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ABOUT COVER Editorial Board Member of *World Journal of Hepatology*, Haruki Komatsu, MD, PhD, Associate Professor, Department of Pediatrics, Toho University, Sakura Medical Center, Sakura 285-8741, Chiba, Japan

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WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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TOPIC HIGHLIGHT

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Primary prevention of bleeding from esophageal varices in patients with liver cirrhosis

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Abstract

Variceal bleeding is a life threatening situation with mortality rates of at least 20%. Prophylactic treatment with non-selective beta blockers (NSBBs) is recommended for patients with small varices that have not bled but with increased risk for bleeding. The recommended treatment strategies on primary prevention of variceal bleeding in patients with medium and large-sized varices are NSBBs or endoscopic band ligation. Nitrates, shunt surgery and sclerotherapy are not recommended in this setting. In this review, the most recent data on prevention of esophageal variceal bleeding are presented. Available data derived from randomized-controlled trials suggest both treatment strategies, and according to Baveno V consensus in portal hypertension "the choice of treatment should be based on local resources and expertise, patient preference and characteristics, side-effects and contra-indications".

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Key words: Cirrhosis; Portal hypertension; Esophageal varices; Primary prevention; β-Blockers; Endoscopic band ligation

Core tip: The significance of primary prevention of bleeding from esophageal varices in patients with liver cirrhosis is major, considering the high mortality rates that accompany the acute bleeding episode. Current management guidelines suggest the use of either non-selective beta-blockers or endoscopic band ligation with same efficacy between them. In this review, we summarize data from randomized clinical trials or prospective studies together with meta-analytical data, when applicable, to present the most updated recommendations on primary prevention of esophageal variceal bleeding.

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INTRODUCTION

Bleeding from esophagogastric varices is a life-threatening condition with an incidence of 5%-15% in patients with liver cirrhosis and mortality rates of at least 20%[1,2], despite improvements in the management of these patients. The term pre-primary prophylaxis is used to define the prevention of development and growth of varices. The term primary prophylaxis refers to the prevention of the first variceal bleeding in patients with liver cirrhosis and consists of two main treatment strategies, non-selective beta blockers (NSBBs) aiming to reduce hepatic venous pressure gradient (HVPG) below 12 mmHg or by 20% from baseline levels, and endoscopic band ligation (EBL) performed until variceal eradication[3].

In this review, we discuss the most recent data on primary prevention of variceal bleeding using data from randomized controlled trials (RCTs), prospective studies or meta-analyses focusing mainly on probability of bleeding, mortality and adverse events. We searched MEDLINE

database, Scopus, and ISI Web of Knowledge search system using the textwords "esophageal varices", or "primary prevention of variceal bleeding", or "management of varices" and major Gastroenterology and Liver meetings.

DIAGNOSIS OF VARICES

The esophagogastroduodenoscopy (EGD) is the gold standard for the diagnosis of esophageal varices and should be performed every 2-3 years in patients with compensated cirrhosis and no varices at initial endoscopy, and every 1-2 years in patients with small varices[4]. In patients with decompensated cirrhosis, EGD should be performed yearly[4]. There is a great interest in identifying non-invasive factors to diagnose esophageal varices but currently there is no evidence that their predictive accuracy is equal to that of EGD. Such factors include platelet count, spleen size, portal vein diameter, Child-Pugh score, presence of ascites, albumin levels and transient elastography[5].

In a recent prospective study[6], spleen stiffness (SS) and liver stiffness (LS) were measured by transient elastography in 200 patients with liver cirrhosis of whom 124 (71%) had esophageal varices. There was a significant difference in median LS (P = 0.001), SS (P = 0.001), LS-spleen diameter to platelet ratio score (LSPS) (P = 0.001), and platelet count to spleen diameter ratio (PSR) (P = 0.001) between patients with and without esophageal varices. LS $\geqslant$ 27.3 kPa had a sensitivity of 91%, specificity of 72%, and a diagnostic accuracy of 86% in predicting esophageal varices. LSPS $\geqslant$ 3.09 had sensitivity and specificity of 89% and 76%, respectively, and a PSR cut-off value of 909 or less had sensitivity of 64%, specificity of 76%, and diagnostic accuracy of 68% in predicting esophageal varices. SS $\geqslant$ 40.8 kPa had a sensitivity of 94%, specificity of 76%, and diagnostic accuracy of 86% for predicting esophageal varices. SS was significantly higher in patients who had large varices (56 *vs* 49 kPa, P = 0.001) and variceal bleeding (58 *vs* 50.2 kPa, P = 0.001).

Capsule endoscopy (CE) has been shown to be an accurate prognostic method for diagnosis of esophageal varices but there is no consensus to recommend its use in this setting. In a meta-analysis of 9 studies including 631 patients[7], the pooled sensitivity and specificity of PILLCAM ESO capsule was 83% and 85%, respectively with positive and negative likelihood ratios of 4.09 and 0.25, respectively. In a recent, prospective study[8], the overall diagnostic yield of CE for esophageal varices was 72% (51 of 71 esophageal varices detected by EGD). The diagnostic yield was significantly greater for F2/F3 esophageal varices than for F1 (87% *vs* 61%, P = 0.03) and for varices located at locus superior or locus medialis than those located at locus inferior (85% *vs* 55%, P = 0.01). The diagnostic accuracy of CE for gastric varices was low (1 of 29 gastric varices detected by EGD), whereas for portal hypertensive gastropathy was 69% (24 of 35). EGD is superior to CE in grading of esophageal varices because capsule lacks air insufflation.

RISK OF FIRST VARICEAL BLEEDING EPISODE

The major predictive factors of first variceal bleeding episode are the size of varices, the severity of liver dysfunction and the endoscopic presence of red wale marks[9]. However, the combination of these, fails to predict all episodes of bleeding. Thus, new and more accurate predictive factors are needed to predict the first bleeding episode considering the importance to identify the cohort of patients who are mostly in need for prophylactic therapy. A significant factor associated with rupture of varices is an HVPG higher than 12 mmHg[10], considering that a high HVPG relates directly to a high variceal wall tension. Goulis *et al*[11] have proposed that, in patients with large varices and a high wall tension, the release of endotoxin into the systemic circulation during episodes of bacterial infection results in a further increase in portal pressure through the induction of endothelin and possibly vasoconstrictive cyclo-oxygenase products. Furthermore, endotoxin-induced nitric oxide and prostacyclin could inhibit platelet aggregation, thus resulting in variceal rupture. Patients with cirrhosis and bacterial infection demonstrate a heparin effect using heparinase I-modified thromboelastography and have anti-Xa activity[12,13]. A heparin effect was reported immediately after the bleeding episode in patients with liver cirrhosis suggesting a possible association with continued variceal bleeding or early rebleeding[14].

