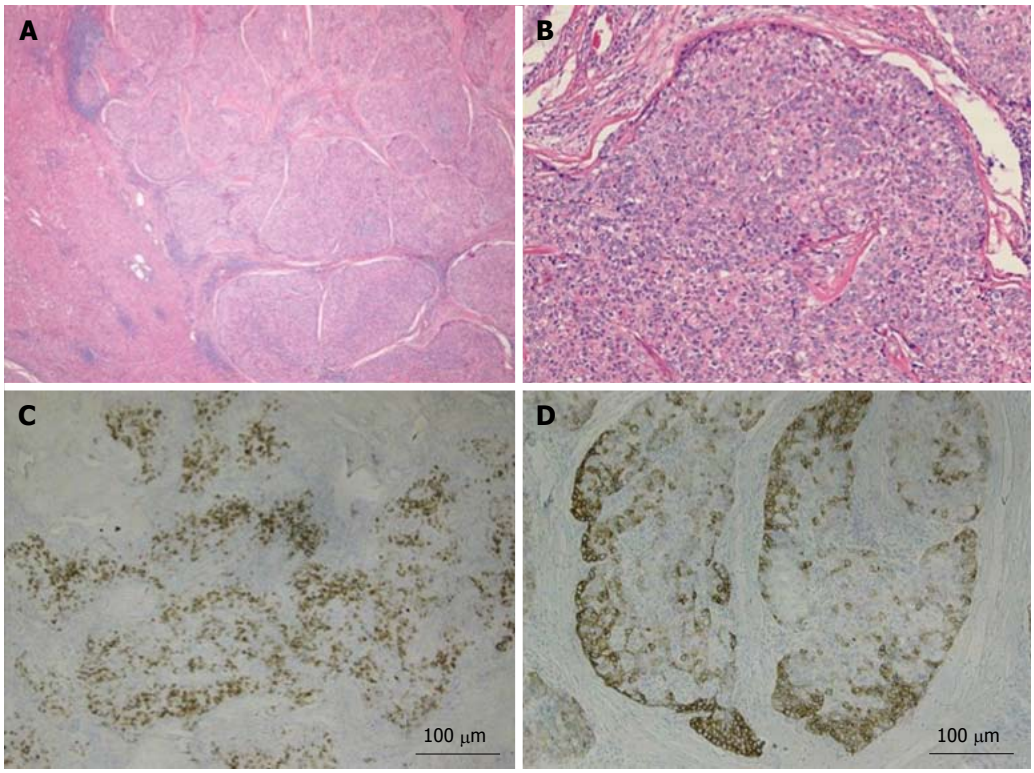

Figure 2 Combined hepatocellular-cholangiocarcinoma with stem cell features, typical subtype. A: H and E, 4 × - tumor nests present on the right side with non-neoplastic liver on the left side; B: H and E, 10 × - peripheral small cells with hyperchromatic nuclei with mature appearing hepatocytes in the center; C: CK7, 4 × - scattered expression of CK7 by tumor cells; D: CK19, 4 × - patchy staining of the tumor and highlighting small tumor cells located at the periphery. Tumor was also positive for Hep-Par1 (not shown). H and E: Hematoxylin and eosin.

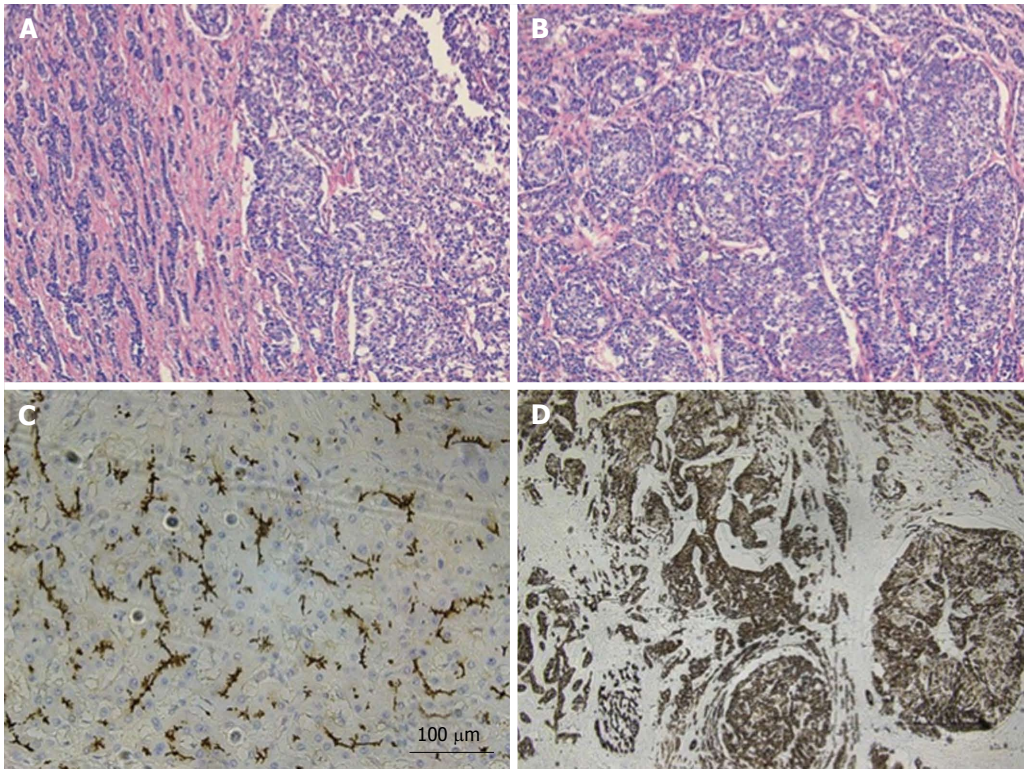

Figure 3 Combined hepatocellular-cholangiocarcinoma with stem cell features, intermediate subtype. A: H and E, 4 × - tumor is present in trabecular/nested pattern on the right side with ill-formed gland like structures seen on the left side; B: H and E, 10 × - tumor cells with intermediate features between hepatocytes and cholangiocytes; C: CD10, 10 × - tumor showing canalicular staining pattern for CD10 (hepatocytic marker); D: CK19, 4 × - tumor cells strongly and diffusely expressing CK19 (chlolangiocytic marker). Focal tumor cells were positive for Hep-Par1 and CD56 (not shown). H and E: Hematoxylin and eosin.

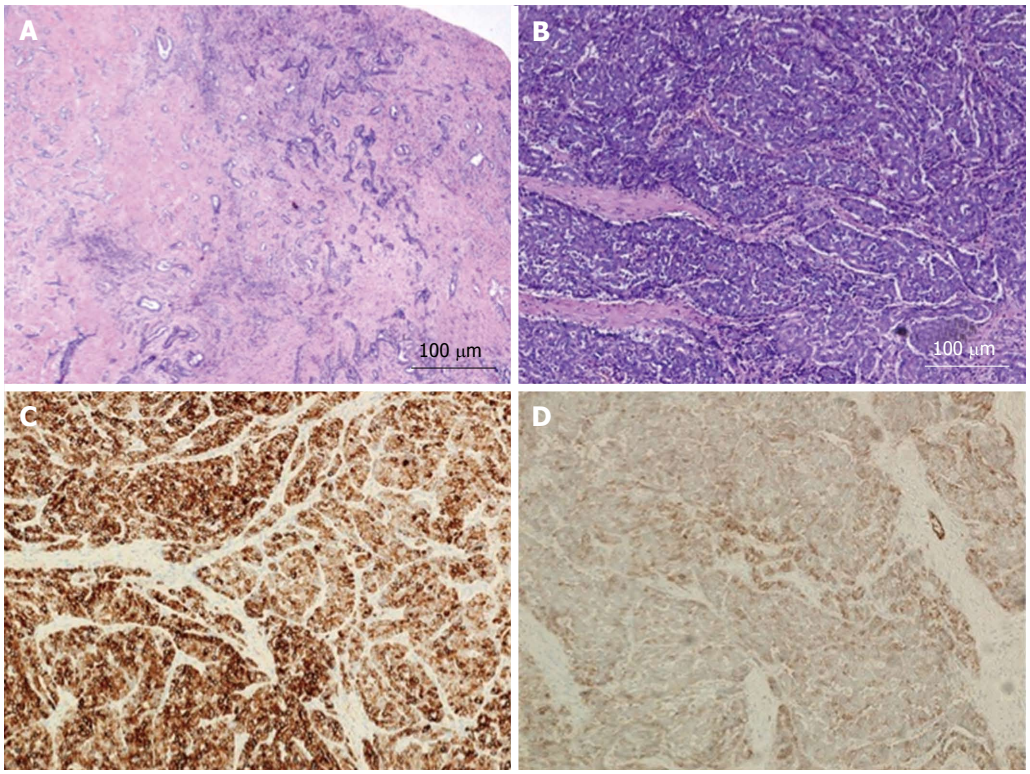

Figure 4 Combined hepatocellular-cholangiocarcinoma with stem cell features, cholangiocellular subtype. A: H and E, 4 × - tumor cells present in tubular, anastomosing (antler-like) pattern; B: H and E, 10 × - small hyperchromatic tumor cells with high nuclear to cytoplasmic ratio present within dense fibrous stroma; C: CK7, 10 × - tumor is diffusely positive for CK7; D: CD56, 10 × - CD56 staining the cholangiolocellular component as well as the tumor cells at the periphery of the trabeculae. The tumor was diffusely positive for CK19 while negative for HepPar-1 and AFP (not shown). H and E: Hematoxylin and eosin.

by small cells with a high nuclear to cytoplasmic ratio and hyperchromatic oval shaped nuclei arranged in a tubular, cord like, anastomosing pattern (also referred to as an "antler-like" pattern) within a dense fibrous stroma. No significant cellular atypia or evidence of mucin production is seen in both intermediate and cholangiolocellular subtypes[13]. Although there are distinct histological features described by the WHO to classify all these types/subtypes, there is no mention of percentage of stem cell area required to categorize CHC with stem cell features. If the stem cells predominate, the tumor is classified as one of the subtype of CHC with stem cell features depending on the histologic appearance. It appears that CHCs with less than 5% stem cell area have better prognosis than those with stem cell areas greater than 5%[30]. Sasaki *et al*[48] proposed certain clinico-pathological findings for CHC with stem cell features. The intermediate subtype was more commonly associated with female patients, larger tumor size, higher histological grade of HCC component, and less fibrosis while cholangiocellular subtype had smaller tumor size and lower histological grade of HCC. The typical subtype has less inflammation in comparison with the cholangiocellular subtype[48].

On cytology specimens, diagnosis of CHCs can be challenging[49,50]. Cell blocks and immunohistochemical stains can prove helpful in reaching a correct diagnosis of CHC. With CHC being an uncommon tumor, arriving at a diagnosis and classification of CHC can be difficult with histology alone.

Special and immunohistochemical stains

Immunohistochemical stains are required for demonstrating hepatic and biliary phenotypes. The hepatocellular component is positive for HepPar1, pCEA, CD10 and glypican[5]. The CC component shows expression of CK7, CK19 and mucin/mucicarmine although HCC components can also express CK7 and CK19[5]. Mucin is essential to demonstrate the biliary component[51]. CAM 5.2 and AE1 can also be useful to differentiate between HCC and CC component; HCC will be positive for CAM5.2 while AE1 will be positive in CC component[49]. CHC with stem cell features expresses stem cell markers including c-kit, NCAM, and EpCAM. Kim *et al*[32] demonstrated similar expression of these stem cell markers in CHC and HCC. Oval cell-like progenitors or small cells (or HPCs) described originally by Theise *et al*[23] are focally positive for AFP and alpha-antitrypsin while negative for HepPar1, c-Kit, vimentin and CHR-A. CD44, which is one of the cancer stem cell markers, is associated with poor prognosis and early recurrence in patients with CHC[32]. DLK1 is another marker of HPCs in adult liver[30]. Survival of patients with high expression of DLK1 is worse[30] suggesting that patients with CHC with stem cell features do worse in comparison to classical type CHC.

CHC with stem cell features, typical subytype stains positively with CK7, CK19 (Figure 2C and D), NCAM1/CD56, cKIT and/or EpCAM.

The intermediate subtype that is characterized by

intermediate cells (between hepatocytes and cholangiocytes), shows simultaneous expression of hepatocyte and biliary markers (Figure 3C and D). Akiba *et al*[52] demonstrated that intermediate cells stain better with Arginase-1 and CK8. Biliary phenotypes (CK7 and CK19) are more commonly positive in intermediate subtype than Hep-Par1[29].

The cholangiolocellular subtype is positive for CK19, and stem cell markers- cKIT, NCAM1/CD56 and EpCAM (Figure 4C and D).

TREATMENT OPTIONS

Currently, minor or major hepatic resection, with or without lymph node dissection, is the consensus recommended treatment for CHC. However, the absence of randomized prospective studies precludes the determination of optimal management strategies in these patients.

The role of liver transplant in the treatment of CHC remains to be defined. Most of the data on transplanted CHC patients comes from patients that were initially misdiagnosed with HCC or from incidentally discovered tumors in the explanted livers. The outcome data of this cohort is mixed, although outcomes are consistently worse when compared with HCC due to associated higher recurrence rates after transplant in CHC[53,54]. A recent study found no survival benefit of transplant over resection in CHC, with 3-year overall survival of 48% and 46% (P = 0.56), respectively[55]. Interestingly, a few studies have reported favorable outcomes in CHC patients undergoing transplantation. Chan *et al*[56] reported three cases, with two of them alive without recurrence 25 and 35 mo post-transplant. The other patient died of metastatic disease 16.5 mo after transplant. Another more recent publication found that transplanted CHC patients have better 5-year overall survival than those treated with major resection (41.1% *vs* 28.1%, P = 0.039)[57]. However, the 5-year overall survival rate of transplanted CHC patients was still much worse than transplanted HCC patients in both studies (41.1% *vs* 67%, P < 0.001 and 48% *vs* 78%, P = 0.01)[55,57]. These findings were further supported by Vilchez *et al*[12] in their UNOS database analysis. They found an overall 5-year survival of 40% in transplanted CHC patients, which was similar to CC (47%) but much worse than HCC (62%, P = 0.002). The authors concluded that currently, liver transplant is not a viable option for these patients. It should be emphasized that these results may be influenced by the fact that majority of CHC patients are misdiagnosed pre-operatively and managed as HCC. Improved initial diagnostic accuracy may allow for optimization and more aggressive neo-adjuvant therapies for CHC patients. This may in turn improve outcomes in this group and make transplant a viable option in the future.

Other treatment modalities that have been reported in CHC include transarterial chemoembolization, radiofrequency ablation, and percutaneous ethanol injection, but the data regarding the benefits of these interventions are inconclusive. The role of chemotherapy and radiotherapy remains to be defined.

PROGNOSIS

Despite the ambiguity and discordance of CHC clinical features reported in the literature, the studies consistently supported that CHC is associated with a more aggressive course and a worse prognosis than HCC. With regards to CC, the studies are varied with some showing CHC to have a worse[9] or similar prognosis while others show an improved outcome. Overall most studies showed that the prognosis of CHC is grim. Reported 3-year and 5-year overall survivals range from (37.3%, 47%, 46%, 12.4%, 10.5%, 34.6%) and (9.2%, 40%, 32%, 23.1%, 33%), respectively[8-12,55,57,58].

Adverse clinicopathologic prognostic factors associated with increased tumor recurrence and worse survival in various studies include large tumor size (> 5 cm), presence of satellite nodules, lymph node involvement, multifocality, vascular invasion, portal vein invasion, high tumor stage, high levels of CA 19-9, decreased capsule formation, free surgical resection margins < 2 cm, and GGT levels > 60 U/L[11,58,59]. However, many of these factors did not reach statistical significance on multivariate analysis. This may be due to the retrospective nature and low number of patients within each study due to the low incidence of CHC.

A recent population level study analyzed the SEER database for patients diagnosed with CHC between 1988-2009 in the United States. Of the 465 cases studied, they founded that a majority of CHC patients were male (66%) and Caucasian (74.9%), and a plurality were 66 years or older (44.3%)[57]. Clinical features of CHC patients fell in between those for HCC and CC, suggestive of the mixed characteristics associated with this tumor and in concordance with its bi-phenotypic differentiation. The authors found that CHC had a worse overall survival when compared to HCC, but better when compared with CC. The reported 5-year overall survival and disease specific survival for HCC, CHC, and CC were 11.7%, 10.5%, 5.7% and 21%, 17.8% and 11.9%, respectively (P < 0.001). The authors of the study concluded that CHC patients have intermediate clinical characteristics, demographics, and prognosis when compared with HCC and CC patients. Another study compared the post-resection outcomes of CHC, HCC and CC and found no significant differences in tumor recurrence rates, but did find worse survival rates when compared to HCC. However, results of this study are not representative of true CHCs as the authors used the Allen and Lisa[7] classification. The majority of patients in this study were classified as "combined type" or type 2 of the Allen and Lisa classification, which is not currently considered a true CHC according to the WHO classification[6].

The intermediate biological behavior of CHC has been further supported by other studies. Multiple series have found clinico-pathologic features in CHC that are commonly associated with either HCC or CC. These

include a high rate of lymph node metastasis, commonly associated with CC, and vascular invasion and portal vein invasion, commonly associated with HCC. Reported rates of lymph node metastasis in CHC cases are as high as 42%[8]. This may be explained by the biphenotypic nature of these malignancies[9].

In summary, CHC is a rare tumor with bad prognosis and overlapping clinical and radiological features with HCC and CC. More studies are required to adequately define its histogenesis including molecular genetics of this tumor.

ACKNOWLEDGMENTS

The authors wish to thank Hongfa Zhu, MD, PhD, at Mount Sinai Hospital, New York, United States, for providing the microphotographs of classic type combined hepatocellular-cholangiocarcinoma.

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P- Reviewer: Tomizawa M, Yoshida K **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Hepatol 2017 February 28; 9(6): 310-317

DOI: 10.4254/wjh.v9.i6.310

ORIGINAL ARTICLE

Retrospective Study

Fibrosis assessment using FibroMeter combined to first generation tests in hepatitis C

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Author contributions: All authors equally contributed to the design of the work, critical revision of important contents of the manuscript and final approval of the version to be published; Chindamo MC conception of the study, acquisition and interpretation of data, article writing; Boursier J, de Mello Perez R, Villela-Nogueira CA analyses and interpretation of data; Luiz RR revision of statistical analysis; Fouchard-Hubert I, Calès P and Coelho HSM critical revision related to important content of the manuscript; Pannain VLN histopathological evaluation of liver biopsies samples; de Araújo Neto JM acquisition of data and liver biopsy procedure.