PRE-PRIMARY PROPHYLAXIS

The rate of development of varices in patients with cirrhosis and no varices at initial endoscopy is 8% per year[4] and the strongest predictor for their development is a HVPG higher than 10 mmHg[4]. In a large RCT[15] of 213 patients with cirrhosis and portal hypertension (minimal HVPG of 6 mmHg), the effect of NSBBs (timolol) on the development of esophageal varices or the occurrence of variceal bleeding was assessed (timolol-group, n = 108; placebo-group, n = 105). During follow-up (mean 54.9 mo), no significant difference was observed between the timolol-group and the placebo-group, regarding development of varices (39% *vs* 40%, respectively; P = 0.89). Serious adverse events were more common in the timolol group (18% *vs* 6%, P = 0.006). However, the development of varices was less frequent in patients with a baseline HVPG lower than 10 mmHg and in those with a decrease of HVPG $\geqslant$ 10% at one year. Thus, NSBBs reduce portal pressure; however, they seem to have no effect on the development of varices. According to current evidence, the use of NSBBs in patients with cirrhosis and no varices is not recommended for the prevention of their development[4]. Treatment of the underlying liver disease may decrease portal hypertension and prevent its clinical complications, according to the recent Baveno consensus[3].

Development of large varices in patients with small

varices at initial endoscopy occurs at a rate of 8% per year[4]. The factors associated to the growth of small varices are decompensated liver cirrhosis (Child-Pugh class B or C), alcoholic etiology of cirrhosis and the presence of red wale marks at initial endoscopy[4]. The efficacy of NSBBs on preventing the progression of small to large varices is debated[16,17]. In a randomized double-blind controlled trial[16] aiming to evaluate propranolol in the prevention of the development of large varices in patients with cirrhosis and small or no varices, 102 patients were randomized to receive propranolol (160 mg/d) and 104 to receive a placebo. The proportion of patients with large varices was 31% in the propranolol group and 14% in the placebo group ($P < 0.05$), at 2 years. However, one third of patients were lost to follow-up after 2 years. In a placebo-controlled trial[17], 161 patients with cirrhosis and small esophageal varices were randomized to nadolol ($n = 83$) or placebo ($n = 78$). The dose of nadolol was adjusted to decrease heart rate by 25%. During follow-up (mean: 36 mo), 9 and 29 patients from nadolol and placebo group respectively, developed large varices. At the end of follow-up, the cumulative risk was 20% *vs* 51% ($P < 0.001$). In addition, the cumulative probability of variceal bleeding was lower in the nadolol group ($P = 0.02$), but there was no difference in survival between groups ($P = 0.33$). Treatment withdrawal because of adverse effects was higher in the nadolol group ($P = 0.01$).

According to current treatment guidelines[4], in patients with cirrhosis and small varices that have not bled but with increased risk of bleeding, NSBBs are recommended. In cases of low risk for variceal bleeding, NSBBs can be used, although their long-term benefit has not been well established[4].

PRIMARY PREVENTION OF VARICEAL BLEEDING

Both shunt surgery and sclerotherapy have been abandoned for primary prevention, mainly because of the high incidence of complications[18-21]. According to Baveno V consensus[3], the current treatment strategies for medium/large-sized varices are NSBBs or EBL, which are both effective in decreasing rates of bleeding and mortality. NSBBs are splachnic vasoconstrictors which reduce portal pressure and increase portal resistance through a decrease in portal venous inflow[4]. Endoscopic treatments have no effect on portal circulation as they act locally by obliteration of varices.

NSBBs vs no intervention

Nine randomized clinical trials enrolling 966 patients compared NSBBs with a non-active treatment[22]. The incidence of bleeding was significantly reduced (OR = 0.54, 95%CI: 0.39-0.74), particularly in patients with medium-sized or large varices or in patients with varices and HVPG higher than 12 mmHg. The number needed to treat (NNT) to prevent one bleeding episode was 11. However, only a trend towards reduced mortality was observed (OR = 0.75, 95%CI: 0.57-1.06). In another meta-analysis[23] which analyzed data from four randomized trials (286 patients received b-blockers-propranolol in 203 and nadolol in 83-and 303 patients received placebo), the mean percentage of patients without upper gastrointestinal bleeding after two years was 78% ± 3% in the treatment group and 65% ± 3% in the placebo group ($P = 0.002$), whereas the 2-year survival rate was 71% ± 3% and 68% ± 3%, respectively ($P = 0.34$). The efficacy of b-blockers in the prevention of bleeding or bleeding-related mortality was the same, independently of the cause and severity of cirrhosis, ascites and size of varices. However, when propranolol is discontinued, the risk of variceal hemorrhage returns to what would be expected in an untreated population[24].

The hemodynamic response to treatment with b-blockers is considered appropriate when HVPG is decreased below 12 mmHg or by ⩾ 20% of baseline values, 1-3 mo after initiation of treatment. The acute hemodynamic response to b-blockers (20 min after administration of propranolol) was shown useful to predict the long-term risk of first bleeding by reducing HVPG ⩾ 10% from baseline values[25,26].

In a recent study[27], patients with esophageal varices with HVPG measurement before and during propranolol treatment were included. HVPG responders were kept on propranolol (PROP group), and non-responders were treated with carvedilol (CARV group). HVPG responders were 36% (37/104), whereas 56% (38/67) non-responders achieved hemodynamic response with carvedilol (the remaining patients were treated with EBL). Carvedilol achieved a greater decrease in HVPG compared to propranolol (-19% ± 10% *vs* -12% ± 11%, respectively, $P < 0.001$). During a 2-year follow-up, bleeding rates were 11%, 5% and 25% for PROP, CARV and EBL, respectively ($P = 0.0429$). Hemodynamic responders showed lower mortality compared to the EBL group patients (PROP 14%/CARV 11% *vs* EBL 31%, $P = 0.0455$). Thus, it seems that carvedilol is more efficient than propranolol to decrease HVPG and it was recently suggested that it might be the beta blocker of choice for portal hypertension[28].

NSBBs have also the potential to protect against spontaneous bacterial peritonitis (SBP) in cirrhotic patients, considering that infection is a risk factor for variceal bleeding[11]. In a meta-analysis of three RCTs and three retrospective studies[29] (including 644 patients, 257 treated with propranolol and 387 receiving no treatment), b-blockers were evaluated against no treatment for the prevention of SBP. There was a statistically significant difference of 12.1% (95%CI: 5.5-18.8; $P < 0.001$) favoring propranolol. The NNT to prevent an additional episode of SBP was 8. In addition, NSBBs can protect against bleeding from portal hypertensive gastropathy by reducing cardiac output and inducing splachnic arterial vasoconstriction[30], whereas endoscopic treatments have no effects on portal inflow or resistance.