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

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: September 25, 2016
Peer-review started: September 27, 2016
First decision: November 22, 2016
Revised: December 15, 2016
Accepted: February 8, 2017
Article in press: February 13, 2017
Published online: February 28, 2017

Abstract

AIM

To evaluate the performance of FibroMeter^{Virus3G} combined to the first generation tests aspartate aminotransferase-to-platelet ratio index (APRI) or Forns index

to assess significant fibrosis in chronic hepatitis C (CHC).

METHODS

First generation tests APRI or Forns were initially applied in a derivation population from Rio de Janeiro in Brazil considering cut-offs previously reported in the literature to evaluate significant fibrosis. FibroMeter^{Virus3G} was sequentially applied to unclassified cases from APRI or Forns. Accuracy of non-invasive combination of tests, APRI plus FibroMeter^{Virus3G} and Forns plus FibroMeter^{Virus3G} was evaluated in the Brazilian derivation population. APRI plus FibroMeter^{Virus3G} combination was validated in a population of CHC patients from Angers in France. All patients were submitted to liver biopsy staged according to METAVIR score by experienced hepatopathologists. Significant fibrosis was considered as METAVIR F $\geqslant$ 2. The fibrosis stage classification was used as the reference for accuracy evaluation of non-invasive combination of tests. Blood samples for the calculation of serum tests were collected on the same day of biopsy procedure or within a maximum 3 mo interval and stored at -70 ℃.

RESULTS

Seven hundred and sixty CHC patients were included (222 in the derivation population and 538 in the validation group). In the derivation population, the FibroMeter^{Virus3G} AUROC was similar to APRI AUROC (0.855 *vs* 0.815, P = 0.06) but higher than Forns AUROC (0.769, P < 0.001). The best FibroMeter^{Virus3G} cut-off to discriminate significant fibrosis was 0.61 (80% diagnostic accuracy; 75% in the validation population, P = 0.134). The sequential combination of APRI or Forns with FibroMeter^{Virus3G} in derivation population presented similar performance compared to FibroMeter^{Virus3G} used alone (79% *vs* 78% *vs* 80%, respectively, P = 0.791). Unclassified cases of significant fibrosis after applying APRI and Forns corresponded to 49% and 54%, respectively, of the total sample. However, the combination of APRI or Forns with FibroMeter^{Virus3G} allowed 73% and 77%, respectively, of these unclassified cases to be correctly evaluated. Moreover, this combination resulted in a reduction of FibroMeter^{Virus3G} requirement in approximately 50% of the entire sample. The stepwise combination of APRI and FibroMeter^{Virus3G} applied to the validation population correctly identified 74% of patients with severe fibrosis (F $\geqslant$ 3).

CONCLUSION

The stepwise combination of APRI or Forns with FibroMeter^{Virus3G} may represent an accurate lower cost alternative when evaluating significant fibrosis, with no need for liver biopsy.

Key words: Chronic hepatitis C; Fibrosis; Liver biopsy; Non-invasive methods; FibroMeter^{Virus3G}; Combination algorithms

Core tip: Liver fibrosis assessment still poses a challenge when prioritizing hepatitis C treatment due to logistical and financial barriers in the use of direct acting antiviral drugs. We introduced a new stepwise combination of first generation fibrosis tests - aminotransferase-to-platelet ratio index (APRI) and Forns-followed by FibroMeter^{Virus3G} whenever results remained unclassified after first generation tests to identify significant fibrosis. This combination presented similar accuracy to FibroMeter^{Virus3G} used as the only test, reduced APRI and Forns grey zone, and spared FibroMeter^{Virus3G} requirement in 50% of cases. This approach represents a lower-cost alternative to assess fibrosis, with no need for liver biopsy.

Chindamo MC, Boursier J, Luiz RR, Fouchard-Hubert I, Pannain VLN, de Araújo Neto JM, Coelho HSM, de Mello Perez R, Calès P, Villela-Nogueira CA. Fibrosis assessment using FibroMeter combined to first generation tests in hepatitis C. *World J Hepatol* 2017; 9(6): 310-317 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i6/310.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i6.310

INTRODUCTION

Fibrosis staging in chronic hepatitis C (CHC) has evolved in recent years with the introduction of blood tests for liver fibrosis as well as physical methods such as elastometry. Although liver biopsy has been classically considered the standard tool to evaluate fibrosis, it presents well-known inconveniences[1-3] and limitations[4-7] which make its use to assess fibrosis staging controversial amongst various authors[8-11]. However, even when considering the recent advances in CHC therapy, the diagnosis of significant fibrosis still represents a challenge to define which patients should have priority in treatment, mainly in resource limited countries. Thus, the development and improvement of alternative methods to identify candidates for an early treatment or intensive fibrosis monitoring is still recommended[11]. Most of the commonly used first generation non-invasive tests such as aspartate aminotransferase-to-platelet ratio index (APRI)[12], FIB-4[13] and Forns index[14] have been constructed and evaluated as binary diagnosis tools aiming to predict or exclude significant fibrosis, advanced fibrosis or cirrhosis at specific cut-offs. Although they are all free of charge, easily accessible and well validated for CHC, these non-invasive tests are limited to classify all patients[12-14].

The interest in detailed fibrosis class classification for non-invasive tests of fibrosis has recently grown[15-18], representing a more comprehensive and sophisticated approach to assess liver fibrosis. In this line, FibroMeters are a group of blood tests providing classifications intended to evaluate liver fibrosis in chronic viral hepatitis, alcoholic liver disease and non-alcoholic fatty liver disease[19-21]. FibroMeter dedicated for viral aetiology has recently evolved from FibroMeter^{Virus2G}[20] to a less costly

hyaluronic acid free test FibroMeter^{Virus3G}[21], which discriminates seven different fibrosis classes. FibroMeters provide scores ranging from 0 to 1 which are correlated with METAVIR staging system[22]. Although this new non-invasive test represents a better strategy to evaluate fibrosis in CHC, it may signify an economic burden hindering easy access mainly in developing countries. Thus, in order to identify patients with significant fibrosis and optimize costs, we evaluated the performance of a stepwise combination using APRI and Forns followed by FibroMeter^{Virus3G} in cases whose results remained unclassified after use of these first generation tests, always considering liver biopsy as reference.

MATERIALS AND METHODS

Patients

A cross-sectional study with prospective inclusion of compensated CHC patients submitted to percutaneous liver biopsy was performed at the Federal University of Rio de Janeiro, Brazil, as part of a pre-treatment routine evaluation. This group represented the derivation population of the study. Patients with concomitant human immunodeficiency virus infection, hepatitis B virus, alcohol abuse, metabolic, autoimmune or biliary diseases, liver transplantation or those who had previously undergone antiviral treatment were excluded. The validation population was composed by an independent cohort of CHC patients from Angers in France, who fulfilled the same inclusion and exclusion criteria. All patients signed an informed consent form and the study was approved by the Ethics Committee of both Institutions.

Liver biopsy

In the derivation population, all consecutive biopsies were guided by ultrasonography using a 14 or 16 G disposable Tru Cut needle (Surecutw, TSK Laboratory, Akasaka, Japan) obtaining a maximum length of 20 mm for each pass. In validation population, Menghini needle was used. Samples were considered inappropriate when length presented < 10 mm or contained < 6 portal tracts. Serial sections 5 μm thick were cut from each paraffin block and routinely stained with hematoxylin and eosin, periodic acid-Schiff diastase, reticulin, Masson Trichrome and Picrosirius red. Liver fibrosis was staged from F0 to F4 according to METAVIR staging system[22] by an experienced hepatopathologist in each centre, blinded for biological and clinical results. METAVIR F ⩾ 2 was considered significant fibrosis. This fibrosis stage classification was used as the reference for accuracy calculation of non-invasive tests.

Blood fibrosis tests

Serum tests of fibrosis were performed with blood samples collected from fasting patients on the same day of biopsy procedure or within a maximum 3 mo interval and stored at -70 ℃. APRI and Forns were selected due to their free accessibility and their good accuracy to discriminate significant fibrosis. The values of APRI[12], Forns[14] and FibroMeter^{Virus3G}[21] tests were calculated according to the original studies: (1) APRI = AST level/ULN)/platelet counts (10^9/L) × 100; (2) Forns index = 7.811 - 3.131 × ln(platelet count) + 0.781 × ln(GGT) + 3.467 × ln(age) - 0.014 × cholesterol; and (3) FibroMeter^{Virus3G} = patented formula including the biologic parameters prothrombin index, AST, ALT, Urea, GGT, alpha-2-macroglobulin and platelets. The calculations were performed by Echosens (Paris, France) laboratory.

Statistical analysis

Quantitative variables were expressed as mean ± SD values or proportions. Student's *t* test or ANOVA were used to compare continuous variables, and McNemar χ^2 test to compare paired proportions. The performance of APRI, Forns and FibroMeter^{Virus3G} to predict significant fibrosis was expressed by the AUROC. In order to evaluate the applicability of FibroMeter^{Virus3G}, considering an economic approach, we determined the best cut-off point of FibroMeter^{Virus3G} to discriminate significant fibrosis using the Youden index that maximizes sensitivity and specificity. The performance of APRI and Forns were separately assessed to exclude or predict significant fibrosis respectively, at cut-offs already established in literature as follows: APRI: cut-off of 0.5 and 1.5[12]; Forns: cut-off of 4.2 and 6.9[14]. FibroMeter^{Virus3G} was then sequentially tested in a stepwise use, considering the results allocated in unclassified APRI values (between 0.5 and 1.5) and Forns values (between 4.2 and 6.9), using histology as reference. The overall accuracy of the aforementioned approaches was calculated considering the sum of true positive and negative cases as a proportion of the total. Sensitivity, specificity, predictive positive (PPV) and negative values (NPV) of first generation tests and sequential use of APRI + FibroMeter^{Virus3G} and Forns + FibroMeter^{Virus3G} were evaluated.

Data were analyzed using the statistics software programs SPSS version 20 for Windows and MedCalc version 14.8.1. A *P* value < 0.05 was considered statistically significant.

RESULTS

Patients' characteristics

The initial series of liver specimens in Rio population consisted of 231 biopsies, of which four (1.7%) were excluded, due to evidence of other hepatic diseases associated with hepatitis C, and five (2.0%) were considered inadequate for analysis. Thus, the final Rio population included 222 biopsies of CHC patients. The validation cohort was represented by 538 French CHC patients. Excepted for gender, demographic characteristics, laboratory data and histological features of derivation and validation cohort were similar and described in Table 1.

The mean length of liver fragments was 24 ± 5 mm in derivation population *vs* 22 ± 10 mm in validation cohort (*P* = 0.315). The prevalence of significant fibrosis

Table 1 Demographic, laboratory and histological features of patients with chronic hepatitis C of both populations

Variables	All (n = 760)	Rio population (n = 222)	Angers population (n = 538)
Females, n (%)	401 (54)	134 (60)	179 (35)
Age (yr, mean ± SD)	46 ± 11	51 ± 11	46 ± 11
AST, IU/L (mean ± SD)	67 ± 58	68 ± 52	66 ± 60
ALT, IU/L (mean ± SD)	101 ± 84	100 ± 67	101 ± 90
Platelet count, 10^6/mm^3 (mean ± SD)	208 ± 68	203 ± 63	210 ± 70
GGT, IU/L (mean ± SD)	144 ± 171	124 ± 135	110 ± 184
APRI	1.0 ± 1.2	0.9 ± 1.2	1.1 ± 1.3
Forns		6.0 ± 1.9	
FibroMeter^{Virus3G}	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3
Biopsy length (mm, mean ± SD)	22 ± 9	24 ± 5	22 ± 10
METAVIR stage, n (%)			
F0	22 (3)	5 (2)	17 (3)
F1	283 (37)	91 (41)	192 (36)
F2	215 (28)	55 (25)	160 (30)
F3	145 (19)	54 (24)	91 (17)
F4	95 (13)	17 (8)	78 (14)

AST: Aspartate transaminase; ALT: Alanine aminotransferase; GGT: Glutamyl transpeptidase; APRI: Aspartate transaminase to platelet ratio index.

Table 2 Rates of correct FibroMeter^{Virus3G} stage classification in comparison to liver biopsy in the Rio population

FibroMeter stage	METAVIR fibrosis classification						Correct fibrosis class classification according to liver biopsy (%)
	0	1	2	3	4	n	
F0/F1	0	10	0	0	0	10	10/10 = 100
F1	0	6	1	0	0	7	6/7 = 86
F1[F1-F2]	3	21	4	2	0	30	25/30 = 83
F2[F1-F2]	1	26	10	4	1	42	36/42 = 86
F2[F1-F3]	1	15	12	10	0	38	37/38 = 97
F2/F3	0	9	17	12	3	41	29/41 = 71
F3[F2-F4]	0	4	8	22	6	40	36/40 = 90
F4[F3-F4]	0	0	3	4	7	14	11/14 = 79
Total	5	91	55	54	17	222	190/222 = 86

was 57% *vs* 61% (P = 0.399) comparing derivation population to validation cohort, respectively, considering liver biopsy as reference.

Rio (derivation) population

FibroMeter^{Virus3G} applied as a class classification test presented an overall rate of correct diagnosis of 86% considering any of the results reported in FibroMeter^{Virus3G} stage class in accordance with fibrosis scored by METAVIR (Table 2). The AUROCs of both tests comparing METAVIR F0-F1 *vs* F2-F4 were similar between FibroMeter^{Virus3G} and APRI [0.855 (0.801-0.898) *vs* 0.815 (0.757-0.864)] but the difference was at the limit of significance (P = 0.06). The FibroMeter^{Virus3G} AUROC was higher in comparison to Forns AUROC [0.855 (0.801-0.898) *vs* 0.769 (0.708-0.823); P < 0.001]. The best cut-off that predicted significant fibrosis was 0.61, demonstrating an accuracy of 80% compared to liver biopsy. The PPV, NPV and accuracy of all tests were shown in Table 3. The stepwise combination of APRI or Forns followed by FibroMeter^{Virus3G} provided an overall accuracy of 79% (Figure 1) and 78% (Figure 2), respectively (P = 0.791), when identifying significant fibrosis. It also enabled 49% (n = 109) and 54% (n = 120) of the total sample, representing the grey zone of APRI and Forns for significant fibrosis, to be correctly classified in 73% and 77% of cases, respectively.

Thus, diagnostic accuracy did not differ comparing the use of FibroMeter^{Virus3G} test alone or combined with APRI or Forns (80% *vs* 79% *vs* 78%, respectively, P = 0.79), but represented a lower cost alternative since this procedure led to a 51% reduction of FibroMeter^{Virus3G} test requirement using APRI + FibroMeter^{Virus3G} and 46% reduction using Forns + FibroMeter^{Virus3G} (P = 0.25). Rates of well classified patients applying the algorithm APRI + FibroMeter^{Virus3G}, using METAVIR score as reference, were as follows: 100% for F0, 81% for F1, 67% for F2, 80% for F3 and 94% for F4.

Angers (validation) population

The cut-off of 0.61 found in derivation population presented an overall accuracy of 75% when discriminating significant fibrosis in the validation cohort in comparison to 80% in derivation population (P = 0.13), considering histology as reference. The diagnostic accuracy of APRI + FibroMeter^{Virus3G} combination in validation population to detect significant fibrosis and advanced fibrosis was respectively 69% and 74%. Rates of correct classification of this algorithm according to METAVIR score were as follows: 100% for F0, 88% for F1, 39% for F2, 64% for F3 and 85% for F4. When analysing the subgroup of false negative patients in this population we observed that 69% are represented by F2, 23% are F3, and only

Table 3 Performance of aspartate transaminase to platelet ratio index, Forns, FibroMeter^{Virus3G} and combination algorithm to discriminate significant fibrosis (F0-F1 *vs* F2-F4) in derivation and validation population

Serum fibrosis tests	AUROC (95%CI)	Cut-off value	Se (%)	Sp (%)	PPV (%)	NPV (%)	OA (%)
Derivation population (n = 222)							
FM score	0.855 (0.801-0.898)	0.61	79	81	85	74	80
APRI	0.815 (0.757-0.864)	0.5[1]	87	53	71	76	72
		1.5[2]	35	98	96	53	62
Forns	0.769 (0.708-0.823)	4.2[3]	94	31	64	79	66
		6.9[4]	41	87	81	53	61
Apri + FM			76	82	85	72	79
Forns + FM			81	75	81	75	78
Validation population (n = 538)							
FM score	0.854 (0.821-0.888)	0.61	67	87	89	63	75
Apri + FM			57	88	87	57	69

[1]APRI ⩽ 0.5 exclude significant fibrosis; [2]APRI > 1.5 predict significant fibrosis; [3]Forns ⩽ 4.2 exclude significant fibrosis; [4]Forns > 6.9 predict significant fibrosis. FM: FibroMeterVirus3G; AUROC: Area under ROC curve; Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; OA: Overall accuracy; APRI: Aspartate transaminase to platelet ratio index.

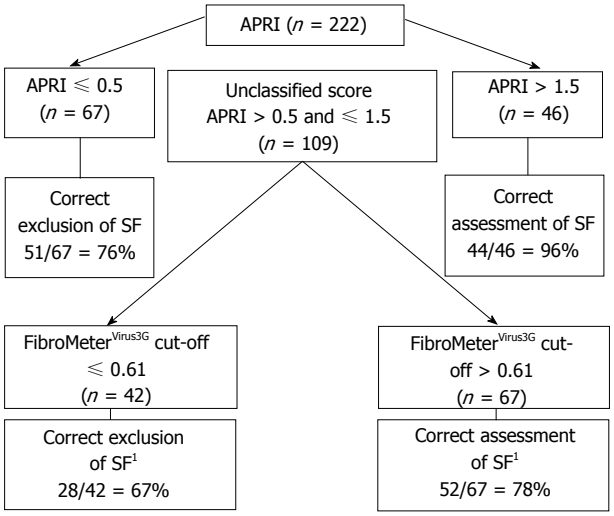

Figure 1 Sequential algorithm of aminotransferase-to-platelet ratio index + FibroMeter^{Virus3G} to predict significant fibrosis, in the Rio population. Accuracy of sequential use of aminotransferase-to-platelet ratio index (APRI) + FibroMeter^{Virus3G} was determined considering: Number of correct assessments of SF (96) + number of correct exclusions of SF(79)/total of liver biopsies (222) = 175/222 = 79%. [1]Considering liver biopsy as reference.