However, there are safety issues on the use of NSBBs in patients with cirrhosis and refractory ascites[31,32]. In a

self-control cross-over study[32], 10 patients with cirrhosis and refractory ascites treated with beta-blockers were evaluated regarding the development of paracentesis-induced circulatory dysfunction (PCID defined as an increase in plasma renin concentrations 1 wk after paracentesis). Patients underwent two clinical and biological assessments: first while receiving NSBBs and second after NSBBs discontinuation. Eight patients (80%) treated with NBBs developed PCID whereas only one patient developed PCID after beta-blocker discontinuation. Thus, a RCT comparing EBL and NSBBs in patients with refractory ascites is needed to determine the use of EBL as preferred prophylactic treatment in this subgroup of patients.

EBL vs no intervention

EBL has substituted sclerotherapy and it is the endoscopic procedure of choice in primary prevention. Meta-analysis of eight RCTs[33] showed that EBL is superior to no intervention in reducing both the risk of first variceal bleeding (OR = 0.3, 95%CI: 0.17-0.53) and mortality (OR = 0.42, 95%CI: 0.3-0.6). However, there are safety issues concerning EBL in primary prophylaxis. In a trial by Triantos *et al*[33], EBL *vs* no treatment was compared in cirrhotics with intolerance or contraindications to b-blockers. The trial had to stop prematurely due to increased bleeding. Sixty percent of the bleeding was probably iatrogenic and the authors suggested that EBL might be as harmful as sclerotherapy regarding primary prevention. However, in a prospective cohort study[34], patients with contraindications, intolerance or not responding to beta-blockers who were treated with EBL achieved protection from variceal bleeding comparable to that of good responders to beta-blockers. Furthermore, in another RCT[35,36], which compared EBL (n = 75) with propranolol (n = 77) for primary prophylaxis in cirrhotic patients with varices > 5 mm, 5 patients (6.7%) bled from ligation ulcers and the treatment-related mortality was 2.6% (n = 2/5).

EBL vs NSBBs

A recent meta-analysis[37] included 19 RCTS with 1504 patients (731 treated with EBL and 773 with NSBBs-propranolol in 17 trials, nadolol in one and carvedilol in one). In total, 24% (n = 176) randomized to EBL *vs* 23% (n = 177) randomized to NSBBs died and meta-analysis showed no difference in mortality between the two treatment groups (RR = 1.09, 95%CI: 0.92-1.30). Upper gastrointestinal bleeding was diagnosed for 14% (n = 103) with EBL and 20% (n = 158) with NSBBs. EBL appeared to be superior to NSBBs for this outcome (RR = 0.68, 95%CI: 0.52-0.90). EBL also had lower rate of variceal bleeding compared to NSBBs [13% (75/590) *vs* 19% (113/611), RR = 0.66, 95%CI: 0.45-0.96]. However, when analysis included trials with adequate randomization or full papers, EBL showed no superiority to NSBBs for gastrointestinal or variceal bleeding. No difference was seen between the two interventions regarding bleeding-related mortality [5.1% (29/567) *vs* 6.3% (37/585); RR = 0.85, 95%CI: 0.53-1.39]. Treatment with NSBBs was associated with dizziness, hypotension, impotence, lethargy, and peripheral edema, whereas EBL was associated with clinically important bleeding and retrosternal pain.

Combined treatment strategies

Gheorghe *et al*[38] randomly assigned 72 patients with high-risk esophageal varices listed for liver transplantation to combined treatment of EBL plus propranolol or propranolol monotherapy. During a mean follow-up of 8 mo, bleeding occurred in 6% patients in the combination group and 31% in the monotherapy group (P = 0.03), with 96% and 69% actuarial probability of bleeding-free survival after follow-up, respectively (P = 0.04). The authors suggested that combined treatment was superior to propranolol monotherapy regarding both bleeding and bleeding-related mortality. On the contrary, Lo *et al*[39] found no differences in upper gastrointestinal bleeding [26% (n = 18) *vs* 18% (n = 13), P = not significant], variceal bleeding [14% (n = 10) *vs* 13% (n = 9), P = not significant], and mortality [22.9% (n = 16) in both treatment groups] between patients treated with EBL combined with nadolol (n = 70) and nadolol alone (n = 70). Patients in the combination group showed a higher rate of adverse events than in nadolol monotherapy (68% *vs* 40%, P = 0.06). Two episodes of variceal bleeding were induced by EBL.

One RCT[40] of 144 patients (11.8% non-cirrhotic portal hypertension), has compared EBL combined with propranolol with EBL monotherapy. In this trial, the probability of bleeding, overall mortality and bleeding-related mortality were comparable between groups. Therefore, according to current evidence, combination treatment of EBL and NSBBs is not recommended for primary prevention.

Isosorbide mononitrate

Isosorbide mononitrate (IsMn) decreases portal pressure by lowering the intra-hepatic resistance through vasodilation and has been evaluated in cirrhosis considering the large number of patients with contraindications or intolerance to b-blockers[41]. The evidence concerning the use of IsMn for primary prevention of variceal bleeding is debatable[42,43]. In a recent meta-analysis[44] the effect of IsMn in primary prevention of variceal bleeding was assessed, comparing IsMn alone *vs* placebo or beta-blockers or EBL and IsMn plus beta-blockers *vs* beta-blockers or EBL. No differences in mortality were observed between IsMn and beta-blockers *vs* β-blockers (49/277 *vs* 50/275; RR = 0.95; 95%CI: 0.68-1.32), or EBL (6/31 *vs* 8/30; RR = 0.73; 95%CI: 0.29-1.84). IsMn increased the risk of bleeding compared to placebo (RR = 2.34; 95%CI: 1.10-4.97) or EBL (RR = 4.33; 95%CI: 1.57-11.92). There were no apparent differences between bleeding rates of patients randomized to IsMn alone or with beta-blockers *vs* beta-blockers or EBL. Meta-analyses of variceal bleeding found a negative effect of IsMn compared to EBL (RR = 3.31; 95%CI: 1.01-10.84), but no apparent dif-

Table 1 Summary on primary prevention of esophageal variceal bleeding

		Management	Goal of treatment
Cirrhosis		Diagnostic endoscopy for the presence of varices	
No varices		Endoscopic surveillance	Surveillance for development of varices (every 2-3 yr in compensated cirrhosis/yearly in cases of decompensation)
Small varices	Low risk of bleeding	Endoscopic surveillance Or NSBBs	Surveillance for progression of varices (every 1-2 yr in compensated cirrhosis/yearly in cases of decompensation)
	Increased risk of bleeding[1]	NSBBs	Decrease in HVPG of at least 20% from baseline or ⩽ to 12 mmHg or resting heart rate of about 55 to 60 beats/min
Medium-large varices		NSBBs Or EBL[2]	NSBBs: Decrease in HVPG of at least 20% from baseline or ⩽ to 12 mmHg or resting heart rate of about 55 to 60 beats/min EBL: Variceal obliteration

[1]Child-Pugh B/C cirrhosis or red signs at initial endoscopy; [2]The choice of treatment should be based on local resources and expertise, patient preference and characteristics, side effects, and contra-indications. NSBBs: Non-selective beta blockers; EBL: Endoscopic band ligation; HVPG: Hepatic venous pressure gradient.

ference in variceal bleeding for the remaining treatment comparisons was observed. No effects on bleeding-related mortality were seen for any of the treatment comparisons assessed. Combination of IsMn and beta-blockers increased the risk of adverse events, compared to beta-blockers monotherapy (RR = 1.65, 95%CI: 1.25-2.17), as well as the number of treatment withdrawal (RR = 2.60, 95%CI: 1.55-4.38). Consequently, current evidence does not support the use of nitrates in primary prevention of variceal bleeding.