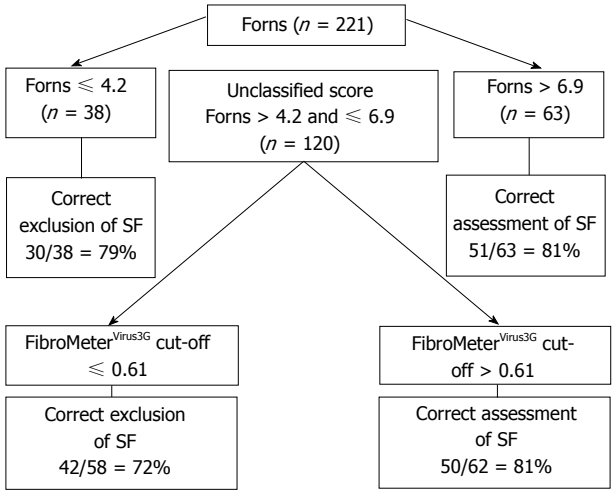

Figure 2 Sequential algorithm of Forns + FibroMeter^{Virus3G} to predict significant fibrosis, in the Rio population. Accuracy of sequential use of Forns + FibroMeter^{Virus3G} was determined considering: Number of correct assessments of SF [(101) + number of correct exclusions of SF (72)]/total of liver biopsies (221) = 173/221 = 78%.

8% are cirrhotic. The PPV, NPV and accuracy of APRI + FibroMeter^{Virus3G} combination on validation population are shown in Table 3.

DISCUSSION

Although new treatment regimens with very high rates of sustained virologic response are now available to treat hepatitis C patients, the logistical and financial barriers to treat all infected patients represent an important limitation, even in resource-replete countries[23]. Thus, it is necessary to determine an optimal and practical approach to prioritize these highly efficacious, but extremely costly therapies, for a selected population at risk of disease progression or for those who require immediate therapy. There is a consensus that non-invasive evaluation of liver fibrosis may be useful to complement or even replace liver biopsy in CHC owing to its low risk of complications and good accuracy. However, non-invasive tests also present some limitations related to cost, fibrosis discrimination and accuracy.

The present study originally evaluated the combination of a more robust patented fibrosis test, FibroMeter^{Virus3G}, with low cost first generation tests to enhance its applicability in the clinical practice. Although APRI and Forns are well established non-invasive tests to assess fibrosis in CHC, their main limitation is that when used alone, almost half of the patients could not be classified according to the possibility of presenting or not significant fibrosis. Thus, using a second test to improve discrimination would enhance the accuracy of these results in order to diagnose significant fibrosis. In the present study, the use of first generation tests combined with FibroMeter^{Virus3G} demonstrated to be a lower cost strategy since it reduced

FibroMeter^{Virus3G} requirement, without loss of accuracy, eliminating the requirement for liver biopsy procedure.

When analyzed as a class classification test, FibroMeter^{Virus3G} presented an overall accuracy of 86% similar to the rate of 87% described in FibroMeter^{Virus3G} original report[21]. The AUROC for significant fibrosis was 0.85, comparable to previous reports ranging from 0.84 to 0.86[16,17,21]. Analysing under a practical point of view, and considering significant fibrosis as the criteria to treat or not the patient, we presented important results that may help hepatologists on clinical decision-making. We found a cut-off of 0.61 as the best numeric value to discriminate significant fibrosis for FibroMeter^{Virus3G}, which is close to the value displayed in the manufacturer's bar graph of FibroMeter^{Virus3G} report of 0.63, representing the transition from F1 to F2 METAVIR stage[24]. The cut-off of 0.61 presented similar performance in comparison to manufacturer's cut-off of 0.63 in the French validation cohort.

FibroMeter^{Virus3G} applied as a numeric score also enables its association to other scores. Sebastiani *et al*[25] provided a sequential algorithm for fibrosis evaluation (SAFE) biopsy combining APRI and Fibrotest, another biomarker based on fibrosis class classification, resulting in a 46% reduction of liver biopsy requirement to identify significant fibrosis. In a more recent study performed with 1785 CHC patients, Boursier *et al*[26] reported that the diagnosis of significant fibrosis using SAFE still required liver biopsy in 64% of the cases. To our knowledge, to date the stepwise combination of APRI or Forns with FibroMeter^{Virus3G} has never been evaluated. The sequential algorithm of either APRI or Forns combined with FibroMeter^{Virus3G} represents a lower cost method with similar accuracy when compared to the isolated use of FibroMeter^{Virus3G} test. This represents an advantage when reducing the number of unclassified APRI and Forns patients in the grey zone, without the need for liver biopsy. This is a useful and alternative approach in countries with less financial resources, considering the easy applicability and low cost of APRI and Forns for significant fibrosis. This procedure may represent a more comprehensive proposal to apply these non-invasive tests in the clinical setting.

Some limitations may be discussed in this study. The prevalence of significant fibrosis in our population was higher than the prevalence reported in a meta-analysis including more than 30000 CHC patients which showed a rate of 48% of significant fibrosis histologically assessed[27]. Our prevalence is greatly influenced by the fact that this study was carried out in a tertiary-care setting. Another limitation is that derivation and validation populations came from different racial ethnic backgrounds. Nevertheless, most patients included in the derivation population were Caucasians and both populations shared similar characteristics regarding laboratorial results and distribution of significant fibrosis.

The diagnostic accuracy of the APRI and FibroMeter^{Virus3G} combination in validation population was 69%. A decrease in diagnostic accuracy is usually expected in the validation population when compared to the derivation population; however some points need to be emphasized. The PPV of the algorithm APRI and FibroMeter^{Virus3G} in the validation population was high (87%). Consequently the algorithm enabled the selection of a subset of patients where very few false positive results were found. In other words, this algorithm allowed treatment to be given to those patients who really required antiviral drugs. The low sensitivity (57%) remains a limitation, since a considerable number of patients who need to be treated will not be correctly identified by the algorithm. Nevertheless, when analysing the subgroup of false negative patients in the validation population, we observed that the majority (69%) were represented by F2 and only 8% were cirrhotic. In the whole validation population, most of the F0 patients (100%), the F1 patients (88%) and the F4 (85%) were well classified by the algorithm, as well as two thirds of the F3. Therefore, the algorithm identifies the more severe patients (F3 and F4) while most of the misclassification concerns the F2 stage. In the derivation population, even though the accuracy of the algorithm was found to be better, the worst result was also observed in F2 stage. And lastly, even when considering a gold standard such as liver biopsy, there is a considerable misclassification of F2 patients[28]. In low income countries, where therapy is offered only to patients with advanced fibrosis, a close follow-up is required until these untreated patients fulfil the criteria for direct antiviral therapy. We may consider following the "missed" patients by reapplying the algorithm to better identify when patients change their fibrosis stage and require treatment[29]. Since most misclassified patients present F2 fibrosis stage, there is sufficient time before they become cirrhotic.

Our findings demonstrated that the association of a more robust non-invasive marker of fibrosis such as FibroMeter^{Virus3G} and first generation tests may represent a useful alternative for fibrosis staging in CHC without loss of accuracy and without the need for liver biopsy. This might be an attractive approach mainly in limited resource countries.

ACKNOWLEDGMENTS

Fibrometer tests results were granted by Echosens, Paris, Fr.

COMMENTS

Background

Despite recent advances in chronic hepatitis C therapy, diagnosis of significant fibrosis still represents a challenge when defining which patients should have priority in treatment, mainly in resource limited countries. Although liver biopsy has been classically considered the standard tool to evaluate fibrosis, it presents well-known inconveniences and limitations which make its use controversial amongst various authors. Thus, the development and improvement of alternative methods to identify candidates for an early treatment or intensive fibrosis monitoring is still recommended. First generation non-invasive tests such as aminotransferase-to-platelet ratio index (APRI), FIB4 and Forns are free of charge, easily accessible and well validated for chronic hepatitis C, however are limited when classifying all patients. Therefore, in order to increase assessment availability for patients

with significant fibrosis, the authors evaluated the performance of a stepwise combination of first generation tests of fibrosis - APRI and Forns - followed by FibroMeter^{Virus3G}, a more robust test, whenever results remained unclassified after first generation tests, always using liver biopsy as reference. This proposed combination allows costs to be optimized with no loss of accuracy and no need of liver biopsy, thus representing a favorable economic approach in resource limited areas.

Research frontiers

This study introduces alternative approaches to evaluate significant fibrosis in chronic hepatitis C using a stepwise algorithm with first generation tests and FibroMeter^{Virus3G} both to improve clinical decision-making and reduce costs. The authors considered this topic of great interest for clinicians and hepatologists in the daily practice management of chronic hepatitis C.

Innovations and breakthroughs

The present study demonstrated that the association of a more robust non-invasive marker of fibrosis such as FibroMeter^{Virus3G} and first generation tests such as APRI and Forns may represent a useful alternative for fibrosis staging in chronic hepatitis C. This might be an attractive non-invasive approach to evaluate liver fibrosis and to optimize the access to potent but expensive direct-acting antiviral agents. To our knowledge, to date the stepwise combination of APRI or Forns with FibroMeter^{Virus3G} has never been evaluated.

Applications

The sequential algorithm of either APRI or Forns combined with FibroMeter^{Virus3G} represents an alternative approach to recognize and prioritize patients with chronic hepatitis C to antiviral therapy. It reduces the number of unclassified APRI and Forns patients allocated in the grey zone and reduces the total FibroMeter^{Virus3G} requirement in 50%, representing an alternative approach in countries with less financial resources, without loss of accuracy, eliminating the requirement for liver biopsy procedure. This procedure may represent a more comprehensive proposal to apply these non-invasive tests in the clinical setting.

Terminology

FibroMeter^{Virus3G} is a non-invasive test to evaluate liver fibrosis in chronic hepatitis C represented by a patented formula including the biologic parameters prothrombin index, AST, ALT, Urea, GGT, alpha-2-macroglobulin and platelets.

Peer-review

It is a carefully planed study, it takes in to consideration the Liver biopsy and the Fibrometer Virus2 and Virus3 and makes a head to head comparison of the three, as to discover the safety profile and the accuracy when it comes to clinical use especially for the group patients with cirhossis and to evaluate the change in fibrosis stage in pts with cirrhosis that are unable to undergo liver biopsy with a non-invasive procedure. The Fibre meter requires more wide use in the clinical setting as to prove its self as a reliable and non-invasive method of estimating the fibrosis stage.

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P- Reviewer: Chiang TA, Samy Kohla MA, Savopoulos CG
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 February 28; 9(6): 318-325

DOI: 10.4254/wjh.v9.i6.318 ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Thrombocytopenia in cirrhosis: Impact of fibrinogen on bleeding risk

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Author contributions: Thakrar SV and Mallett SV contributed equally to this work in designed and coordinated the research and writing the manuscript.

Institutional review board statement: This work was reviewed and approved by the Royal Free London Research and Development department and was deemed not to require ethics approval.

Informed consent statement: All data retrieved from our transplant database was anonymised. The data has been previously collected (prior to the study commencing), and was collected as part of standard care. Only those persons involved in clinical care had access to our database. No patient interventions were performed as part of this study. As a result, it was deemed unnecessary to require patient consent.

Conflict-of-interest statement: There are no conflicts of interest to declare.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at s.thakrar1@nhs.net. Participant onsent was not obtained but the presented data are anonymised and risk of identification is low.

Manuscript source: Invited manuscript

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Received: July 27, 2016
Peer-review started: July 29, 2016
First decision: September 28, 2016
Revised: December 23, 2016
Accepted: January 11, 2017
Article in press: January 14, 2017
Published online: February 28, 2017

Abstract

AIM

To investigate the relationship between baseline platelet count, clauss fibrinogen, maximum amplitude (MA) on thromboelastography, and blood loss in orthotopic liver transplantation (OLT).

METHODS

A retrospective analysis of our OLT Database (2006-2015) was performed. Baseline haematological indices and intraoperative blood transfusion requirements, as a combination of cell salvage return and estimation of 300 mls/unit of allogenic blood, was noted as a surrogate for intraoperative bleeding. Two groups: Excessive transfusion (> 1200 mL returned) and No excessive transfusion (< 1200 mL returned) were analysed. All data analyses were conducted using IBM SPSS Statistics version 23.

RESULTS

Of 322 OLT patients, 77 were excluded due to fulminant disease; redo transplant or baseline haemoglobin (Hb) of < 80 g/L. One hundred and fourteen (46.3%) were classified into the excessive transfusion group, 132 (53.7%) in the no excessive transfusion group. Mean age and gender distribution were similar in both groups.

Baseline Hb ($P \leqslant 0.001$), platelet count ($P = 0.005$), clauss fibrinogen ($P = 0.004$) and heparinase MA ($P = 0.001$) were all statistically significantly different. Univariate logistic regression with a cut-off of platelets < 50 × 10^9/L as the predictor and Haemorrhage as the outcome showed an odds ratio of 1.393 (95%CI: 0.758-2.563; $P = 0.286$). Review of receiver operating characteristic curves showed an area under the curve (AUC) for platelet count of 0.604 (95%CI: 0.534-0.675; $P = 0.005$) as compared with AUC for fibrinogen level, 0.678 (95%CI: 0.612-0.744; $P \leqslant 0.001$). A multivariate logistic regression shows United Kingdom model for End Stage Liver Disease ($P = 0.006$), Hb ($P = 0.022$) and Fibrinogen ($P = 0.026$) to be statistically significant, whereas Platelet count was not statistically significant.

CONCLUSION

Platelet count alone does not predict excessive transfusion. Additional investigations, *e.g.*, clauss fibrinogen and viscoelastic tests, provide more robust assessment of bleeding-risk in thrombocytopenia and cirrhosis.

Key words: Thrombocytopenia; Cirrhosis; Haemostasis; Fibrinogen; Liver transplantation

Core tip: Current literature describing bleeding risk in thrombocytopenia and cirrhosis does not take into account the impact of fibrinogen. The minimal platelet count to form a clot with normal strength is unknown, and would be influenced by fibrinogen. Viscoelastic testing, particularly maximum amplitude (MA, thromboelastography) or maximum-clot-firmness (MCF, thromboelastometry), reflects platelet-fibrinogen interaction and allows assessment of clot strength. Low platelet count and low fibrinogen levels lead to low MA/MCF and correlate strongly with increased bleeding tendency. Assessment of platelet count alone does not accurately predict bleeding, but is useful in conjunction with other indices such as clauss fibrinogen and MA/MCF.

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INTRODUCTION

Thrombocytopenia is a common finding in patients with advanced liver disease. In most instances it is well tolerated but it is traditionally thought to increase the likelihood of surgical or traumatic bleeding. Moderate thrombocytopenia (defined as platelet count < 50 × 10^9/L) occurs in approximately 13% of those with liver disease and is associated with significant morbidity[1]. A number of factors contribute to thrombocytopenia in liver disease, including low thrombopoietin levels, and sequestration of platelets in hypersplenism as a result of portal hypertension[2].

Derangements of other haematological indices in cirrhosis include prolongation of prothrombin time (PT), prolongation of activated thromboplastin time (APTT) and dysfibrinogenaemia. Conventionally, these changes were thought to lead to an increased bleeding risk. Over the last 10 years, however, a new paradigm of haemostasis in liver disease has been described. There is now considered to be a "rebalancing" with a reduction in procoagulant molecules being accompanied by a reduction in anticoagulant molecules. Thrombin generation is normal, or even increased and patients with cirrhosis are now considered to have an elevated risk of thrombosis rather than have complications of bleeding[3].

Standard tests of coagulation such as PT and APTT do not accurately reflect coagulation status *in vivo*, as they cannot assess cellular contributions or the effects of anticoagulant molecules. *In-vitro* studies in cirrhosis have shown a compensatory increase in levels of Von Willebrand Factor (vWF) - a platelet adhesion protein and reductions in ADAMTS-13, the cleavage enzyme responsible for the breakdown of vWF[4]. Additionally, platelet hyperactivity has been reported in cholestatic liver disease[5]. A systematic review evaluating platelet function concluded that in patients with cirrhosis, primary haemostasis is not defective[6].

Whole blood viscoelastic testing provides valuable information about dynamic clot formation. It measures changes in clot tensile strength with time and is used in goal-orientated algorithms to target transfusion. Clot strength is assessed by maximum amplitude (MA) or maximum clot firmness (MCF) and is influenced by both platelet count and by fibrinogen level. MA or MCF can be maintained in the face of low platelet counts by normal or increased levels of fibrinogen[7]. Whole blood global viscoelastic tests such as thromboelastography (TEG®) or thromboelastometry (ROTEM®) may provide more clinically relevant information about coagulation profiles in liver disease. Increasingly observed blood transfusion free orthotopic liver transplantation (OLT) suggests that conventional tests of coagulation are inadequate in predicting bleeding.

Studies of low platelet count in cirrhosis suggest that thrombin generation may be reduced in cases of severe thrombocytopenia[8]. *In-vitro* studies, however, have shown that a platelet count of 20-30 × 10^9/L is likely to be adequate to initiate haemostasis and generate enough thrombin to allow normal MA on TEG[9]. Despite a reduction in thrombin production, clot strength is likely to be adequate if the appropriate substrates for clot formation are present. Moderate reductions in platelet count, therefore, do not necessarily indicate an increased risk of bleeding in liver disease.

British Haematology Society guidelines for the use of platelet transfusions and consensus guidelines for percutaneous image guided interventions[10,11] recommend the prophylactic transfusion of platelets to a count of > 50

$\times 10^9$/L prior to liver biopsy to prevent complications of bleeding. In view of current knowledge of coagulation and haemostasis in cirrhosis, the objectives of this study were to investigate the relationship between baseline platelet count, clauss fibrinogen, MA on TEG and the volume of blood transfused in patients undergoing orthotopic liver transplantation.

MATERIALS AND METHODS

Study design

A retrospective study of patients who had undergone OLT at the Centre for Hepatobiliary Surgery, Royal Free London between 2006 and 2015 was conducted. The cohort of patients reviewed had transplantation for chronic end stage liver disease, with or without hepatocellular carcinoma. Data from their intraoperative course was retrieved from a database formed as part of standard care. Those with acute fulminant liver failure, paracetamol overdose or redo transplantation were excluded. Patients with starting haemoglobin of less than 80 g/L were also excluded in view of an increased risk of intraoperative blood transfusion. Data was anonymised and institutional research and development departmental approval was obtained for its use.

Patient demographic data, baseline haematological results, number of packed red cell units transfused intra-operatively and volume of cell salvaged blood returned to patients was retrieved electronically.

Measurements

Baseline variables were retrieved from the OLT database and included patient characteristics such as age, gender, diagnosis and severity scoring with United Kingdom model for End Stage Liver Disease (UKELD score). Baseline clinical measurements were point-of-care (*i.e.,* Medical diagnostic testing at the point of care) samples taken at the time of anaesthesia for liver transplantation from arterial catheters and measurements included haemoglobin concentration (Hb) and platelet count by pocH-100i full blood count analyser (Sysmex Europe GmbH). TEG variables were from TEG® 5000 (Haemonetics, Braintree, MA, United States. United Kingdom TD in particular MA on heparinase TEG was assessed. Heparinase TEGs were used for analysis to remove any influence that may have been exerted by endogenous heparinoids and to standardize results. Laboratory Clauss fibrinogen levels using ACL-TOP 700 (Werfen, United Kingdom) were obtained prior to transplantation and did not exceed 24 h prior to anaesthetic start time. All assays are controlled and monitored using laboratory quality assurance processes.

As a surrogate for intraoperative blood loss, an estimation of 300 mL of blood in a packed red cell unit given to patients was made, and the volume summated with cell salvage return volume to give a total volume of blood returned. Patients were divided into 2 groups according to total volume of blood returned: $\leqslant$ 1200 mL (no excessive transfusion) and > 1200 mL (excessive transfusion).

Statistical analysis

Descriptive statistics were performed on baseline variables and comparisons made between excessive transfusion (ET) and no excessive transfusion (NET) groups. Univariate logistic regression was performed for each variable independently as the predictor, and ET as the binary outcome. Receiver operating characteristic (ROC) curves for baseline platelet count and for baseline clauss fibrinogen were also constructed and area under the curves calculated. Binomial logistic regression with each variable as the predictor and ET as the outcome was also performed. Predicted probabilities from the binomial logistic regression were used for further ROC analysis. The relationship between platelet count and blood volume returned as well as fibrinogen and blood volume returned was further investigated by linear regression modeling. All data analyses were conducted using IBM SPSS Statistics version 23. All statistical analyses were reviewed by Ms Fatima Jichi, a trained biostatistician with the department of Biostatistics, University College London.

RESULTS

Baseline demographics

Results for 323 patients were reviewed and of these 37 patients had either acute, fulminant liver failure or a redo-liver transplant and were excluded. A further 40 patients had a baseline Hb less than 80 g/L and were also excluded. Of the remaining 246 patients, 114 (46.3%) had excessive transfusion and 132 (53.7%) had no excessive transfusion.

Mean patient ages were 53 years (± 1 SD 10.04 years) and were similar in ET and NET groups. The gender distribution of patients was 72.8% male and 27.2% female with a similar divide in both groups. Mean UKELD was 56 (± 10.55) in the ET group and 51 (± 5.08) in the NET group ($P \leqslant 0.001$). Liver disease due to Infection (33.3%) or Alcohol (29.6%) was the commonest aetiology. Interestingly, primary sclerosing cholangitis (PSC) was more common in the NET group (18.9%) *vs* the ET group (7.9%). χ^2 analysis of aetiologies in both groups revealed $\times \chi^2 = 18.81$, $P = 0.016$. Baseline Hb, platelet count, clauss fibrinogen and hep MA were all statistically significantly different between the two groups (Table 1).

A comparison of patient demographics and baseline measurements between those with platelet count < 50 and those $\geqslant 50 \times 10^9$/L is described in Table 2. Baseline fibrinogen was statistically significantly different between those with low platelet count (mean = 1.78 ± 0.62) and those without (2.45 ± 1.15, $P \leqslant 0.001$). Baseline hep MA was also significantly different in the 2 groups (35.28 ± 9.49 *vs* 47.85 ± 11.93, $P \leqslant 0.001$). The total volume of blood returned was not significantly different between

Table 1 Baseline demographics for patients with chronic liver disease who have undergone orthotopic liver transplantation[1]

	Excessive transfusion	No excessive transfusion	Total	*P* value
n	114 (46.3%)	132 (53.7%)	246	
Age (yr)				
Mean	53.12	52.72	52.91	0.757
SD	9.68	10.38	10.04	
Gender				
Female	28 (24.6%)	39 (29.5%)	67 (27.2%)	0.381
Male	86 (75.4%)	93 (70.5%)	179 (72.8%)	
UKELD				
Mean	56.11	51.11	53.02	< 0.001
SD	10.55	5.08	6.22	
Diagnosis				
ALD	44 (38.6%)	29 (21.9%)	73 (29.6%)	
Infectious	40 (35.1%)	42 (31.8%)	82 (33.3%)	
NASH	4 (3.5%)	6 (4.5%)	10 (4.1%)	
PSC	9 (7.9%)	25 (18.9%)	34 (13.8%)	
PBC	3 (2.6%)	6 (4.5%)	9 (3.7%)	
AIH	4 (3.5%)	3 (2.3%)	7 (2.8%)	
Wilsons	1 (0.8%)	0 (0%)	1 (0.4%)	
Haemochromatosis	1 (0.8%)	0 (0%)	1 (0.4%)	
Misc	8 (7.0%)	21 (15.9%)	29 (11.8%)	
Hb (g/L)				
Mean	101.90	109.71	106.09	< 0.001
SD	16.89	15.38	16.53	
Platelets ($\times 10^9$/L)				
Mean	83.18	107.29	96.12	0.005
SD	55.03	75.14	67.53	
Median	67	84.5	76.0	
IQR	42.5	77.5	61.25	
Fibrinogen (g/L)				
Mean	1.96	2.60	2.3	0.004
SD	0.91	1.15	1.09	
Hep MA (mm)				
Mean	42.15	47.71	45.14	0.001
SD	12.68	11.87	12.54	
Total blood returned (mL)				
Mean	3323	487	1802	
SD	2536	419	2251	

[1]Excessive transfusion defined as blood volume returned > 1200 mL. SD: Standard deviation; UKELD: United Kingdom end stage liver disease score; ALD: Alcoholic liver disease; NASH: Non-alcoholic steato-hepatitis; PSC: Primary sclerosing cholangitis; PBC: Primary biliary cirrhosis; AIH: Autoimmune hepatitis; Hb: Haemoglobin; Hep MA: Maximum amplitude on heparinase thromboelastography.

Table 2 Comparison of baseline demographics according to baseline platelet count cut off value of 50 × 10^9/L

	Platelet count < 50	Platelet count ⩾ 50	*P* value
n	53 (21.5%)	193 (78.5%)	
Age (yr)			
Mean	51.23	53.37	0.13
SD	10	9.04	
Median	52.38	55.26	
Range	52	50	
Gender			
Female	40 (75.5%)	139 (72%)	0.62
Male	13 (24.5%)	54 (28%)	
UKELD			
Mean	54.16	52.72	0.09
SD	5.06	6.47	
Diagnosis			
ALD	10 (19%)	63 (33%)	
Infectious	30 (56%)	52 (27%)	
NASH	3 (6%)	7 (4%)	
PSC	5 (9%)	29 (15%)	
PBC	1 (2%)	8 (4%)	
AIH	2 (4%)	5 (2%)	
Wilsons	0	1 (0.5%)	
Haemochromatosis	0	1 (0.5%)	
Misc	2 (4%)	27 (14%)	
Hb (g/L)			
Mean	106.34	106.03	0.89
SD	15.08	16.94	
Fibrinogen (g/L)			
Mean	1.78	2.45	< 0.001
SD	0.62	1.15	
Hep MA (mm)			
Mean	35.28	47.85	< 0.001
SD	9.49	11.93	
Total blood returned (mL)			
Mean	1692.13	1831.6	0.69
SD	1426.72	2431.54	
Median	1500	1110	
Range	6305	21237	

SD: Standard deviation; UKELD: United Kingdom end stage liver disease score; ALD: Alcoholic liver disease; NASH: Non-alcoholic steato-hepatitis; PSC: Primary sclerosing cholangitis; PBC: Primary biliary cirrhosis; AIH: Autoimmune hepatitis; Hb: Haemoglobin; Hep MA: Maximum amplitude on heparinase thromboelastography.

the 2 groups ($P = 0.69$).

A logistic regression analysis (Table 3) was performed to ascertain the independent effects of age, gender, UKELD, baseline Hb, baseline platelet count, baseline platelet count < 50 × 10^9/L or ⩾ 50 × 10^9/L as a binary value, baseline clauss fibrinogen level and baseline heparinise MA on likelihood of excessive transfusion. UKELD ($P \leqslant 0.001$), HB ($P \leqslant 0.001$), platelet count ($P = 0.007$), clauss fibrinogen ($P \leqslant 0.001$) and Hep MA ($P = 0.001$) were all statistically significant. A cut off value of platelet count less than 50 was not a good predictor of excessive transfusion ($P = 0.286$).

Review of ROC curves showed an area under the curve (AUC) for platelet count of 0.604 (standard error: 0.036; 95%CI: 0.534-0.675; $P = 0.005$). AUC for fibrinogen level was 0.678 (standard error: 0.034; 95%CI: 0.612-0.744; $P \leqslant 0.001$) (Figure 1).

A multivariate logistic regression with all covariates with $P \leqslant 0.1$ from univariate logistic regression added to a model, shows UKELD ($P = 0.006$), Hb ($P = 0.022$) and Fibrinogen ($P = 0.026$) to be statistically significant. Platelet count was not statistically significant (Table 4).

The AUC from ROC curve analysis of predicted probabilities from multivariate logistic regression was 0.749 (standard error: 0.032; 95%CI: 0.686-0.812; $P \leqslant 0.001$), suggesting that variables need to be considered together to better predict excessive transfusion (Figure 2).

Further investigation of the relationship between baseline platelet count and total volume of blood returned to patients showed that for every 1 point increase in platelet count, a 4.9 mL ($P = 0.19$) reduction in total

Table 3 Univariate logistic regression

	Odds ratio	95%CI	*P* value
Age	1.004	0.979-1.029	0.76
Gender	1.288	0.730-2.271	0.382
UKELD	1.130	1.076-1.188	< 0.001
Hb	0.97	0.954-0.986	< 0.001
Platelet count	0.994	0.990-0.998	0.007
Platelets (Binary cut off < 50 and ⩾ 50)	1.393	0.758-2.563	0.286
Fibrinogen	0.523	0.388-0.703	< 0.001
Hep MA	0.963	0.942-0.984	0.001

UKELD: United Kingdom end-stage liver disease score; Hb: Haemoglobin; Hep MA: Maximum amplitude on heparinise TEG.

Table 4 Multivariate logistic regression with covariates *P* < 0.1 from univariate logistic regression

	OR	95%CI	*P* value
UKELD	1.081	1.023-1.143	0.006
Hb	0.977	0.958-0.997	0.022
Platelets	0.999	0.994-1.004	0.700
Fibrinogen	0.682	0.487-0.955	0.026
Hep MA	0.986	0.957-1.015	0.338

OR: Odds ratio; UKELD: United Kingdom end-stage liver disease score; Hb: Haemoglobin; Hep MA: Maximum amplitude on heparinise TEG.

Table 5 Relationship between baseline platelet count and baseline fibrinogen and blood returned to patients (linear regression)

Model	Regression coefficient B	95%CI	*P* value
Platelet count	2280.72-4.99	-9.142-0.829	< 0.001
Fibrinogen	3014.63-525.95	-776.88-275.02	< 0.001

blood volume returned was achieved. For every 1-point increase in fibrinogen level, however, a reduction of 525.95 mL ($P \leqslant 0.001$) of blood returned to the patient was achieved (Table 5).

DISCUSSION

Principle findings

Guidelines recommend the prophylactic transfusion of platelets to achieve a count of 50 × 10^9/L prior to invasive procedures such as liver biopsy. Understanding of haemostasis in the cirrhotic population has altered with the concept of a "rebalanced" haemostatic profile in liver disease. This study evaluated differences in baseline platelet count, fibrinogen levels, viscoelastic tests and blood transfusion requirements in those undergoing OLT for chronic liver disease. Patients in our study were divided according to whether they received excessive blood transfusion or not. On comparison, the 2 groups were well matched for gender and age, although UKELD was found to be significantly different. Severity of liver disease, in the form of childs-pugh score and Model for end stage liver disease (MELD) scoring, is associated with a prediction of increased intraoperative transfusion requirement[12]. This may explain the difference in UKELD between the 2 groups.

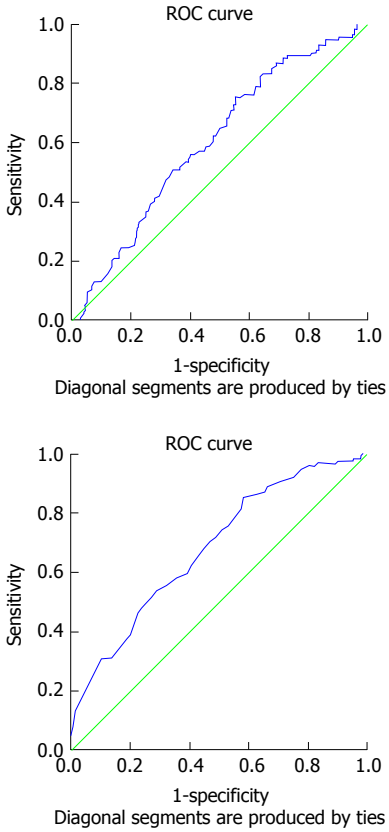

Figure 1 Receiver operating characteristics for baseline platelet count and baseline clauss fibrinogen. A: Platelets; B: Fibrinogen; ROC: Receiver operating characteristic.

It was interesting to note a higher preponderance of PSC as the aetiology of liver disease in those not requiring excessive transfusion. Hypercoagulable haemostatic profiles have been described for those with biliary cirrhotic disease and in general this population does not have thrombocytopenia or low fibrinogen[5].

A statistically significant difference in baseline Hb, platelet count, Clauss fibrinogen and MA on heparinase TEG was observed between those who received excessive transfusion *vs* those who did not. This highlights an association with bleeding risk and indicates that possibly all of these measurements would be useful in predicting increased blood transfusion requirements.

Logistic regression performed to evaluate the probability of excessive transfusion with each variable shows clearly that a platelet threshold value of 50 × 10^9/L is not a good predictor of blood transfusion in this population. Although there has been a previously described association between a reduction in thrombin generation with a reduction in platelet count[8], the cut off value of platelets requiring transfusion in cirrhosis is likely to lie significantly below 50 x 10^9/L described in guidelines. One small prospective study of liver biopsy in severe thrombocytopenia associated

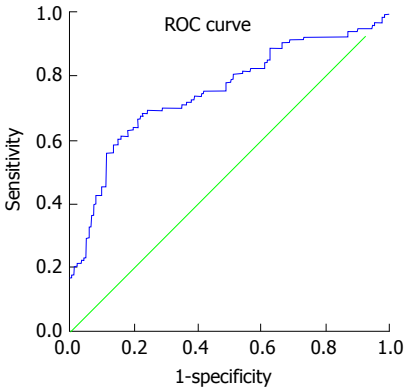

Figure 2 Receiver operating characteristics with predicted probabilities from multivariate regression. ROC: Receiver operating characteristic.

with haematological malignancy suggests the likely cut off value lies below 30 × 10^9/L[13].

Much of the literature describing bleeding risk in cirrhosis and thombocytopenia does not take into account fibrinogen level. The minimal platelet count required for normal clot strength is unknown and is markedly affected by fibrinogen. MA on TEG is a composite reflection of platelet-fibrinogen interaction and can be used to assess clot strength. Assessment of MA shows that even in the face of a low platelet count, adequate clot strength may still be achieved if fibrinogen is normal or raised. A combination of low platelet count and low fibrinogen level always results in low MA and is strongly associated with an increased bleeding tendency[14,15]. Platelet count alone is not a true indicator of clot strength; therefore, if baseline platelet count is low, assessment of MA is useful in guiding whether to replace fibrinogen or to transfuse platelets. Thrombocytopenia predominately leads to a reduction in blood clot strength displayed as MA on TEG[16], but fibrinogen also contributes to clot firmness. The effect of the administration of fibrinogen concentrates in thrombocytopenia, at a count of 30 × 10^9/L, in the pig model has been studied. Velik-Salchner *et al*[17] showed an improvement in impaired clot formation and a reduction in blood loss in thrombocytopenia with the addition of fibrinogen.

The impact of fibrinogen on bleeding risk can be observed in the results of this study. Baseline clauss fibrinogen level is likely to have a greater protective effect than the other baseline haematological variables (OR: 0.52; 95%CI: 0.388-0.705; $P \leqslant 0.001$). Similarly, Odds ratios for fibrinogen on multivariate analysis are the lowest when compared with other variables (0.682, 95%CI: 0.487-0.955) (Table 4). On comparison of AUCs on ROC curve analysis, baseline fibrinogen level is a better predictor of excessive transfusion than platelet count. Interestingly, linear regression analysis shows a 525.95 mL reduction in blood returned to patients with each 1-unit increase in baseline fibrinogen level (*i.e.,* 1 g of fibrinogen factor concentrate) (Figure 1). In comparison, 1 pool of platelets (one adult therapeutic dose) increases platelet count by 20 × 10^9/L[11], equating to a 99.8 mL reduction in blood transfusion if the linear model is used. ROC analysis of predicted probabilities on multivariate analysis show an AUC greater than that of platelet count alone, indicating a better predictive value on assessing all the demographic and haematological variables simultaneously (Figure 2).

Strengths and weaknesses of the study

Although there are a number of *in-vitro* investigations into the associations between thrombocytopenia and fibrinogen concentration and clot strength, there is a lack of evidence relating to the influence of the two *in vivo*. There is also a lack of substantial evidence to validate a cut off value for prophylactic platelet transfusion in the cirrhotic population. This study highlights the contribution of fibrinogen in reducing the risk of excessive blood transfusion, and therefore bleeding risk.

Excessive transfusion was used as a surrogate for intraoperative bleeding in this study. Measurement of blood loss in suction and weight of swabs would provide more accurate information with regard to blood loss, but this information was unavailable retrospectively. Furthermore, OLT is complex surgery with other influences on haemorrhage apart from the haemostatic picture. These include presence of portal hypertension and varices, difficult operative dissection with multiple adhesions, surgical technique (*i.e.*, Caval replacement surgery or "piggy back" technique for reperfusion) and the volume of fluid given to the patient[12,18]. Additionally, baseline low haemoglobin values increase the likelihood of requiring intraoperative blood transfusion. Low haematocrit also has an impact on laminar flow in blood vessels and therefore a disturbance in primary haemostasis may occur in anaemia[19]. We excluded those with baseline haemoglobin of < 80 g/L for this reason.

Although the results of our study point to the usefulness of measuring baseline clauss fibrinogen in conjunction with platelet count and assessment of TEG, we are unable to assess for specific cut off values for baseline platelet count and fibrinogen level. Results would require validation against external data sets to allow for cut off values, requiring further prospective research.

Implications of study

The transfusion of platelets is not without risk. Complications of platelet transfusion include allergic or anaphylactic reactions, haemolytic and non-haemolitic transfusion reactions, transfusion related acute lung injury and septic transfusion reactions of bacterial origin[20]. Furthermore, in liver transplantation, platelets have been shown to be involved in ischaemic reperfusion injury by interactions with activated sinusoidal endothelium and induction of apoptosis. Perioperative platelet transfusion has been identified as an independent risk factor for adverse post-operative outcomes[21]. In a large retrospective analysis of patients undergoing cardiac surgery, those receiving platelets were at an increased risk of postoperative infection, stroke and multiorgan failure[22].