CONCLUSION

Baveno V[3] recommends both EBL and NSBBs for the prevention of first variceal bleeding (Table 1); however, there is a controversy on which one should be the first choice. Both therapies are equally effective and have no survival difference. Thus, other issues should be considered in order to determine the best therapeutic approach. Prophylactic treatment should have few adverse events, be easy to administer and inexpensive. EBL can cause fatal iatrogenic bleeding, is accompanied by increased expense, needs specialized staff and cannot prevent bleeding from portal hypertensive gastropathy. NSBBs could probably be the first choice in primary prevention, whereas EBL could be reserved for patients with contra-indications, not response, intolerance to NSBBs or lack of compliance to life-time use of drugs. The potent benefit of EBL on patients with refractory ascites should be further investigated.

Lastly, there are issues on the primary prevention of variceal bleeding that require further study including the use of carvedilol, the advancement in ligation devices with better endoscopic field of view and the evaluation of novel therapeutic agents.

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REVIEW

Management of cytomegalovirus infection and disease in liver transplant recipients

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Abstract

Cytomegalovirus (CMV) is one of the most common viral pathogens causing clinical disease in liver transplant recipients, and contributing to substantial morbidity and occasional mortality. CMV causes febrile illness often accompanied by bone marrow suppression, and in some cases, invades tissues including the transplanted liver allograft. In addition, CMV has been significantly associated with an increased predisposition to acute and chronic allograft rejection, accelerated hepatitis C recurrence, and other opportunistic infections, as well as reduced overall patient and allograft survival. To negate the adverse effects of CMV infection on transplant outcome, its prevention, whether through antiviral prophylaxis or preemptive therapy, is an essential component to the management of liver transplant recipients. Two recently updated guidelines have suggested that antiviral prophylaxis or preemptive therapy are similarly effective in preventing CMV disease in modest-risk CMV-seropositive liver transplant recipients, while antiviral prophylaxis is the preferred strategy over preemptive therapy for the prevention of CMV disease in high-risk recipients [CMV-seronegative recipients of liver allografts from CMV-seropositive donors (D+/R-)]. However, antiviral prophylaxis has only delayed the onset of CMV disease in many CMV D+/R- liver transplant recipients, and such occurrence of late-onset CMV disease was significantly associated with increased all-cause and infection-related mortality after liver transplantation. Therefore, a search for better strategies for prevention, such as prolonged duration of antiviral prophylaxis, a hybrid approach (antiviral prophylaxis followed by preemptive therapy), or the use of immunologic measures to guide antiviral prophylaxis has been suggested to prevent late-onset CMV disease. The standard treatment of CMV disease consists of intravenous ganciclovir or oral valganciclovir, and if feasible, reduction in pharmacologic immunosuppression. In one clinical trial, oral valganciclovir was as effective as intravenous ganciclovir for the treatment of mild to moderate CMV disease in solid organ (including liver) transplant recipients. The aim of this article is to provide a state-of-the art review of the epidemiology, diagnosis, prevention, and treatment of CMV infection and disease after liver transplantation.

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Key words: Cytomegalovirus; Outcome; Hepatitis; Transplantation; Valganciclovir; Prophylaxis; Treatment

Core tip: This paper summarizes the current state in the management of cytomegalovirus disease after liver transplantation, including a review of recently updated guidelines for diagnosis, prevention and treatment.

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INTRODUCTION

Cytomegalovirus (CMV) is the single most common viral pathogen that influences the outcome of liver transplantation[1,2]. CMV is a ubiquitous herpes virus that, depending on the population studied, infects 50%-100% of humans[1,2]. Primary CMV infection in immune competent individuals presents most commonly as an asymptomatic illness or less commonly as a benign infectious mononucleosis-like syndrome. When CMV infection occurs in individuals with compromised immunity, such as liver transplant recipients, clinical disease with high morbidity may develop and, occasionally, may lead to death if untreated[1,2].

Primary infection results in viral latency in various cells, and ensures the persistence of the virus throughout the life of the host[1,2]. Such characteristic plays an important role in how liver recipients develop CMV infection. First, cellular sites of viral latency become reservoirs for reactivation during periods of inflammation (such as allograft rejection and critical illness). And second, cellular sites of viral latency serve as vehicles for transmission to susceptible hosts (*i.e.*, during blood transfusions and transplantation of liver allografts latently infected with CMV)[1-5].

CLINICAL IMPACT OF CMV ON LIVER TRANSPLANTATION

Direct CMV effects

The classic illness caused by CMV after liver transplantation is manifested most commonly as fever and bone marrow suppression (most commonly, leukopenia and neutropenia, termed CMV syndrome). CMV syndrome accounts for over 60% of CMV diseases after liver transplantation. Less commonly, CMV infection may clinically manifest as tissue-invasive disease (which may involve any organ system) (Table 1)[1]. The most common organ system involved is the gastrointestinal tract (in the form of CMV gastritis, esophagitis, enteritis, and colitis). Gastrointestinal CMV disease accounts for over 70% of tissue-invasive CMV disease cases in liver and other solid organ transplant recipients[6]. The transplanted liver allograft is also predisposed to develop tissue-invasion by CMV (*i.e.*, CMV hepatitis), and this is often manifested with symptoms that may be clinically indistinguishable from acute rejection[7].

CMV disease among liver recipients who are not receiving antiviral prophylaxis occur most commonly during the first 3 mo after transplantation[8]. Overall, it is estimated that 18%-29% of all liver transplant recipients will develop CMV disease in the absence of prevention strategy (Table 2)[4,5,9-11]. However, this incidence varies depending upon donor and recipient CMV serologic status; it may be as high as 44%-65% in CMV D+/R-, or as low as 1%-2% among CMV D-/R- patients (who may still acquire the virus from natural transmission or through blood transfusion). The incidence is between 8%-19% among CMV-seropositive (CMV R+) liver transplant recipients[4,9,11].