The availability of platelets, largely due to short storage life, can also be limited. By demonstrating a lack

of association between excessive blood transfusion and a threshold platelet count of 50 × 10^9/L, the question of unnecessary prophylactic platelet transfusion arises. If an increase in fibrinogen levels by 1 g reduces the volume of blood transfused significantly, the usefulness of fibrinogen concentrate rather than platelet transfusion should be considered. Fibrinogen concentrate appears to have a better safety profile than cryoprecipitate and fresh frozen plasma, particularly when considering the risk of blood borne infection. Other advantages of its use include the accuracy and rapidity of its administration[23].

In conclusion, further prospective evaluation to assess for the true baseline platelet count at which bleeding risk is increased needs to be performed. Studies have described an increase likelihood of bleeding associated with invasive procedure with low platelet counts (< 75 × 10^9/L), but it is important to note that these studies failed to assess the contribution of fibrinogen to clot strength in cases of thrombocytopenia[24]. Additional haematological indices such as Clauss fibrinogen and the use of viscoelastic testing may provide a more robust assessment of bleeding risk in thrombocytopenia associated with cirrhosis.

ACKNOWLEDGMENTS

We would like to appreciate Dr. Eleanor Galtrey and Dr. Simon Goddard, Anaesthetic fellows, for their contribution in obtaining and processing data for this study. We also appreciate Felicity Blake, Point of Care laboratory manager for her continuing support in facilitating point of care assessment in patients undergoing OLT.

COMMENTS

Background

Thrombocytopenia is a common finding in patients with advanced liver disease. Concomitantly an increase in value of conventional tests of coagulation including prothombin time and activated partial thromboplastin time occurs. Anew paradigm of haemostasis in liver disease has been described and there is now considered to be a "rebalancing" of haemostasis in cirrhosis. As a result, the authors investigated the relationship between platelet count, clauss fibrinogen, thromboelastography, and blood loss in orthotopic liver transplantation (OLT).

Research frontiers

Much of the literature describing bleeding risk in cirrhosis and thombocytopenia does not take into account fibrinogen level. There have been recent studies that show the effect of the administration of fibrinogen concentrates in thrombocytopenia, at a count of 30 × 10^9/L, in the pig model. Velik-salchner *et al* showed an improvement in impaired clot formation and a reduction in blood loss in thrombocytopenia with the addition of fibrinogen. Further studies evaluating the contribution of fibrinogen to clot strength in thrombocytopenia in humans are required.

Innovations and breakthroughs

Currently, there are limited studies evaluating the contribution of fibrinogen to bleeding risk. The increased use of thromboelastography and in particular the evaluation of clot strength with other haematological indices could provide a more robust method of evaluating bleeding risk and requires further investigation.

Applications

The transfusion of platelets does not come without risks to patients. The availability of this valuable resource is also limited. The study highlights that platelet count alone is not a true indicator of clot strength and the threshold at which bleeding occurs in the face of a normal fibrinogen level needs further evaluation. Many current guidelines suggest prophylactic transfusion of platelets up to a count of 50 × 10^9/L, when in fact the threshold for bleeding risk is likely to be lower.

Peer-review

Interesting study with respectable sample size. This article evaluated retrospectively the association between coagulation parameters and need for transfusion in patients undergoing OLT. The study is well written with a clear message.

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P- Reviewer: Manolakopoulos S, Procopet B, Wong GLH
S- Editor: Kong JX **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 February 28; 9(6): 326-332

DOI: 10.4254/wjh.v9.i6.326 ISSN 1948-5182 (online)

SYSTEMATIC REVIEWS

Sarcopenia and non-alcoholic fatty liver disease: Is there a relationship? A systematic review

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Author contributions: Tovo CV and Fernandes SA perfomed the data collection; all the authors wrote the paper and approved the final version.

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

Data sharing statement: All available data can be obtained by contacting the corresponding author.

Manuscript source: Invited manuscript

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Received: September 7, 2016
Peer-review started: September 9, 2016
First decision: October 20, 2016
Revised: January 3, 2017
Accepted: February 8, 2017
Article in press: February 13, 2017
Published online: February 28, 2017

Abstract

AIM

To perform a systematic review to evaluate the incidence and prevalence of non-alcoholic fatty liver disease (NAFLD) in adult patients with sarcopenia.

METHODS

Randomized clinical trials, cross-sectional or cohort studies including adult patients (over 18 years) with sarcopenia were selected. The primary outcomes of interest were the prevalence or incidence of NAFLD in sarcopenic patients. In the screening process, 44 full-text articles were included in the review and 41 studies were excluded.

RESULTS

Three cross-sectional studies were included. The authors attempted to perform a systematic review, but due to the differences between the studies, a qualitative synthesis was provided. The diagnosis of NAFLD was made by non-invasive methods (image methods or any surrogate markers) in all three evaluated studies. All the studies suggested that there was an independent association between sarcopenia and NAFLD.

CONCLUSION

Sarcopenia is independently associated with NAFLD and possibly to an advanced fibrosis.

Key words: Metabolic syndrome; Obesity morbid; Sarcopenic obesity; Steatohepatitis; Skeletal muscle

Core tip: The aim of the present study was to perform a systematic review evaluating the incidence and prevalence of non-alcoholic fatty liver disease (NAFLD) in adult patients with sarcopenia. Randomized clinical trials, cross-sectional or cohort studies including adult patients (over 18 years) with sarcopenia were selected. The primary outcomes of interest were the prevalence or incidence of NAFLD in sarcopenic patients, and three cross-sectional studies were finally included. There was an independent association between sarcopenia and NAFLD in all the studies. In conclusion, sarcopenia is independently associated with NAFLD and possibly to an advanced fibrosis.

Tovo CV, Fernandes SA, Buss C, de Mattos AA. Sarcopenia and non-alcoholic fatty liver disease: Is there a relationship? A systematic review. *World J Hepatol* 2017; 9(6): 326-332 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i6/326.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i6.326

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as a set of liver diseases that can range from simple steatosis to steatohepatitis (NASH), which can progress to fibrosis or even cirrhosis[1] and complications such as hepatocellular carcinoma[2,3]. It will soon become the most common liver disease worldwide[4], with an estimated prevalence in the general population of Western countries about 20% to 30%[5]. In specific populations, its prevalence can be much higher and may reach 90% in morbidly obese patients eligible for bariatric surgery, 69% in type 2 diabetes mellitus patients and 50% in dislipidemic ones[4]. In our experience, the prevalence of NASH when obese individuals without diabetes mellitus with high aminotransferases levels were evaluated in a nutrition outpatient clinic was 88%[6]. On the other hand, when we evaluated morbidly obese patients submitted to bariatric surgery, the prevalence of steatosis was 90.4% and NASH 70.4%[7]. NAFLD patients present higher mortality than the general population, being the cardiovascular disease the most common cause of death. In patients presenting NASH, however, the mortality is associated more often to hepatic causes[4].

Sarcopenia is well characterized by the progressive loss of strength and skeletal muscle mass, generally associated with functional limitations, morbidity, and mortality[3,8,9]. The European consensus on definition and diagnosis of sarcopenia recommends using the low muscle mass and muscle function (strength or performance) for its diagnosis. Assessment of different stages of sarcopenia may help to establish the best treatment to be administered in different contexts and set appropriate recovery targets[9].

There is some concern about whether NAFLD results in sarcopenia through the activation of myostatin in the skeletal muscle, or if is sarcopenia the initial abnormality resulting in the activation of the stellate cells with fibrogenic properties in the liver. Considering the hypothesis that myostatin increases adipose tissue mass that will result in the decrease of adiponectin secretion, the original defect may actually begin in the skeletal muscle[10].

Sarcopenia may occur simultaneously with obesity, particularly the accumulation of visceral fat, which can be related to inflammation, insulin resistance (IR), and further reduction in the skeletal muscle mass, consequently causing muscle catabolism[11]. In some conditions, lean body mass is lost while fat mass may be preserved or even increased[12]; this state is called sarcopenic obesity[9]. The prevalence of sarcopenic obesity increases with age, depending on definitions and reference populations[13-15].

Although sarcopenia has been independently related to an increased risk of NAFLD and advanced fibrosis, and that sarcopenia may be associated with worse liver related clinical outcomes, this is an understudied issue, and its role on NAFLD or NASH has not been fully established[16]. The aim of this study was to perform a systematic review identifying original studies that evaluated the association between sarcopenia and NAFLD in adults.

MATERIALS AND METHODS

Protocol and registration

This systematic review was registered at the international prospective register of systematic reviews platform (PROSPERO) (https://www.crd.york.ac.uk/PROSPERO/), number CRD42015027083. This study followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[17].

Eligibility criteria

Randomized clinical trials (RCTs), cross-sectional or cohort studies including adult patients (over 18 years) with sarcopenia were selected.

The primary outcomes of interest were the prevalence or incidence of NAFLD in sarcopenic patients, liver fibrosis and NASH activity index assessed by biopsy or non-invasive methods. Studies in which one or more of these outcomes were assessed were included in the present systematic review.

Search and study selection

The search for eligible studies was performed in PubMed, Lilacs, EMBASE and Cochrane in October, 2016, without a limiting period. The search strategy included the following set of keywords: "Sarcopenia"(Mesh) OR "Sarcopenia"

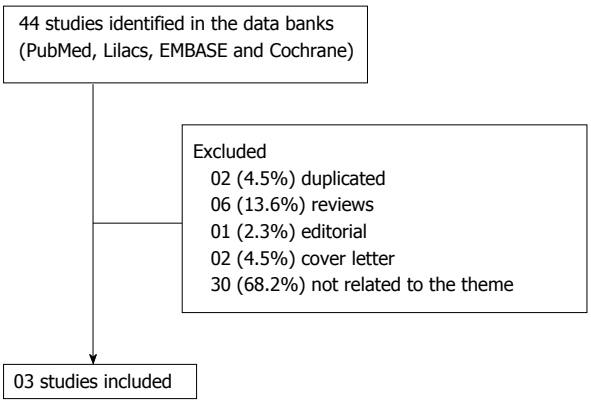

Figure 1 Screening process.

OR "Loss of skeletal muscle" OR "Loss of muscle mass and strength" OR "Reduced muscle mass and strength" OR "Intra-abdominal fat" OR "Muscle wasting" OR "Sarcopenic obesity" and (additional keyword). The last gap was changed at each search using the keywords "Non-alcoholic fatty liver disease"(Mesh) OR "Non-alcoholic fatty liver disease" OR "NAFLD" OR "NASH" OR "Non-alcoholic fatty liver disease" OR "Nonalcoholic fatty liver disease" OR "Nonalcoholic fatty liver" OR "Nonalcoholic fatty livers" OR "Nonalcoholic steatohepatitis" OR "Nonalcoholic steatohepatitides" OR "Fatty liver index". The searches were performed without limiting the types of articles (RCTs, clinical trial, comparative study). The selection of eligible studies was performed by title and abstract reading. When abstracts regarding subjects or outcomes of interest were not clear, the full text of the article was read.

Data collection process

Data was collected by two independent investigators for the following variables: Design of the study, age and sex of participants, and the presence of NAFLD. The methodological quality assessment criteria followed the guidelines according to the study design - CONSORT[18] or STROBE[19].

RESULTS

In the initial screening process (Figure 1), 44 full-text articles were included in the present review, of which 41 studies were finally excluded, remaining three cross-sectional studies for analysis. The authors attempted a systematic review with meta-analysis, but due to the variance amongst the three studies, a qualitative synthesis is provided. The main results of the studies with the respective comparisons within and between groups (when available) are shown in Table 1.

The diagnosis of NAFLD was made by non-invasive methods (image methods or any surrogate markers) in all three evaluated studies. The liver attenuation index (LAI) was evaluated by computed tomography in the study of Hong *et al*[15]. The fatty liver index (FLI) was calculated from waist circumference, body mass index, gama-glutamyl transpeptidadase and triglyceride levels in the study of Moon *et al*[20]. The NAFLD fibrosis score (NFS), hepatic steatosis index and the liver fat score were non-invasive scores used in the studies of Lee *et al*[16].

The diagnosis of sarcopenia was defined by the skeletal muscle mass index (SMI) as follow: Total skeletal muscle mass (kg)/weight (kg) × 100, and was evaluated by dual energy X-ray absorptiometry (DXA) in three of the studies[15,16] or by bioelectric impedance analysis (BIA) in one[20].

Moon *et al*[20] evaluated the effects of skeletal muscle mass to visceral fat area ratio by BIA on NAFLD (diagnosed using FLI). Of all the 9565 individuals who underwent a routine health examination, 1848 (19.3%) presented NALFD (FLI $\geqslant$ 60). The group with low FLI showed the lowest visceral fat area and highest skeletal muscle mass, and the SMI presented inverse correlations with FLI. In the multivariate analysis, skeletal muscle mass to visceral fat ratio was negatively associated with FLI. Considering the quartiles of the skeletal muscle mass to visceral fat ratio, the highest one showed the lowest risk of NAFLD, adjusted for age, gender, diabetes mellitus, hypertension, C-reactive protein and lipid profile (odds ratio, 0.037).

The study of Hong *et al*[15] performed a cross-sectional analysis between sarcopenia and NAFLD in the Korean Sarcopenic Obesity Study, a prospective observational cohort study. The authors included 452 healthy adults by LAI (evaluated by computed tomography), used as a parameter for the diagnosis of NAFLD. Both SMI and LAI were negatively correlated with the homeostasis model assessment of insulin resistance ($P < 0.001$). After using the multiple logistic regression analysis, the odds ratio for NAFLD was 5.16 in the lowest quartile of SMI (adjusting for potential confounding factors).

Lee *et al*[16] used a representative sample of 15132 subjects from the Korea National Health and Nutrition Examination Surveys (2008-2011), a population-based study. Non-invasive scores as the body mass index, aspartate aminotransferase/alanine aminotransferase ratio and diabetes mellitus (BARD) and fibrosis-4 (FIB-4) were used to define advanced fibrosis in subjects with NAFLD. The prevalence of NAFLD in non-sarcopenic patients ranged from 4% to 14% (non-obese) and from 50% to 72% (obese), depending on the hepatic steatosis score employed. The prevalence of NAFLD in sarcopenic patients ranged from 9% to 30% (non-obese) and from 61% to 83% (obese). The SMI was inversely correlated with the NAFLD predicting scores ($P < 0.001$). Sarcopenic subjects had an increased risk of NAFLD regardless of obesity (odds ratio 1.55-3.02; $P < 0.001$) or metabolic syndrome (odds ratio 1.63-4.00; $P < 0.001$) than those non-sarcopenic. Furthermore, it was demonstrated an independent association between sarcopenia and NAFLD when analysed by multiple logistic regression analysis.

Table 1 Characteristics and outcomes of the included studies

Ref.	Year	Design of the study	Sample size	Mean age (± SD)	Gender	Method of diagnosis of sarcopenia	Independent variable	Method of diagnosis of NAFLD	Frequency of NAFLD	Results of the studies
Hong *et al*[15]	2014	Cross-sectional	452	49.5 ± 10.3	285 women (63.1%)	DXA	SMI/weight (quartiles)	CT (LAI)	Prevalence	OR of having NAFLD by quartiles of SMI after adjusting for potential confounding factors: OR = 5.16 (95%CI: 1.63-16.33) P = 0.041 after adjustment for age, sex, smoking status, phisical activity, HOMA-IR, hsCRP and 25[OH]D levels
Lee *et al*[16]	2015	Cross-sectional	15132	49.7 ± 16.5	9515 women (62.9%)	DXA	SMI: < 32.2% for men and < 25.5% for women	HSI, CNS and LFS BARD and FIB-4 for advanced fibrosis	Prevalence: 22%-29%	Sarcopenic *vs* non-sarcopenic patients according to the NAFLD assessment method: OR = 1.18-1.22 (95%CI: 1.02-1.39) P < 0.001 when adjusted for age, sex, regular exercise, HOMA-IR, smoking and HT
Moon *et al*[20]	2013	Cross-sectional	9565	47 ± 10.3	5293 men (55.3%)	BIA multi frequencies	SVR (quartiles)	Surrogate marker: FLI ⩾ 60	Prevalence: 19.32%	OR for NAFLD among the quartiles of SVR using multiple logistic regression analysis: OR = 0.037 (95%CI: 0.029-0.049) P < 0.001 when adjusted for age, sex, total cholesterol, low-density lipoprotein cholesterol, DM, HTN, hsCRP

BIA: Bioelectric impedance analysis; CNS: Comprehensive NAFLD score; CT: Computed tomography; DM: Diabetes mellitus; DXA: Dual energy X-ray absorptiometry; FLI: Fatty liver index; HOMA-IR: Homeostasis model of insulin resistance; hsCRP: High sensitivity C-reactive protein; HIS: Hepatic steatosis index; HTN: Systemic hypertension; LAI: Liver attenuation index; LFS: Liver fat score; NAFLD: Non-alcoholic fatty liver disease; 25[OH]D: 25-hydroxyvitamin D; OR: Odds ratio; SMI: Skeletal muscle mass index; SVR: Skeletal muscle mass to visceral fat area ratio.

Among the individuals with NAFLD, the lower the SMI, the more chance of advanced fibrosis when compared with the non-sarcopenic (P < 0.001).

DISCUSSION

In the present review, all the studies[15,16,20] concluded that that there was an independent association between sarcopenia and NAFLD. The association of sarcopenia with NAFLD seems to be independent of IR[15,16] or obesity[16]. However, it is not possible to establish whether the association between sarcopenia and NAFLD is a cause or an effect. The skeletal muscle is now recognized as an endocrine organ secreting myokines, and this fact may help to understand its role in the pathogenesis of NAFLD[21] as well as contribute to the development of effective therapeutic options[10].

The association between fat accumulation in the liver and in the muscle has recently been established. The fat content in the paravertebral muscles analyzed by computed tomography may be correlated with aging and steatosis, and a reduction in muscle fat may be associated with an decrease of the liver fat content[22].

Insulin resistance and metabolic syndrome has been consistently associated with sarcopenia and NAFLD, as both conditions may share pathophysiological mechanisms[23-26]. However, the association between sarcopenia and NAFLD seems to be independent of IR, raising the possibility that the loss of muscle mass may contribute to the development of NAFLD[27].

The study of Moon *et al*[20] showed that the FLI was lower in the group with higher skeletal muscle mass, and the group with NAFLD (high FLI) presented lower SMI and higher visceral fat area when compared with the lower FLI group, suggesting that the incidence of NAFLD increases as the muscle mass relative to visceral fat decreases. Therefore, this fact could support a favorable role for skeletal muscle in IR and in the development of NAFLD.