Table 1 Direct and indirect clinical effects of cytomegalovirus after liver transplantation

Direct effects	Indirect effects
CMV syndrome	Acute allograft rejection
Fever	Chronic allograft rejection
Myelosuppression	Vanishing bile duct syndrome
Malaise	Chronic ductopenic rejection
Tissue-invasive CMV disease[1]	Hepatitis C virus recurrence
Gastrointestinal disease (colitis, esophagitis, gastritis, enteritis)	Allograft hepatitis, fibrosis
	Allograft failure
	Opportunistic and other infections
Hepatitis	Fungal superinfection
Pneumonitis	Nocardiosis
CNS disease	Bacterial superinfection
Retinitis	Epstein-Barr virus and PTLD
Mortality	HHV-6 and HHV-7 infections
	Vascular thrombosis
	New onset diabetes mellitus
	Mortality

[1]Any organ system may be affected by cytomegalovirus (CMV). Data adapted from Ref. [104]. PTLD: Post-transplant lymphoproliferative disease; HHV: Human herpes virus.

Table 2 Estimated incidence of cytomegalovirus disease during the first 12 mo after liver transplantation

	Use of anti-CMV prophylaxis for 3-6 mo	
	Yes[1]	No
CMV D+/R-	12%-30%	44%-65%
CMV D+/R+	2.70%	18.20%
CMV D-/R+	3.90%	7.90%
CMV D-/R-	0%	1%-2%
All patients	4.80%	18%-29%

[1]Most cases occur as delayed-onset cytomegalovirus (CMV) disease. CMV disease occurs rarely during prophylaxis with oral valganciclovir. Data adapted from Ref. [4,5,92,104]. D: Donor; R: Recipient.

The incidence of CMV disease is markedly reduced in liver transplant recipients who received 3 mo of valganciclovir or oral ganciclovir prophylaxis. The CMV disease incidence rates are 12%-30% in CMV D+/R-, and < 10% of CMV R+ liver transplant recipients who received 3 mo of antiviral prophylaxis[3,4,9,11-13]. The onset of disease in these patients occurs during first 3-6 mo after completing antiviral prophylaxis; hence, the term late-onset CMV disease[3]. To reduce the incidence of late onset CMV disease, there have been efforts to prolong prophylaxis to 6 mo in CMV D+/R- liver recipients. There is limited data available on the incidence of late-onset CMV disease after 6 mo of prophylaxis, although this is estimated to be further reduced by half (*e.g.*, about 15% of CMV D+/R- liver recipients).

Indirect CMV effects

CMV has a variety of indirect effects that are believed to be mediated by the ability of the virus to modulate the immune system (Table 1)[1,2]. CMV is a potent up-regu-

Table 3 Actors associated with increased risk of cytomegalovirus disease after liver transplantation

CMV D+/R- > CMV R+
Allograft rejection
High viral replication
Mycophenolate mofetil
Anti-thymocyte globulin
Alemtuzumab
Human herpesvirus-6
Human herpesvirus-7
Renal insufficiency
Deficiency in CMV-specific CD4+ T cells
Deficiency in CMV-specific CD8+ T cells
Toll-like receptor gene polymorphism
Mannose binding lectin deficiency
Chemokine and cytokine defects (IL-10, MCP-1, CCR5)
Expression of immune evasion genes
Programmed cell death 1 expression
Others[1]

[1]Others include re-transplantation, volume of blood transfusion, sepsis and other factors associated with high tumor necrosis factor-α secretion. D: Donor; R: Recipient; IL-10: Interleukin-10; MCP-1: Monocyte chemotactic protein-1; CCR5: Chemokine (C-C motif) receptor 5; CMV: Cytomegalovirus.

lator of alloantigens, which increases the risk of acute rejection and chronic allograft dysfunction[14]. CMV has been associated with vanishing bile duct syndrome and ductopenic rejection that leads to chronic cholestasis and allograft failure[15-17]. A higher incidence of vascular and hepatic artery thrombosis has been reported in liver recipients with CMV disease, and this effect is postulated to result from infection of the vascular endothelial cells[18,19].

The immunomodulatory effects of CMV may account for a higher predisposition to develop opportunistic infections due to fungi, other viruses, and bacteria[20,21]. CMV-infected transplant recipients are more likely to develop Epstein-Barr virus-associated post-transplant lymphoproliferative disorders, or develop co-infections with other viruses such as human herpesvirus (HHV)-6 and HHV-7[20-22]. Co-infection with HHV-6 and HHV-7 is significantly associated with an increased predisposition to CMV disease[23-25]. Similarly, there is a significant association between CMV and hepatitis C virus (HCV) recurrence after liver transplantation[26-31], and this is clinically manifested as a more accelerated clinical course of HCV recurrence[29,31]. A recent retrospective study of 347 HCV-infected liver recipients observed that CMV infection increased by 1.5 times the risk of allograft fibrosis, while CMV disease increased by 3.4 times the risk of allograft inflammation[32]. A significant association between CMV infection and metabolic disease such as post-transplant diabetes mellitus has been reported. In a recent study of 169 non-diabetic liver recipients, CMV infection was a significant risk factor for development of new-onset diabetes after transplantation[33].

Impact on mortality

Through direct, indirect and possibly immunomodulatory mechanisms, CMV is associated with higher risk of death after liver transplantation[20,34,35]. The use of intravenous (IV) and oral ganciclovir has reduced the incidence of CMV disease and the risk of death due to CMV[20,36-38]. Despite these improvements in CMV prevention with use of antiviral drugs, late-onset CMV disease continues to occur, particularly among CMV D+/R- liver transplant recipients. Notably, late-onset CMV disease remains significantly associated with increased risk of mortality after liver transplantation[35]. In an analysis of 437 liver transplant recipients, CMV disease occurred in 37 patients (8.5%) and its occurrence was independently associated with a 5-fold increased risk of all-cause mortality, and 11-fold increased risk of infection-related mortality[35].

RISK FACTORS FOR CMV DISEASE AFTER LIVER TRANSPLANTATION

Lack of pre-existing CMV-specific humoral immunity

The most important risk factor for CMV disease after liver transplantation is a lack of effective CMV-specific immunity. In the clinical setting, this is best measured by serology to detect immunoglobulin G against CMV. Specifically, CMV D+/R- patients are at highest risk of CMV disease[4,20], while CMV R+ patients have modest and CMV D-/R- have the lowest risk of CMV disease after liver transplantation (Table 3).

Drug-induced suppression of immune function

Drug-induced immunosuppression impairs the ability of liver recipients to mount an effective immune response against CMV, thereby predisposing to higher risk of CMV disease[4,20]. Immune dysfunction is particularly intense with the use of lymphocyte-depleting drugs, as either induction or rejection therapy[39,40]. When alemtuzumab, an anti-CD52 lymphocytic antibody, is used for short-course induction therapy, the risk of CMV disease is not significantly increased[41,42]. However, when alemtuzumab is used as treatment for rejection, the risk of CMV disease is higher suggesting that rejection per se also increases the risk[42]. Basiliximab and daclizumab are associated with lower risk of CMV disease compared to anti-thymocyte globulin[43].