Hong *et al*[15] evaluated the relationship between sarcopenia and NAFLD, demonstrating a higher risk of NAFLD in those with lower muscle mass after adjusting for confounding factors as IR and inflammation. The individuals with sarcopenia presented more metabolic syndrome, higher C-reactive protein levels and higher body fat mass when compared to those without sarcopenia.

The study of Lee *et al*[16] compared sarcopenic and non-sarcopenic patients within obese and non-obese groups of patients. The analysis made it possible to control the effect of obesity on NAFLD and it was the only study that clearly presented an association of sarcopenia

and hepatic steatosis. The prevalence of NAFLD in non-obese sarcopenic patients was more than twice as high as in non-obese non-sarcopenic patients. The proportion of increase in the prevalence of NAFLD comparing obese sarcopenic patients and obese non-sarcopenic patients was remarkably lower. This demonstrates the strong association of sarcopenia and NAFLD in non-obese patients, as well as with fibrosis.

It is worth noting that all three studies included representative samples and performed differing methods of analysis of the outcome, *i.e.*, the relationship between sarcopenia and NAFLD. Even though all three presented multivariable logistic regression analysis, the predictive models were different in all of them, illustrating the complexity and lack of consensus on the factors affecting NAFLD risk. Regardless the model, all of them showed increased risk of NAFLD in the presence of sarcopenia.

More recently, Lee *et al*[28] investigated whether sarcopenia was associated with significant liver fibrosis in the same population. Liver fibrosis was assessed by non-invasive scores as Forns, FIB-4 and NFS. It was observed that sarcopenia was significantly associated with significant liver fibrosis (odds ratio 0.52-0.67; $P < 0.01$) in subjects with NAFLD, independently of obesity and IR.

As possible limitations of the studies, the use of a cross-sectional design limits the possibility to infer causality between skeletal muscle mass loss and NAFLD or NASH[15,16,20]; and there was no information regarding the use of smoking status or alcohol consumption[15], which may allow for a bias. Also, no study performed liver biopsy to establish the diagnosis of NAFLD, considered the gold standard in the respective diagnosis[4,9,15,20]. Furthermore, the BMI of the patients included in the studies was not so high, varying from 21.4[20] to 27.9[16], characterizing overweight and not obesity, and being lower than the BMI of the occidental population[29]. This point may be explained by the local ethnic characteristics (all three studies reviewed are Korean studies), limiting the external validity of such studies.

The European consensus[9] defined that the CT scan and the magnetic resonance imaging are considered the gold standard to estimate muscle mass. DXA is considered the preferred alternative method, and BIA is a portable alternative to DXA. All the three studies included in the present analysis used the gold standard methods for the diagnosis of sarcopenia, being BIA[20] or DXA[15,16].

Of the three articles included in the present systematic review, only the one of Lee *et al*[16] reported the exclusion of approximately 25% of the patients because of missing information about the main variables evaluated (skeletal muscle mass and NAFLD).

Two additional studies were published in 2016, however they were excluded of the present systematic review because of the different primary outcomes of interest. The first was the cross-sectional study of Kim *et al*[30], evaluating 3739 Korean people, showing that the risk of NAFLD is associated with a low SMI independent of metabolic risk factors, and may differ according to the age or menopausal status. The other study, of Koo *et al*[31], evaluated 309 Korean subjects, where the prevalence of sarcopenia was 8.7%, 17.9% and 35.0% in subjects without NAFLD, with NAFLD and with NASH respectively ($P < 0.001$).

There is an independent association between sarcopenia and NAFLD and possibly to an advanced fibrosis. A higher skeletal muscle mass may have a beneficial effect in the prevention of NAFLD, which might be explored by future standardized experimental studies.

COMMENTS

Background

Non-alcoholic fatty liver disease (NAFLD) is becoming the most common liver disease worldwide, presenting a higher mortality than the general population. Sarcopenia has been related to an increased risk of NAFLD and advanced fibrosis, and may be associated with worse liver related clinical outcomes. However, this is an understudied issue, and its role on NAFLD has not been fully established. The aim of this study was to perform a systematic review identifying original studies that evaluated the association between sarcopenia and NAFLD in adults.

Research frontiers

Sarcopenia may occur simultaneously with obesity, particularly the accumulation of visceral fat, which can be related to inflammation, insulin resistance and further reduction in the skeletal muscle mass, consequently causing muscle catabolism. In some conditions, lean body mass is lost while fat mass may be preserved or even increased; this state is called sarcopenic obesity. The skeletal muscle is now recognized as an endocrine organ secreting myokines, and this fact may help to understand its role in the pathogenesis of NAFLD as well as contribute to the development of effective therapeutic options.

Innovations and breakthroughs

In the present review, all the studies concluded that there was an independent association between sarcopenia and NAFLD. The association of sarcopenia with NAFLD seems to be independent of insulin resistance or obesity. However, it is not possible to establish whether the association between sarcopenia and NAFLD is a cause or an effect.

Applications

The association between fat accumulation in the liver and in the muscle has just recently been established. The fat content in the paravertebral muscles analyzed by computed tomography may be correlated with aging and steatosis, and a reduction in muscle fat may be associated with an decrease of the liver fat content.

Terminology

Dual energy X-fay absorptimetry and bioelectric impedance analysis are methods of diagnosis of sarcopenia. Computed tomography using liver attenuation index, as well as the comprehensive NAFLD score, the hepatic steatosis index, the liver fat score and the fatty liver index are non-invasive methods of diagnosis of NAFLD.

Peer-review

This review is timely as there is emerging evidence and understanding of the association between NAFLD and sarcopenia.

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P- Reviewer: Hamaguchi M, Qu BG, Tan CK **S- Editor**: Song XX
L- Editor: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 February 28; 9(6): 333-342

DOI: 10.4254/wjh.v9.i6.333 ISSN 1948-5182 (online)

META-ANALYSIS

Vitamin E for the treatment of children with hepatitis B e antigen-positive chronic hepatitis: A systematic review and meta-analysis

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Author contributions: Fiorino S conceived the study, coordinated and supervised the search activity, evaluated the quality of the selected studies, wrote the final draft of the manuscript and checked the accuracy of data collection; Bacchi-Reggiani ML and Andreone P extracted and tabulated all relevant data from the included studies by means of a standardized method, evaluated the quality of the selected studies; Bacchi-Reggiani ML also analyzed the data; Leandri P and Loggi E performed the literature search, identified and screened the articles by consulting the above-reported database and evaluated the quality of the selected studies; Andreone P commented on drafts of the manuscript and was responsible for the final approval of the manuscript; all authors approved the final version of the article, including the authorship list.

Conflict-of-interest statement: Nothing to declare.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: October 3, 2016
Peer-review started: October 8, 2016
First decision: November 10, 2016
Revised: December 24, 2016
Accepted: January 16, 2017
Article in press: January 18, 2017
Published online: February 28, 2017

Abstract

AIM

To assess vitamin E efficacy, defined as its ability to induce hepatitis B e antigen (HBeAg) seroconversion, in children with HBeAg-positive persistent hepatitis.

METHODS

In July 2016, we extracted articles published in MEDLINE and the Cochrane Library using the following search terms: "chronic hepatitis B", "children", "childhood", "therapy", "treatment", "vitamin E", "tocopherols", "tocotrienols". Only randomized controlled trials (RCTs) published in English language were collected.

RESULTS

Three RCTs met inclusion criteria and were considered in the present meta-analysis. Overall, 23/122 children in the treatment group underwent HBeAg seroconversion *vs* 3/74 in the control group (OR = 3.96, 95%CI: 1.18-13.25, P = 0.025).

CONCLUSION

Although our meta-analysis has several limits, including the very small number of available studies and enrolled children with HBeAg positivity-related hepatitis, it suggests that vitamin E use may enhance the probability to

induce HBeAg seroconversion in these patients. Further well designed and adequately sized trials are required to confirm or deny these very preliminary results.

Key words: Hepatitis B; Pediatric hepatology; Viral hepatitis; Immunology

Core tip: Treatment of chronic hepatitis B in children is still an area of uncertainty. Vitamin E, based on immunostimulatory anti-inflammatory activity, has been evaluated in the treatment of pediatric hepatitis B virus infection. These few experiences seem to be encouraging as they suggest a potential role of vitamin E in inducing HBeAg seroconversion, but they need to be confirmed in well-designed and adequately-sized trials.

Fiorino S, Bacchi-Reggiani ML, Leandri P, Loggi E, Andreone P. Vitamin E for the treatment of children with hepatitis B e antigen-positive chronic hepatitis: A systematic review and meta-analysis. *World J Hepatol* 2017; 9(6): 333-342 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i6/333.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i6.333

INTRODUCTION

Chronic hepatitis B virus (HBV) infection continues to represent a very serious health problem worldwide, both in adults and in children[1], despite several efforts to prevent its spread and to reduce its disease burden, such as vaccination programs[2], the use of safe injection techniques and blood donor screening[3], as well as the introduction, in our therapeutic arsenal, of new and more advanced and effective antiviral treatments[4]. The clinical relevance of this pathogen depends on its ability to insidiously induce, in a large proportion of infected individuals, a necro-inflammatory hepatic disease with different patterns of severity and course, including cirrhosis and hepatocellular carcinoma. Several factors have been demonstrated to influence the prevalence[5], severity and outcome of patients with HBV-related chronic hepatitis. In particular, ethnicity, mode of acquisition and mainly, age at the time of HBV infection represent the major risk factors for the development of a persistent liver disease[6]. Whereas approximately 90%-95% of acutely HBV-infected immunocompetent adults experience a self-limiting hepatitis with the establishment of protective long-lasting immunity, and only the remaining 5%-10% develop chronic hepatitis, neonatal transmission of HBV causes a higher rate of chronic infection[7]. Approximately 90% of infected children in highly endemic countries, where vertical transmission from mother to child is predominant, become persistent carriers[8]. According to the current knowledge, the natural history of long-lasting HBV infection is characterized by three chronological phases, including immune tolerance, immune clearance and low replication status.

Despite a large series of available studies, long-term outcomes of HBV infection acquired in infancy are still unclear. However, the spontaneous or therapy-induced hepatitis B e antigen (HBeAg) seroconversion with HBe antibody development is generally considered a key event in patients with long-lasting HBV-related infection[9], because it is often accompanied by remission of liver disease and confers a favorable course over a long-term follow-up[10]. After HBeAg loss, serum HBsAg persists, but serum aminotransferase levels usually decrease, reaching normal or low values and a significant reduction in HBV replication is observed in a large part of the subjects undergoing HBeAb development[11]. Nevertheless, in some of these patients, the achievement of HBeAg seroconversion is not associated with the improvement of hepatic disease, rather these individuals still present with liver damage with different grades of severity and course and they are at higher risk of developing complications later in life[12]. Persistent HBV-related infection is considered as the result of an impaired immune response against this pathogen, consequently the boosting of antiviral immune response has become an innovative strategy in the attempt to obtain the remission of the infection, which still occurs at very low rate[13,14]. Starting from this assumption,a randomized controlled pilot trial was performed in 2001 in adult patients with HBV-related hepatitis, showing beneficial effects in anti-viral activities in these individuals[15].

Therefore, on the basis of these findings, we aimed to perform a systematic review and meta-analysis to identify and summarize the current evidence on the potential efficacy of vitamin E in the treatment of children with HBeAg-positive persistent hepatitis, defined as its ability to induce HBeAg loss and HBeAb development.

MATERIALS AND METHODS

Search strategy

A systematic computer-based search of published articles, according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis Statement, issued in 2009[16], was conducted through Ovid interface, in order to identify relevant studies on the vitamin E use for the treatment of children with HBeAg-positive chronic hepatitis. The literature review was performed in July 2016. The following electronic databases were used: MEDLINE (1950 to June 30, 2016), the Cochrane Library (until the second quarter of 2016) and EMBASE (1980 to June 30, 2016) for all relevant articles. The search strategy and the search terms were developed with the support of a professional research librarian. The text word sused for the search were identified by means of controlled vocabulary, such as the National Library of Medicine's MESH (Medical Subject Headings) and Keywords. The used MESH terms and keywords were: "chronic hepatitis B", "children", "childhood", "therapy", "treatment", "vitamin E", "tocopherols", "tocotrienols". The PubMed "related articles"

feature as well as the reference lists of retrieved articles was also searched to find additional pertinent studies. If a study was considered potentially eligible by either of the two reviewers, the full-text of this study was further evaluated. Full-text assessment was performed according to eligibility criteria developed to systematically include studies into this review. Selected studies were considered eligible if all the following predefined criteria were met: (1) the research was designed to assess the tolerability and efficacy of vitamin E use for the treatment of children with HBeAg-positive chronic hepatitis; (2) the research studies were designed as randomized studies; (3) the studies were reported in the English language, as peer-reviewed, full-text publications, whereas articles that were not published as full reports, such as conference abstracts, case reports, and editorials were excluded; and (4) sufficient data for the evaluation of HBeAg seroconversion rate were available (Table 1).

Study selection

Data extraction: Two authors (Leandri P and Loggi E), independently and in a parallel manner, performed the literature search, identified and screened relevant articles, based on title or title and abstract. If a study was considered potentially eligible by either of the 2 reviewers, the full article was collected for further assessment. Other two authors (Bacchi-Reggiani ML and Andreone P) independently extracted and tabulated all relevant data from included studies by means of a standardized method, according to the Cochrane handbook section 7.3a checklist of domains. The following information was obtained from each study, by means of a predefined data extraction form, including: First author's name, study design, inclusion and exclusion criteria, year of publication, country of origin, ethnicity, number of cases and controls, diagnostic methods for HBV markers and genome detection. We contacted the authors of the three studies to obtain additional information, including children's seroconversion rate by age groups and by HBV genotype. Unfortunately, access to patients' database was possible only for one study, whereas the above mentioned data, concerning the other two trials, were no longer available for the time period elapsed since their publication. The accuracy of data collection was checked by SF and any disagreements concerning the results were settled by consensus between all authors.

Quality score assessment: Three investigators (Fiorino S, Bacchi-Reggiani ML and Andreone P) independently evaluated the quality of the selected studies, on the basis of the Jadad scale[17] (Table 2). It includes the assessment of the following three items: Randomization (2 points as maximum score), blinding (2 points as maximum score) and an account for all patients (1 point as maximum score).

Any disagreement was resolved by discussion with the other authors. Total score ranges from 0 to 5. Included studies were classified into higher quality ($\geqslant 3$) and lower quality (< 3), on the basis of the total scores.

Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were used as the summary statistic. The pooled OR was calculated with both fixed effect (inverse variance weighting) and random effect (Der Simonian and Laird) models. To avoid the exclusion of one among the eligible studies[18], a 0.5 zero-cell correction was used. The variability, expressed in percentage across studies and attributable to heterogeneity beyond chance, was estimated with I^2 statistic. We assessed the extent of small study effects by Egger's test. *P*-values $\leqslant 0.05$ were considered significant for all included studies. Statistical analyses were carried out using STATA/SE version 14.1 (STATA Corp., College Station, TX, United States).

RESULTS

Study selection

We searched in MEDLINE, EMBASE, and Cochrane Library to retrieve works assessing vitamin E use in children with HBeAg-positive chronic hepatitis, our systematic review identified 2471 potential studies, and 2458 of them were excluded after a preliminary review of the titles and/or abstracts. The full texts of the remaining 13 articles were retrieved for a more detailed assessment. Of these, 10 studies did not meet the eligibility criteria, as described, because they were reviews, clinical studies carried out in adults or they were not published in the English language. Therefore, three studies were selected and included in the current meta-analysis. A flow-chart of the study search and selection process is shown in Figure 1.

Study characteristics

Three randomized controlled studies, assessing the use of tocopherols in children with HBV-related chronic infection and rate of HBeAg seroconversion, met inclusion criteria and were considered in the present meta-analysis[18-20].

Overall, the three trials involved 122 children, randomized to receive treatment according to an intention-to-treat protocol with different doses of vitamin E, and 74 controls. One study was performed in Turkey, one in Germany and one in Italy. The baseline characteristics of these included studies and their participants are summarized in Table 1. All considered studies described serological assays, which were employed to detect viral infection markers. Serum samples were tested for the presence of both viral antigens and host antiviral antibodies, using both enzyme-linked immunosorbent assayor radioimmunoassay, as well as of HBV-DNA, by means of liquid hybridization[18] or real time PCR[19,20].

Overall, according to the Jadad scale, two trials were classified into higher quality[19,20] and one into lower quality[18] studies (Table 1).