The combined effects of drugs for maintenance immunosuppression have been associated with CMV disease[1,2,20], although specific agents such as mycophenolate mofetil, when used at high doses has also been implicated to increase the risk[44,45]. In contrast, some of the newer immunosuppressive drugs such as sirolimus and everolimus [mammalian target of rapamycin (mTOR) inhibitor] have been associated with lower risk of CMV disease[46,47]. These observations have generated special interest in the use of the mTOR agents for patients at high risk of CMV disease.

Defects in innate immunity

Inherent defects in innate immunity, such as mutations

in innate immunity-associated genes, increase the risk of CMV disease (Table 3). In a pilot study in 92 liver recipients with chronic HCV, the R753Q single nucleotide polymorphism (SNP) in the Toll-like receptor 2 (*TLR2*) gene was associated with a higher CMV replication and higher incidence of CMV disease. TLR2 is a pattern recognition receptor that senses the presence of CMV and signals the immune cells to produce antiviral peptides and cytokines; the R753Q SNP impairs this immunologic cascade[48]. A larger study of 737 liver recipients confirmed that TLR2 R753Q SNP was significantly and independently associated with CMV disease after liver transplantation, especially for tissue-invasive disease[49].

The lectin pathway of complement activation is also important in the innate immune response to CMV. Mannose binding lectin levels or mutation in its gene has been assessed as prognostic indicators of CMV disease after transplantation[50]. In a study of 295 liver recipients, whose donors were also genotyped for SNPs in mannose-binding lectin (MBL2), Ficolin-2 (FCN2) and MBL-associated serine protease genes, the risk of CMV infection was 2.77 fold higher with the gene profile of the donor and 4.57 fold higher for the combined MBL2 and FCN2 donor-recipient mismatch profile. These results were independent from donor-recipient CMV serostatus[51].

Other immune measures, such as programmed death-1 expression[52] have also been assessed for their association with CMV infection. In one study, programmed death-1 receptor up-regulation was significantly associated with incipient and overt CMV disease and with CMV viremia[52].

Lack of CMV-specific cell-mediated immunity

Cell-mediated immunity are the most essential components to the control of CMV after liver transplantation[40]. Hence, measuring CMV-specific cell-mediated immunity is a promising strategy in CMV management after transplantation[53]. In one study, secretion of interferon-γ by $CD8^+$ T cells during *in vitro* stimulation with CMV peptides was associated with a lower incidence of CMV disease in solid organ transplant recipients (including liver recipients)[54]. A variety of CMV-specific T-cell assays are currently being developed including QuantiFERON-CMV assay, ELISpot assay, and intracellular cytokine staining for IFN-γ using flow cytometry. The principle of these assays relies on the detection of cytokine (most commonly interferon-γ) production following *in vitro* stimulation with CMV antigens[55]. Recently, QuantiFERON-CMV assay was studied in a multi-center study that enrolled 124 high-risk (D+/R-) solid-organ transplant (including liver) recipients. Twenty five percent of patients had positive result, 65.3% had a negative result, and 9.7% had an indeterminate result. At 12 mo follow-up, patients with a positive QuantiFERON-CMV assay had a significantly lower risk of CMV disease (6.4%) compared to those with negative (22.2%) and indeterminate result (58.3%). The assay provides a positive and negative predictive values for protection from CMV disease of 0.90 (95%CI: 0.74-0.98) and 0.27 (95%CI: 0.18-0.37), respectively[53,56]. Collectively, these studies indicate that immune monitoring of CMV-specific T-cell responses may have a potential to predict individuals at increased risk of CMV disease, and may be useful in guiding the use of prophylaxis.

Allograft rejection

Allograft rejection can trigger CMV reactivation after transplantation[13]. The cytokines released during acute rejection, particularly tumor necrosis factor-α[57], could transactivate CMV from latency[58,59]. Subsequent therapy for allograft rejection (intensified immunosuppression with the use of high doses of steroids or lymphocyte-depleting drugs) enhances viral replication by impairing the generation of an effective CMV-specific cell-mediated immunity[60]. In a bidirectional relationship, CMV increases the risk of allograft rejection[61].

Virus-to-virus interactions

Interactions among reactivated viruses have been proposed to enhance the risk of CMV disease after liver transplantation[22,23,27-31]. HHV-6 increases the risk of CMV disease after liver transplantation[22,23,25]. Likewise, HCV-infected liver transplant patients have a higher incidence of CMV disease[62], although the data in the era of valganciclovir prophylaxis has refuted this observation[26].

Viral burden and other factors

The risk of CMV disease after liver transplantation is associated, in direct proportion, with viral burden and the degree of CMV replication[9,24,63,64]. Other factors associated with CMV disease after liver transplantation include cold ischemia time, bacterial and fungal infections and sepsis, the amount of blood loss, fulminant hepatic failure as the indication for liver transplantation, age, female gender, and renal insufficiency[2,3,20,65].

PREVENTION OF CMV DISEASE AFTER LIVER TRANSPLANTATION

There are two major strategies for CMV disease prevention after liver transplantation: (1) preemptive therapy; and (2) antiviral prophylaxis. For preemptive therapy, patients are monitored for evidence of CMV replication by sensitive assays, most commonly using quantitative nucleic acid amplification tests by PCR and less commonly by detection of pp65 antigenemia, and upon the detection of asymptomatic CMV replication, antiviral therapy is administered preemptively to prevent progression to symptomatic clinical disease. In contrast, antiviral prophylaxis entails the administration of antiviral drugs such as valganciclovir to all patients at risk of CMV disease after liver transplantation[20]. Both of these strategies are similarly effective in preventing CMV disease after liver transplantation[4,5,66-69]. However, there has not been a large prospective well-controlled randomized trial directly comparing preemptive therapy and prophylaxis in liver

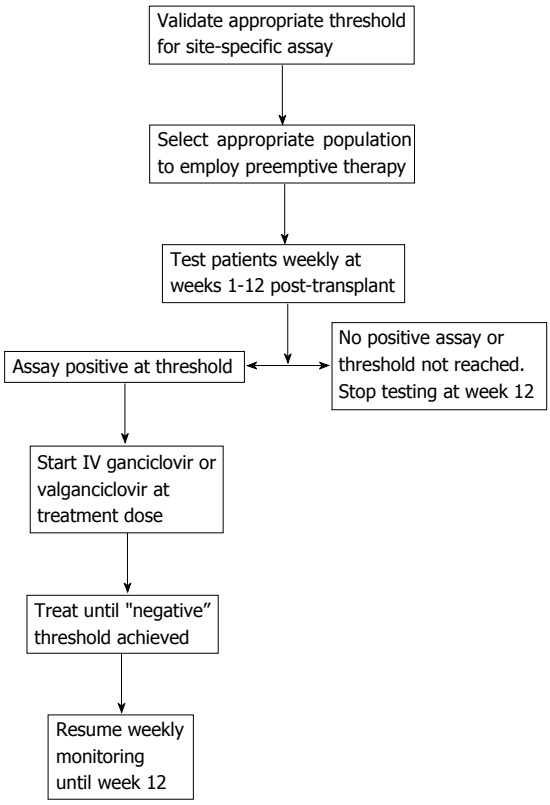

Figure 1 Suggested algorithm for preemptive therapy. Figure adapted from Ref. [62].