The end-point of this meta-analysis was to estimate the potential antiviral efficacy of vitamin E, defined as its

Table 1 Characteristic of included studies evaluating the use of vitamin E in children with hepatitis B e antigen-positive chronic hepatitis

First author/ year	Study design/ country of origin	Sample size	Treat and VE dose	Treat period/ follow-up	End-points	Main results	Quality score[3]	Tolerability
Dickici B, 2007	PRT (1:1) Turkey	58 enrolled children in the immune-tolerant phase (1) 30 treated patients M/F: 21/9 (2) Age (yr): (9.0 ± 3.8) No data concerning age 28 untreated patients M/F: 23/5 Age (yr): (8.5 ± 4.5) No data available for children's seroconversion rates by age groups and by HBV genotype	100 mg/d *vs* no treatment	3 mo/6 mo	HBV-DNA clearance HBeAg seroconversion	No antiviral-effects induced by vitamin E treatment	1	No side effects
Gerner P, 2008	PRT (3.1) Germany	92 enrolled children # (1) 69 in treatment group (2) 23 in placebo group 76 children completed the study (1) 56 in treatment group M/F: 34/22 Age (yr): 10.4 (2) 20 in placebo group M/F: 12/8 Age (yr): 11.8 No data available for children's seroconversion rates by age groups and by HBV genotype	From 200 to 600 IU/d depending on body weight *vs* placebo	6 mo/12 mo	HBV-DNA clearance HBeAg loss HBeAg seroconversion	VE may induce HBeAg seroconversion, but further studies are required	5	Well-tolerated Self-limited gastroenteritis cases
Fiorino S, 2016	PRT (1:1) Italy	46 enrolled patients (1) 23 in treatment group (18 in immune-tolerant phase and 5 in immune-reactive group) (2) 23 in placebo group (17 in immune-tolerant phase and 6 in immune-reactive group) 40 children completed the study (1) 20 in treatment group M/F: 15/5 Age (yr): (11.9 ± 3.8) (2) 20 in placebo group M/F: 16/4 Age (yr): (10.2 ± 3.5) HBeAg seroconversion in vitamin E Age (yr)/number pts/ genotype 2/0 3/0 4/1 (D[1]) 5/0 6/2 (1A, 1D)	15 mg/kg per day *vs* no treatment	12 mo/ 12 mo	(1) safety and tolerability (2) HBeAg loss/anti-HBe seroconversion (3) efficacy of VE in inducing: (1) $\geqslant 2 \log_{10}$ sustained decrease in serum HBV-DNA *vs* baseline	VE may induce HBeAg seroconversion, but further studies are required	3	Generally good safety profile Self-limited gastroenteritis (nausea, vomiting, upper abdominal pain, diarrhoea), headache, fatigue Adverse events: ALT flare

7/0																			
8/3 (1C, 2D)																			
9/0																			
10/2 (1C, 1D)																			
11/0																			
12/1 (1D)																			
13/6 (1A, 1C, 4D[2])																			
14/3 (1A[1], 1D, 1NA[1])																			
15/0																			
16/2 (1D, 1F)																			
17/3 (3D[2])																			
HBeAg seroconversion in the control group																			
Age (yr)/number pts/ genotype																			
2/1 (B)																			
3/0																			
4/1 (D)																			
5/1 (A)																			
6/1 (D)																			
7/0																			
8/3 (1C, 2F[1])																			
9/1 (D)																			
10/1 (C)																			
11/5 (1A, 2C, 2D)																			
12/2 (1A, 1E)																			
13/2 (1B, 1D)																			
14/4 (2A, 2NA)																			
15/1 (D)																			
16/0																			
17/0																			

[1]Identifies 1 patients undergoing HBeAb seroconversion in a subgroup of patients; [2]Identifies 2 patients, who underwent HBeAg seroconversion; [3]According to the Jadad scale. PRT: Prospective randomized trial; IT: Immune-tolerant; IR: immune-reactive; VE: Vitamin E; CVR: Complete virological response, defined as sustained HBeAg loss/anti-HBe seroconversion together with serum HBV-DNA reduction < 2000 IU/mL; ALT: Alanine-aminotransferase; NA: Not available; HBeAg: Hepatitis B e antigen; M: Male; F: Female.

ability to induce HBeAg loss and HBeAb development. A pooled estimate was performed on the basis of the considered studies. Overall, we observed that vitamin E use induced HBeAg seroconversion in 18.8% patients (23 seroconversion among 122 patients in the treated children *vs* 3 among 74 controls, OR = 3.96, 95%CI: 1.18-13.25, P = 0.025, I^2 = 0.0%) (Figure 2). Children's seroconversion rates by age groups and by HBV genotype were available only for one of the three trials (Table 1).

Sensitivity analysis and publication bias risk

To evaluate the effect of each individual study on the overall meta-analysis estimate, one study at a time was excluded, but the exclusion of any single research did not cause a significant alteration of the final decision and did not suggest the possibility of publication bias, but the number of considered studies is very small and this factor suggests caution in data interpretation.

DISCUSSION

The natural history of HBV-related persistent infection in childhood as well as the indications for the appropriate use of the antiviral therapy still represent an area of considerable uncertainty in this subset of patients, although an expert panel consensus[21] and some meta-analyses[22,23] have been proposed to guide physicians in the treatment decision-making process. As no definitive guidelines exist, according to available recommendations, the criteria for the management of these subjects in the real-life clinical setting have to be carefully evaluated before initiating any type of antiviral therapy. In particular, treatment is considered appropriate in HBeAg-positive children with persistently elevated (about 1.5-2.0 times the laboratory upper limit of normal), or exceeding 60 IU/L serum alanine aminotransferase and moderate/severe inflammation/fibrosis on liver biopsy[8]. Some drugs, such as interferon-α (IFN-α)[24], lamivudine[25], entecavir[26], adefovir[27] and tenofovir[28] have been approved for these patients. However, their use is limited by important side effects, risk of resistance development, high costs and, mainly, age of the treated subjects[29]. Most of these antiviral compounds have been licensed for children over 12 years old and only IFN-α as well as entecavir have been approved for a pediatric population aged between 2 and 18 years[24,26]. In addition, to date, no therapy is considered appropriate in HBeAg-positive children in the immune tolerance phase. A risk of resistance development to antiviral drugs exists in this group of patients[21]. To date, on the basis of these results, a long-term follow-up is the only approach currently applied to these children and further therapeutic approaches or treatment schedules are required[30].

Therefore, according to preliminary observations on the beneficial role of vitamin E as therapy in patients with chronic hepatitis B in a randomized controlled pilot

Table 2 Jadad scale is a procedure for assessing the methodological quality or risk of bias in randomized controlled trials, adapted from Jadad *et al*[17]

Item	Maximum points	Description
Randomization	2	1 point if randomization is mentioned 1 additional point if the method of randomization is appropriate 1 point has to be deducted if the method of randomization is inappropriate (minimum 0)
Blinding	2	1 point if blinding is mentioned 1 additional point if the method of blinding is appropriate 1 point has to be deducted if the method of blinding is inappropriate (minimum 0)
An account of all patients	1	The fate of all patients in the trial is known, if no data are reported the reason is stated

trial several years ago[15], we had the aim to understand whether this compound could exert useful and safe therapeutic effects against HBV in childhood. We performed a systematic review and meta-analysis to search studies available in the literature assessing vitamin E ability to induce HBeAb seroconversion in children with HBeAg-positive persistent hepatitis. We found only three trials[18-20] meeting inclusion criteria for our meta-analysis, but, surprisingly, two of them reported high rates of HBeAg loss and HBeAb development[19,20].

In particular, in Fiorino's trial a very high percentage of children (7/23, 30.4%), receiving vitamin E supplementation for 12 mo with a follow-up period of further 12 mo, obtained HBeAg clearance *vs* 1/23 (4.3%), in the control arm at the end of the follow-up period (24 mo)[20]. However, it has to be considered that a substantial percentage of HBeAg loss (23.2%) was also observed in the group of vitamin E-treated children for 6 mo with an additional follow-up period of 12 mo in comparison to the placebo arm (8.7%) in Gerner's research[19]. HBeAg seroconversion rates obtained in the above mentioned studies are higher in comparison to those observed in trials enrolling patients who were treated with nucleotide/nucleoside analogues. A recent research performed by Chan *et al*[31], using TDF (tenofovir disoproxil fumarate) or TDF + FTC (emtricitabine) in adult immune-tolerant patients, showed low percentage of HBeAg loss and HBeAb development (5% and 0% respectively) over 192 wk. The reasons explaining these high seroconversion rates in children supplemented with vitamin E in comparison to subjects treated with TDF analogs, mainly in Fiorino's study, are not completely understood and deserve further investigations. Nevertheless, some very interesting issues have to be considered: (1) different mechanisms of vitamin E and nucleotidic/nucleosidic analogue action; (2) duration of the treatment period; and (3) vitamin E dosage used.

Nucleos(t)ide analogues exert antiviral activities by means of well-known direct as well as indirect mechanisms: (1) suppression of HBV replication mainly through the inhibition of reverse transcription process in the viral lifecycle[32]; (2) partial restoration of the impaired

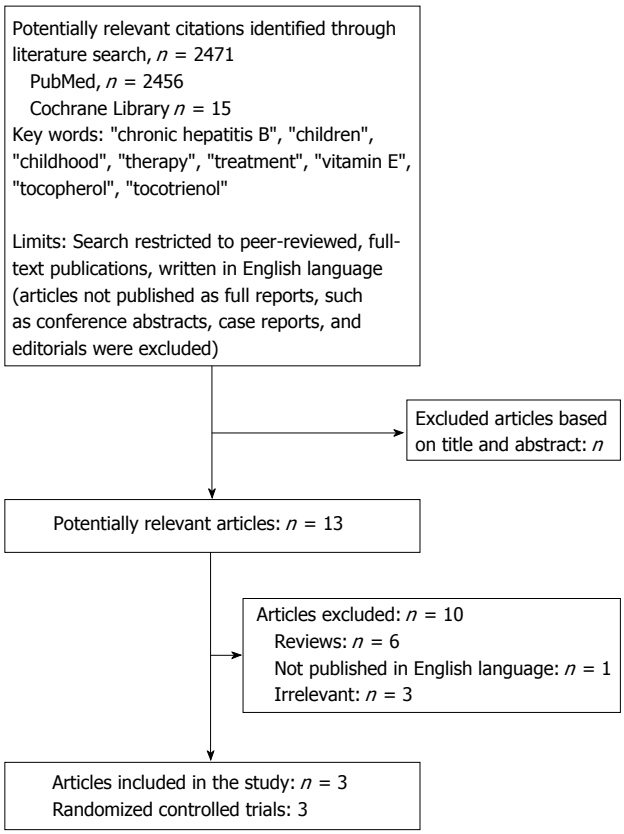

Figure 1 Study flow diagram concerning vitamin E use in children with hepatitis B e antigen positive chronic hepatitis.

immune response, as shown by significant reductions in the percentages of $CD4^+CD25^{high}$ T regulatory cells, programmed death-ligand 1 (PD-L1) receptor expression on $CD4^+$ T cells and pro-inflammatory cytokine production[33,34]. Therefore, antigen-viral burden reduction is associated with the improvement of anti-HBV activity of immune cells. According to our current knowledge, vitamin E exerts its beneficial effects by means of similar mechanisms. It is well known that vitamin E plays critical roles for normal cellular functions[35]. This essential lipid-soluble compound, acting as a free radical "scavenger", exerts potent antioxidant effects[35] in all cellular membranes[36] and contributes to their protection from oxidative injury[37,38]. In addition, *in vitro* and *in vivo* studies have suggested that vitamin E is also able to improve immune-system functions[39], mainly by increasing cell-mediated-immunity[40,41]. Mechanisms involved in these complex processes remain poorly understood, but, a recent systematic review has summarized its possible intracellular targets and suggested its potential direct antiviral or immunostimulating activities against HBV[42]. Vitamin E is able to influence the transcriptional function of some cellular genes by directly interacting with their promoter sequences[42-44] and it may modulate the post-transcriptional regulation of protein synthesis[45-47]. In particular, very preliminary studies have demonstrated that vitamin E is able to regulate the expression profiles of some microRNAs, but it is conceivable that it interacts with a larger number of these molecules[41,43,44]. In par-

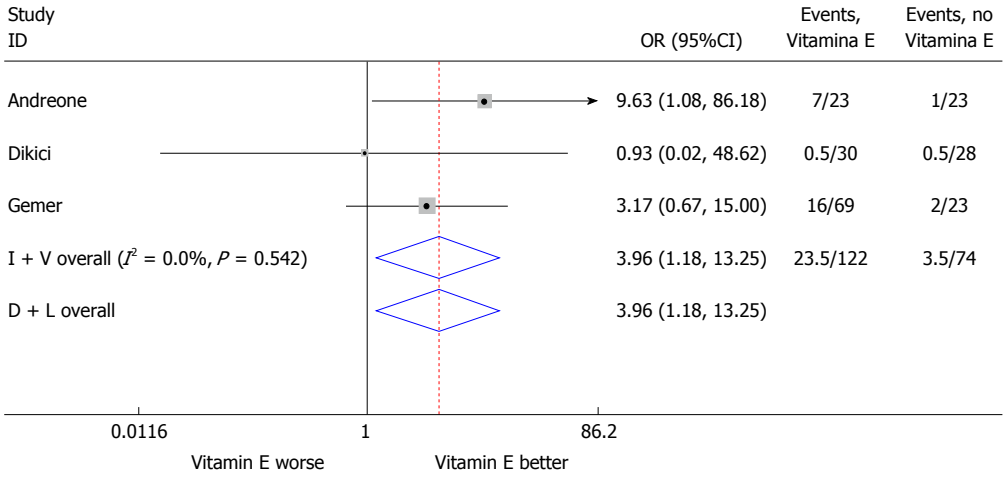

Figure 2 The relationship between vitamin E use and hepatitis B e antigen seroconversion in published studies. The area of each black square is proportional to the statistical size and the centre of the square is placed at the point of estimate. Error bars indicate 95%CIs for the estimate for each study. I + V: Inverse variance method; D + L: DerSimonian and Laird method.

ticular, it has been reported that vitamin E increases the expression of 8 oxidative stress-associated miRs, including miR-16, miR-21, miR-122, miR-125b, miR-146a, miR-155, miR-181a, miR-223[44]. The tight modulation of the synthesis of these miRs, due to their pleiotropic effects, might increase the protective immune response against the virus. Some microRNAs, which are modulated by vitamin E, possess a double function. They are required not only to preserve the normal function of the immune system[48], mainly of the cell-mediated arm[49], and to prevent the development of a redundant and abnormal inflammatory response[50,51], but they also exert a direct anti-HBV activity[52]. These premises may help us to explain the positive preliminary results of our meta-analysis. It is conceivable to think that vitamin E supplementation may contribute to the improvement of the anti-HBV activity of the immune system by means of a direct anti-viral action both by decreasing its replication abilities and by boosting the host's immune cell responses. The slow but progressive decline of HBV replication associated with HBeAg loss/HBeAb seroconversion, as described in 2 of the 3 studies included in our meta-analysis, corroborates the hypothesis that vitamin E acts as an immunomodulator resulting in a global antiviral activity. Interestingly, a delayed response has already been reported, with the use of immunomodulatory drugs for the treatment of adults with CHB[53]. In Fiorino's study, HBeAg seroconversion was also observed in additional 7 of 11 previously non-responder patients in the vitamin E supplemented group, with an extended follow-up for an additional period of 12 mo[20]. This observation underlines the importance of the duration of the treatment period and might contribute to explaining, at least in part, the absence of children responding to vitamin E therapy in Dickici's study[18]. In this trial, the period of supplementation with this compound was only three months long, in addition the dosage used was equal to 100 mg/d for three months, probably not enough to induce an improvement of anti-viral immune responses. Therefore, both dosage and duration of vitamin E supplementation represent very important factors that must be considered when its anti-viral efficacy is evaluated. Taking into considerable account all the described factors, our study seems to suggest that vitamin E use may effectively enhance the probability to induce HBeAg seroconversion in these patients, even in children in the immune-tolerant phase. However, it has to be considered that our meta-analysis has several limits. First, the very small number of available studies enrolling children with HBeAg positivity-related hepatitis, as well as the small size of these trials may increase publication and selection biases; second, one research study has been carried out in Asia[18] and 2 studies in Europe[19,20], it is not known whether differences in response rates, following vitamin E use, may exist among children belonging to different ethnicities or depending on the different prevalence of HBV genotypes; third, the study design did not include the use of placebo in control groups in two studies[18,20]; fourth, it has to be taken into account that HBeAg seroconversion rate in children in the immunetolerant phase is < 2% among children younger than 3 years and 4%-5% among older children[54] and that data concerning HBV genotypes as well as children's seroconversion rates by age groups were available only for one of the studies considered for the meta-analysis. Therefore, this limit precludes further proper assessments of the potential vitamin E benefits in children under 14 years as well as of its potential efficacious dose. However, in the study by Fiorino *et al*[20], patients who responded to vitamin E treatment were respectively 4 (1 child), 13 (2 children), 14 (2 children), and 17 (2 children) years old at the enrollment (Table 1).

In addition, it has to be considered that some meta-analyses[55,56] have reported that long-term administration of vitamin E, at dosages exceeding 150 UI once a day, is associated with serious negative outcomes, such as an increase in all-cause mortality. However, although the conclusions of these studies are rather questionable[57-60]

because of the meta-analytic approaches used and no severe side effects have been described in the three included trials, with the exception of some adverse events, represented by flares of transaminases, these results suggest caution in the generalization of vitamin E administration in the pediatric population before confirmation of the effectiveness and safety of this compound. Certainly, vitamin E, which was administered at high dosage to the children in the reported trials, has to be considered as a drug, with possible benefits as well as with potential risks. Therefore, to date these limiting factors prevent the formulation of definitive recommendations on the role of this type of treatment in children with HBeAg-positive hepatitis. However, to our knowledge this is the first attempt to quantitatively assess the therapeutic effects and efficacy of vitamin E in the treatment of these subjects. These patients suffer from a viral-related liver disease, a condition associated with a deficiency in immune system responses. Therefore, the use of vitamin E in patients with HBV chronic infection might represent an approach combining the direct antiviral effect of nucleoside/nucleotide analogues on HBV replication together with the immune-enhancing role of this fat-soluble compound. Therefore, further well-designed and adequately-sized trials are required to confirm or deny these preliminary, but apparently very interesting and promising, results with the aim to establish the potentially useful dose of vitamin E to induce HBeAg seroconversion.

ACKNOWLEDGMENTS

The authors thank Dr. Simonetta Righi, Biblioteca Centralizzata, Policlinico S Orsola-Malpighi, Università di Bologna, Bologna, Italy for her support in the search of scientific bibliography and Dr. Cecilia Baroncini IRCCS - Institute of Neurological Sciences of Bologna, Italy for her support in the linguistic revision of the manuscript.

COMMENTS

Background

Hepatitis B virus (HBV) chronic infection in childhood continues to represent a very important clinical problem in several countries worldwide and the treatment indications for this subset of patients are still an area of considerable uncertainty. Clear-cut guidelines are lacking, therefore, to date, therapeutic decisions are only based on the consensus of expert panels.

Research frontiers

A defective immune response is considered a crucial factorin maintaining a persistent infection in subjects with HBV. Drugs able to restore anadequate immune activity could contribute to promote an effective control of this infection.

Innovations and breakthroughs

In the real-life clinical setting, a wide proportion of children with persistent HBV infection remain untreated and a conservative approach is generally applied in these subjects. Therefore, the introduction of additional therapies and different strategies in clinical practiceis required. Since several years ago, vitamin E has emerged as a compound with a large spectrum of immune-stimulatory activities and its use could improve the defective immune activity detectable in several diseases, including chronic viral infections. This research field could be very interesting, but this kind of application requires caution. Some reports, although rather questionable, report possible harmful effects, when this compound is used at high dose.

Applications

Vitamin E administration could represent one among the options for the treatment of children with HBV-chronic infection, either as mono-therapy in the developing countries with limited economic resource or, for the future, in combination with standard antiviral drugs. The decrease in viral replication and antigen burden as well as the restoration of an adequate immune function might represent an effective approach for the management of patients with HBV persistent infection.

Peer-review

The authors underwent a meta-analysis to evaluate the role of vitamin E administration to children with immune tolerant HBV infection. Results showed a nearly four-fold likelihood of achieving HBeAg seroconversion in those children receiving vitamin E. Sensitivity analysis showed that exclusion of any of the three studies did not change results.

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P- Reviewer: Fernandez-Rodriguez CM **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Hepatol* 2017 February 28; 9(6): 343-348

DOI: 10.4254/wjh.v9.i6.343 ISSN 1948-5182 (online)

CASE REPORT

Hyperammonemia crisis following parturition in a female patient with ornithine transcarbamylase deficiency

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Supported by A Grant-in-Aid for Guidelines and Lifetime Medical Support Systems for inborn errors of metabolism found by newborn screening; a Grant-in-Aid for the Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and Development, AMED.