transplant recipients. In a retrospective study comparing the two approaches in liver transplant recipients, antiviral prophylaxis was more effective in prevention of CMV disease in high risk D+/R-, but there were no differences in acute rejection, opportunistic infections, or rate of mortality[40,70]. Another retrospective study reported the incidence of CMV viremia was 4.9% and 50.0% ($P < 0.001$) at 3 mo in the antiviral prophylaxis and preemptive therapy groups, respectively, but the rates were expectedly reversed, at 24.6% and 8.3% ($P = 0.026$), respectively at 6 mo; the reversal of the rates during the latter period accounts for the higher rates of late onset CMV disease with antiviral prophylaxis[71]. An NIH-sponsored prospective study is being conducted in six transplant centers in the United States to compare the efficacy and safety of antiviral prophylaxis *vs* preemptive therapy in CMV D+/R- liver transplant recipients.

According to the recently updated American Society of Transplantation (AST) and The Transplantation Society (TTS) guidelines, preemptive therapy may be an option in CMV D+/R- liver transplant recipients, however, many authorities prefer to use antiviral prophylaxis in this high-risk population and reserve preemptive therapy for lower-risk populations[39,72]. The main reason for this preference for antiviral prophylaxis is the rapidity of CMV replication in CMV D+/R- liver recipients, which may escape detection with once weekly CMV surveillance. Indeed, antiviral prophylaxis has been used by the majority of American and European transplant centers in preventing primary CMV disease in high-risk CMV D+/R- liver transplant recipients[73,74]. Moreover, primary antiviral prophylaxis has the added benefit of reduction in bacterial and fungal opportunistic infections and mortality[34,35,37,75].

Preemptive therapy

The basic principle of preemptive therapy is to detect the presence of early CMV replication prior to the onset of clinical symptoms, so that antiviral therapy is administered early in order to prevent the progression of asymptomatic infection to clinical disease[64,66,67,69,76]. An example of a preemptive algorithm is shown in Figure 1. Preemptive therapy has the potential advantage of targeting therapy to the highest risk patients and thereby decreasing drug costs and toxicity. The success of this approach relies on several aspects including: (1) the optimal laboratory test and frequency and duration of monitoring; (2) selection of the appropriate population for preemptive therapy; and (3) choosing the type, dose and duration of an antiviral drug.

The two laboratory methods used for CMV surveillance for preemptive therapy are pp65 antigenemia assay and nucleic acid testing (NAT). During the past decade, clinical laboratories have been moving towards preference for NAT over antigenemia, mainly for assay sensitivities, performance and logistics. The pp65 antigenemia assay, a semi-quantitative assay based on detection of CMV pp65 antigen in infected leukocytes, has comparable sensitivity to CMV NAT[77], but it needs to be processed within 6-8 h of blood collection, it requires a large sample volume, it has subjective interpretation of results, and is labor-intensive. Accordingly, quantitative NAT is now the preferred method for detecting CMV after transplantation[78]. The assay has a better precision and faster turnaround time[79]. Because of its quantitative ability, the assay can distinguish between active viral replication (typically with high-level viremia) from latent virus (low-level viremia if using highly sensitive tests)[78]. In the past, NAT lacked standardization, and this prevented the generation of widely applicable viral load thresholds for various clinical applications. In 2011, CMV viral load standardization was made possible with the release of the World Health Organization (WHO) calibrator standard. A recent study applied this assay in the plasma samples of 267 solid organ (including liver) transplant recipients. This study demonstrated that patients with pretreatment CMV DNA of less than 18200 [4.3 log (10)] IU/mL have 1.5 fold higher chance for CMV disease resolution. Likewise, CMV suppression to less than 137 [2.1 log (10)] IU/mL is predictive of clinical response to antiviral treatment[80].

The optimal interval and duration of monitoring for preemptive therapy is still unknown, but guidelines recommend once weekly CMV NAT for 12 wk after liver transplantation. If a patient shows viremia above a defined threshold during the surveillance period, antiviral therapy (with oral valganciclovir or intravenous ganciclovir) should be initiated and continued until CMV

Table 4 Currently available antiviral drugs for cytomegalovirus prophylaxis and treatment in liver transplant recipients

Drug	Route	Usual adult prophylaxis dose	Usual adult treatment dose	Comments on use and major toxicity
Ganciclovir	Intravenous	5 mg/kg once daily	5 mg/kg twice daily	Intravenous access; leukopenia
Ganciclovir	Oral	1 g three times daily	Not applicable	Low oral bioavailability; high pill burden
Valganciclovir	Oral	900 mg once daily	900 mg twice daily	Ease of administration; leukopenia
Foscarnet	Intravenous	Not recommended	60 mg/kg every 8 h (or 90 mg/kg every 12 h)	Second-line drug Intravenous access; nephrotoxicity
Cidofovir	Intravenous	Not recommended	5 mg/kg once weekly × 2 then every 2 wk thereafter	Third-line drug Intravenous access; nephrotoxicity

viremia is no longer detectable[55,72]. Several studies have reported the success of IV ganciclovir or oral valganciclovir for preemptive treatment of CMV infection in liver transplant recipients, including high-risk CMV D+/R- patients[68,76]. However, some studies have indicated that preemptive therapy may not be completely effective in CMV D+/R- liver recipients since the replication kinetics of CMV in immune-deficient individuals is so rapid[63] that it may escape detection with once weekly surveillance[9,58,66]. Indeed, in our clinical experience, nearly 25% of CMV D+/R- liver recipients who developed CMV disease were not identified early despite weekly CMV PCR assay[9,58,66]. Accordingly, the recently updated AST and TTS guidelines prefer antiviral prophylaxis in CMV D+/R- liver recipients. In contrast, preemptive therapy is recommended for preventing CMV disease in CMV-seropositive liver recipients[55,72].

Clinical trials have demonstrated the efficacy of preemptive therapy in CMV disease prevention[66-68,76]. Three meta-analyses that collectively analyzed data from prospective clinical trials demonstrated the benefits of preemptive therapy in preventing CMV disease[35,36,68]. When conducted properly, preemptive therapy, with the use of IV ganciclovir or oral valganciclovir resulted in the reduction of CMV disease by about 70%[37,38,75]. Moreover, preemptive therapy is much less likely associated with late onset CMV disease (unlike in antiviral prophylaxis, as discussed below)[66,67]. Currently, valganciclovir is the most commonly used drug for preemptive therapy[73], and in one non-controlled study, it was demonstrated to be as effective in terms of clinical and virologic response, when compared to IV ganciclovir)[66,67]. In addition, preemptive therapy may be beneficial in reducing the indirect effects of CMV, although to a much lesser degree compared to antiviral prophylaxis. In one study, the incidence of major opportunistic infections, bacteremia, bacterial infection, HCV recurrence, and rejection were not significantly different between liver transplant patients who received preemptive therapy and those who did not have CMV reactivation[81].