Institutional review board statement: This study was approved by the ethical committee of the Faculty of Life Science, Kumamoto University.

Informed consent statement: Written informed consent was obtained from the patient and her husband.

Conflict-of-interest statement: None.

Manuscript source: Unsolicited manuscript

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Received: November 5, 2016
Peer-review started: November 9, 2016
First decision: December 15, 2016
Revised: January 2, 2017
Accepted: January 11, 2017
Article in press: January 14, 2017
Published online: February 28, 2017

Abstract

Ornithine transcarbamylase deficiency (OTCD) is an X-linked disorder, with an estimated prevalence of 1 per 80000 live births. Female patients with OTCD develop metabolic crises that are easily provoked by non-predictable common disorders, such as genetic (private mutations and lyonization) and external factors; however, the outcomes of these conditions may differ. We resuscitated a female patient with OTCD from hyperammonemic crisis after she gave birth. Hyperammonemia after parturition in a female patient with OTCD can be fatal, and this type of hyperammonemia persists for an extended period of time. Here, we describe the cause and treatment of hyperammonemia in a female patient with OTCD after parturition. Once hyperammonemia crisis occurs after giving birth, it is difficult to improve the metabolic state. Therefore, it is important to perform an early intervention before hyperammonemia occurs in patients with OTCD or in carriers after parturition.

Key words: Brain image; Delivery; Glutamine; Amino acid; Ornithine transcarbamylase deficiency; Urea cycle disorders; Uterus; Hyperammonemia

Core tip: Hyperammonemia crisis after parturition in patients with ornithine transcarbamylase deficiency (OTCD) is often fatal and difficult to predict. It is important to perform early intervention before hyperammonemia occurs in patients with OTCD or in carriers after parturition.

Kido J, Kawasaki T, Mitsubuchi H, Kamohara H, Ohba T, Matsumoto S, Endo F, Nakamura K. Hyperammonemia crisis following parturition in a female patient with ornithine transcarbamylase deficiency. *World J Hepatol* 2017; 9(6): 343-348 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i6/343.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i6.343

INTRODUCTION

Urea cycle disorders (UCDs) are one of the most common inherited metabolic diseases in Japan, with an estimated prevalence of 1 per 50000 live births. The urea cycle is the metabolic pathway that eliminates excess endogenous and exogenous nitrogen from the body by modifying ammonia into urea, thereby reducing its toxicity. This cycle comprises 6 different enzymes, including ornithine transcarbamylase (OTC; EC 2.1.3.3).

OTC deficiency (OTCD; Mc Kusick No. 311250) is an X-linked disorder, with an estimated prevalence of 1 per 80000 live births[1]. The traditional treatment for OTCD is a low-protein diet. Sodium benzoate and/or sodium phenylbutyrate are significant as an alternative pathway therapy[2,3].

In female patients with OTCD, metabolic crises can be easily provoked by non-predictable common disorders, such as genetic (private mutations and Lyonisation) and external factors, and sometimes may be fatal.

We resuscitated a female patient with OTCD, who maintained a relatively stable condition using a self-restricted protein diet, from hyperammonemic crisis after parturition. Hyperammonemia after giving birth in a female patient with OTCD can be fatal[4], and this type of hyperammonemia persists for an extended period of time. If patients with OTCD develop hepatic coma with hyperammonemia $\geqslant$ 300 μmol/L, hemodialysis as well as treatments that target alternative nitrogen metabolism pathways and arginine or/and citrulline treatments should be used to control blood ammonia levels immediately to avoid damage to the brain[5-7]. We discontinue hemodialysis and control blood ammonia levels using the alternative pathway therapy, and arginine or/and citrulline treatments when their blood ammonia levels decrease to < 180 μmol/L[8,9]. Hemodialysis is excellent for the removal of ammonia in the body; however, this treatment does not suppress the production of ammonia and removes useful medications from the body. Moreover, although a high-caloric infusion that largely consists of glucose is important, the early administration of essential amino acids and low-protein is useful for preventing protein catabolism in the body and for controlling ammonia levels[10].

Here, we discuss the cause and treatment of hyperammonemia in a female patient with OTCD after parturition.

CASE REPORT

A 37-year-old female patient who was diagnosed with late-onset OTCD was followed by her doctor, and was introduced to our institute after pregnancy. She was the first child of nonconsanguineous parents and had no family history of hyperammonemia. She presented with seizures at the age of 7 and developed hyperammonemia following the administration of valproic acid as treatment for the seizure. She was diagnosed with OTCD by liver biopsy examination (liver OTC enzyme activity that was 30% the level of healthy patients) (Table 1). She had visited the emergency room previously due to hyperammonemia and had undergone hemodialysis for impaired consciousness at the age of both 18 years and 21 years. Despite this, she followed a self-restricted protein diet and L-carnitine treatment. She naturally became pregnant at the age of 37 years and delivered an unaffected male baby by vacuum extraction at 41 wk and 1 d of gestation. Her blood ammonia value was 68 μmol/L at delivery, 59 μmol/L at 10 h after delivery, and 54 μmol/L at 24 h after delivery. She developed hyperammonemia (194 μmol/L) 4 d after delivery, but was discharged 6 d after delivery because her blood ammonia level decreased to 60 μmol/L following arginine and citrulline treatment. Upon discharge, she consumed a hamburger at a fast food restaurant and 250 g of beef at her home. She was hospitalized on an emergency basis at midnight because of hyperammonemia (180 μmol/L) with impaired consciousness (Table 2). Blood ammonia levels instantly decreased to 82 μmol/L by the continuous administration of arginine. She then underwent hemodialysis and continuous hemodiafiltration under respirator management in the intensive care unit because her blood ammonia level increased to 339 μmol/L, with a grade Ⅲ hepatic coma. Moreover, she received a high-calorie infusion (2500 kcal/d), arginine (80 mg/kg per day), citrulline (150 mg/kg per day), sodium benzoate (150 mg/kg per day), and sodium phenylbutyrate (140 mg/kg per day) as an alternative pathway therapy. Following this, her hyperammonemia improved.

Although extubation was attempted because of stable blood ammonia levels following these treatments (Figure 1), she was reintubated because she could not maintain respiration. Her ammonia levels increased again to 210 μmol/L after experiencing physical stress during extubation, before gradually decreasing to 60 μmol/L. She developed sepsis and was managed at the

Table 1 Data at diagnosis

	Serum AA (nmol/mL)	Uric AA (nmol/mg Cre)	Cerebrospinal fluid AA (nmol/mL)
Amino acids			
Glutamine	1754.6	282.9	534.1
Glutamic acid	90.8	277.9	TR
Ornithine	60.3	TR	5.8
Citrulline	19.7	ND	ND
Arginine	54.4	ND	14.2
Lysine	162.8	ND	15.9
Urinary orotic acid	13.3 μg/mg Cre		
Liver enzyme assay	Patient (μmol/mg protein/min)	Control	
CPS1	0.024	0.023-0.074	
OTC	0.17 (30% of the control)	0.51-1.51	

AA: Amino acids; Cre: Creatinine; TR: Trace; ND: Not determined; CPS1: Carbamoyl-phosphate synthetase 1; OTC: Ornithine transcarbamylase.

Table 2 Laboratory data upon admission

AST	28 (IU/L)
ALT	34 (IU/L)
γGTP	19 (IU/L)
LDH	369 (IU/L)
ALP	338 (IU/L)
CHE	219 (IU/L)
T-Bil	0.6 (mg/dL)
TP	6.8 (g/dL)
Alb	3.3 (g/dL)
BUN	12.3 (mg/dL)
Cre	0.44 (mg/dL)
NH3	180 (μmol/L)
Amy	90 (IU/L)
CK	111 (IU/L)
CRP	0.3 (mg/dL)
WBC	12000 (/μL)
Hb	10.7 (g/dL)
Plt	26.4×10^4 (/μL)
PT	112 (%)
APTT	97 (%)
P-FDP	37.2 (μg/mL)
Fib	347 (mg/dL)
AT Ⅲ	134 (%)

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGTP: γ glutamyl transpeptidase; LDH: Lactase dehydrogenase; ALP: Alkaline phosphatase; CHE: Choline esterase; T-Bil: Total bilirubin; TP: Total protein; Alb: Albumin; BUN: Blood urea nitrogen; Cre: Creatinine; Amy: Amylase; CK: Creatine kinase; CRP: C-reactive protein; WBC: White blood cell count; Hb: Hemoglobin; Plt: Platelets; PT: Prothrombin time; APTT: Activated partial thromboplastin time; P-FDP: Plasma fibrin degradation products; Fib: Fibrinogen; AT Ⅲ: Antithrombin Ⅲ.

intensive care unit for 43 d.

The cause of hyperammonemia for this case was considered to be: (1) physical stress experienced during vaginal parturition and fasting while withstanding pain for a prolonged period of time; (2) excessive protein intake after discharge; and (3) metabolic changes during puperium following anabolism for the repair of the parturient canal, including the uterus, after delivery and

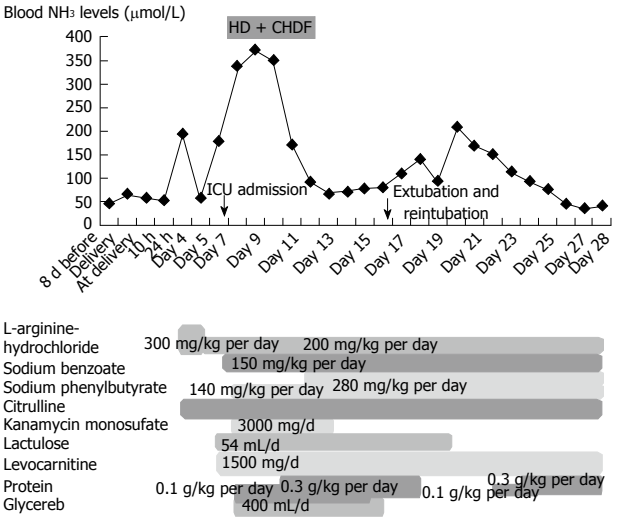

Figure 1 Blood ammonia levels after delivery. The patient was admitted on emergency basis because of impaired consciousness 7 d after delivery (day 7) and was intubated. She then underwent HD and CHDF under ICU management (from day 8 to day 12). HD: Hemodialysis; CHDF: Continuous hemodiafiltration; ICU: Intensive care unit.

catabolism for producing maternal milk.

Presently, she uses medication and has been able to raise her child following hospital discharge despite her brain magnetic resonance imaging and single-photon emission computed tomography indicating atrophy of the bilateral frontal and temporal lobes with decreased bold flow (Figure 2).

DISCUSSION

We resuscitated a female patient with late-onset OTCD who developed hyperammonemia crisis after giving birth. We previously reported the long-term outcome for patients with OTCD in Japan[8,9,11]. The expected survival rate at 35 years of age in late-onset OTCD patients was less than 30% for both male and female patients according to data obtained from 1978-1995[8], and 89% for male patients and 84% for female patients according to data obtained from 1999-2009[9]. The more recent long-term outcome improved compared to previous outcomes due to improved medication, hemodialysis, and liver transplantation; however, the long-term survival of patients is not guaranteed. These patients always have the potential to develop hyperammonemia due to metabolic stress during infection, surgery or delivery, even if the hyperammonemia is resolved at the time of onset and their medical state is well controlled thereafter.

It has been reported that female patients with OTCD are likely to develop hyperammonemia that persists for 6 to 8 wk at 3 to 14 d after delivery[12,13]. Although the cause of hyperammonemia after delivery in female patients with OTCD is not clear, it is considered to be related to increased protein load for collagen catabolism following involution of the uterus[14]. We also consider that hyperammonemia is related to metabolic changes in

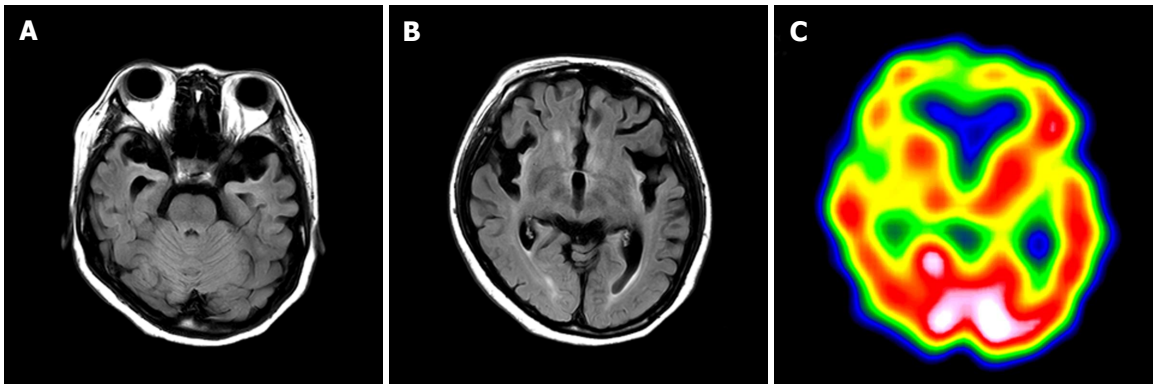

Figure 2 Brain magnetic resonance imaging and single-photon emission computed tomography 7 mo after delivery. A and B: FLAIR demonstrates atrophy of the bilateral frontal and temporal lobes, and a high signal for the cortex and subcortex on both sides of insula, the ventral tamporal lobe and the frontal lobe bottom. The signal is elevated bilaterally in the putamen, caudate nucleus, and front globus pallidus; C: Single-photon emission computed tomography shows bilaterally decreased blood flow in the frontal lobe, ventral temporal lobe, basal ganglia, and thalamus.

their bodies during puerperium.

She developed hepatic coma that required respiratory management, which required 40 d to resume breathing without assistance and to regain improved level of consciousness. She continued to produce maternal milk a month after the onset of hyperammonemia, and hyperammonemia persisted even after maternal milk production stopped. The cause of long-term hyperammonemia is not only puperium. Mitochondrial abnormalities within the liver of patients with OTCD has been previously demonstrated[15], and hyperammonemia itself disrupts the function of mitochondria[16]. Therefore, it has been considered that the impaired mitochondria in cases of hyperammonemia aggravates OTCD and results in long-term hyperammonemia.

In this case, her blood ammonia levels remained at 60 μmol/L from during pregnancy to 3 d after delivery and elevated at 4 d after delivery. She did not receive sodium benzoate or sodium phenylbutyrate, which are utilized as alternative pathway therapies, because her blood ammonia levels soon decreased after arginine and citrulline treatment. We consider that even female patients with OTCD who do not receive sodium benzoate or sodium phenylbutyrate therapy are recommended to start receiving sodium benzoate or sodium phenylbutyrate treatment from the start of labor to immediately after delivery, if they have developed severe hyperammonemia[17]. Benzoate is conjugated with glycine to form hippurate, which is rapidly excreted in the urine. For each mole of benzoate administered, 1 mole of nitrogen is removed. Phenylbutyrate is activated to the CoA ester, which is metabolized by β-oxidation in the liver to form phenylacetyl-CoA, which is then conjugated with glutamine. The resulting phenylacetylglutamine is excreted into the urine, and 2 moles of nitrogen are excreted for each mole of phenylbutyrate[5].

Therefore, it is effective for OTCD patients who have never received sodium phenylbutyrate or sodium benzoate to preliminarily receive such a medication when the blood ammonia level is expected to increase during excessive stress, such as during delivery. Because ammonia is a final product in amino acid metabolism, it is believed that there is a time lag from stress load to the increased blood ammonia levels. It may be effective to measure blood glutamine levels to predict whether the blood ammonia levels will increase or to estimate the change in body condition in patients with OTCD because glutamine is a supplier of ammonia and one of the markers of the medical condition[18,19]. Furthermore, the blood value is high, even in OTCD carriers[20]. Moreover, it may be useful to adjust the amount of sodium phenylbutyrate or sodium benzoate based upon blood glutamine levels.

The patient should have been monitored without discharge from our hospital because hyperammonia crisis was likely to develop anytime within 2 wk after parturition and she could not consume protein-rich food in our hospital. Moreover, we consider that hyperammonemia crisis could have been avoided if she had received sodium benzoate or sodium phenylbutyrate therapy immediately after delivery. Caesarean section and the halting maternal milk production might contribute to preventing a hyperammonemia crisis.

Moreover, this patient was a candidate for liver transplantation (LT) since she was susceptible to developing hyperammonemia crisis for non-predictable common disorders. If female patients with OTCD who have had hyperammonemia crisis are going to be married and give birth, LT before pregnancy may be a treatment option because LT prevents recurrent hyperammonemia attacks and contributes to improved quality of life in patients with UCD[21-24].

In conclusion, we treated a female patient with late-onset OTCD, who developed hyperammonemia crisis after delivery. Hyperammonemia crisis after delivery in patients with OTCD is often fatal and difficult to predict. It is important to perform early intervention before hyperammonemia occurs in patients with OTCD or carriers after delivery. Once hyperammonemia crisis occurs following parturition, it is difficult to improve the

metabolic state of the patient.

COMMENTS

Case characteristics

Hyperammonemia crisis with hepatic coma after delivery.

Clinical diagnosis

Hyperammonemia crisis with hepatic coma.

Differential diagnosis

Carbamoyl-phosphate synthetase 1 deficiency, arginosuccinate synthetase deficiency, arginosuccinate lyase deficiency and arginase 1 deficiency were considered to be a differential diagnosis.

Laboratory diagnosis

Increased blood glutamine and urinary orotic acid levels as well as impaired liver ornithine transcarbamylase enzyme activity.

Imaging diagnosis

Atrophy of the bilateral frontal and temporal lobe, as indicated by brain magnetic resonance imaging.

Treatment

Arginine, citrulline, sodium benzoate, sodium phenylbutyrate, and hemodialysis.

Related reports

A peak ammonia concentration less than 180 μmol/L was shown to be a marker of a good neurodevelopmental prognosis, and a peak ammonia concentration of more than 360 μmol/L was a marker of a bad prognosis.

Term explanation

UCDs: Urea cycle disorders; OTCD: Ornithine transcarbamylase deficiency; LT: Liver transplantation.

Experiences and lessons

It is important to perform early intervention before hyperammonemia occurs in patients with OTCD or carriers following parturition.

Peer-review

Hyperammonemia after delivery in a female patient with OTCD can be fatal. In this case report, authors have discussed the cause and treatment of hyperammonemia in a female patient with OTCD after delivery. This case indicates that it is important to perform early intervention before hyperammonemia occurs in patients with OTCD or carriers after delivery.

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P- Reviewer: Cao WK, Morioka D **S- Editor**: Ji FF **L- Editor**: A
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