Antiviral prophylaxis

Antiviral prophylaxis is highly effective in preventing the direct effects, and there is increasing evidence that it reduces the indirect effects of CMV after liver transplantation[4,5,37,38,75]. Compared to placebo or no treatment, patients who received antiviral prophylaxis had lower incidence of CMV disease (58%-80% reduction) and CMV infection (about 40% reduction)[75]. In one meta-analysis, a 25% reduction in the incidence of acute allograft rejection was observed[37]. In two studies, a reduction in all-cause mortality was observed[37,75], mainly due to a decline in CMV-related death[75]. A reduction in the incidence of other herpes viruses, bacterial, and protozoan infections were also observed[37]. Because of these additional benefits, liver transplant centers prefer the use of antiviral prophylaxis over preemptive therapy in the prevention of CMV disease, particularly in CMV D+/R- liver transplant recipients[73]. Table 4 shows the currently available antiviral drugs for CMV prophylaxis and treatment in liver transplant recipients.

Valganciclovir vs ganciclovir prophylaxis

Ganciclovir-based regimen is more effective than acyclovir or immunoglobulins in reducing the incidence of CMV disease after liver transplantation. In one study, the administration of IV ganciclovir for 90-100 d reduced the incidence of CMV disease in CMV D+/R- liver transplant recipients to 5.4% (compared to 40% in patients who received less than 7 wk of prophylaxis)[44]. Oral ganciclovir, administered at 1000 mg PO three times daily, was compared to placebo, and there was a significant reduction in the 6-mo incidence of CMV infection (51.5% *vs* 24.5%, $P < 0.001$), and CMV disease (19% *vs* 5%, $P < 0.001$) in liver transplant recipients[4], including CMV D+/R- patients (44% *vs* 15%, $P = 0.02$) and patients who received antilymphocyte antibodies (33% *vs* 5%; $P = 0.002$)[4]. Among CMV R+ liver transplant recipients, oral ganciclovir for 12 wk reduced the incidence of CMV disease to 1% (compared to 7% in patients who received acyclovir)[82]. Oral ganciclovir, however, is poorly absorbed, and its oral administration results in low systemic ganciclovir levels[83].

Valganciclovir provides systemic ganciclovir levels that are comparable to IV ganciclovir[83,84]. Pharmacokinetic studies indicate that a 900 mg dose of valganciclovir achieves a similar daily area under the concentration time curve (AUC_{24}) as an IV dose of 5 mg/kg of ganciclovir[83]. The role of valganciclovir in the prevention of CMV disease after liver transplantation was evaluated in a multicenter randomized non-inferiority clinical trial that compared it with oral ganciclovir in a cohort of 364 CMV D+/R- solid organ (including liver) transplant recipients (Figure 2). Among all solid organ transplant

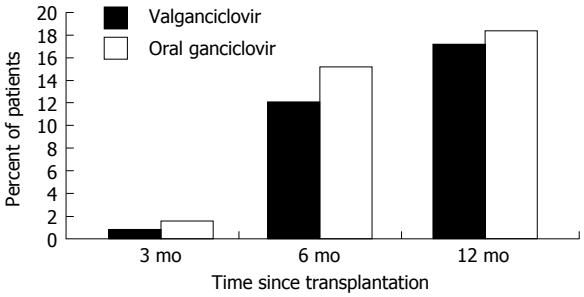

Figure 2 Time to the onset of cytomegalovirus disease in solid organ transplant recipients who received three mo of oral ganciclovir or valganciclovir prophylaxis. Data obtained from the study by Paya *et al*[5].

recipients, the 6-mo incidence of CMV disease was 12% and 15% in the valganciclovir and oral ganciclovir groups, respectively. Follow-up at one year, demonstrated that the incidence of protocol-defined CMV disease in all patients was 17% and 18% with valganciclovir and oral ganciclovir, respectively[5].

However, in a subgroup analysis of the 177 liver transplant recipients, the incidence of CMV disease was 19% in the valganciclovir group as opposed to only 12% in the ganciclovir group. There was also a higher incidence of tissue-invasive CMV disease in the valganciclovir group[5]. As a result of these findings, valganciclovir did not gain approval from the United States food and drug administration (US-FDA) for prophylaxis against CMV disease after liver transplantation. A recent meta-analysis of 5 controlled clinical studies, including 380 liver transplant recipients who received valganciclovir (450 or 900 mg daily) prophylaxis, showed the overall CMV disease rate was 12%, and the rate among D+/R- patients was 20%. The risk of CMV disease with valganciclovir was 1.8-fold higher than oral ganciclovir. For CMV D+/R- patients, the risk of CMV disease was 2-fold higher than oral ganciclovir. The risk of CMV disease remained significant with valganciclovir 900-mg daily dose, but not with the 450 mg dose. The risk of leukopenia with valganciclovir was 1.9-fold higher than those using oral ganciclovir[85]. Despite these findings, and even if not FDA-approved for this indication, valganciclovir remains as the most widely used drug for CMV prophylaxis after liver transplantation[73].

Maribavir prophylaxis

Maribavir, an investigational oral benzimidazole riboside with *in vitro* activity against CMV, was compared to oral ganciclovir, for prophylaxis in 303 high-risk liver transplant recipients. In this randomized, double blind, multicenter controlled trial, maribavir was less effective than oral ganciclovir for the prevention of CMV disease. Significantly fewer patients who received oral ganciclovir prophylaxis had confirmed CMV disease or CMV infection compared to maribavir at 100 d (20% *vs* 60%, $P < 0.0001$) and at 6 mo (53% *vs* 72%, $P = 0.0053$) after liver transplantation. Because of this finding (and the results of the bone marrow transplant trial), the clinical development of maribavir for CMV management is on hold[86].

CMV immunoglobulin

A combination of anti-CMV drugs and CMV immunoglobulin has been used in a clinical practice for prophylaxis. A pooled analysis of previous studies revealed a combination regimen may reduce severe CMV disease and mortality in solid organ transplant recipients; however the finding has been debated[87,88].

Late-onset CMV disease

In many high-risk CMV D+/R- individuals, the use of antiviral prophylaxis for 100 d has only delayed the onset of CMV disease to 3-6 mo after liver transplantation[3,5,13]. In our analysis of 67 CMV D+/R- liver transplant recipients who received 3 mo of oral ganciclovir and valganciclovir prophylaxis, the two-year incidence of CMV disease was 29%, and was similar between the two drugs (22% *vs* 28%, $P = 0.63$)[3]. The most common presentation of late-onset CMV disease was CMV syndrome, with fever and bone marrow suppression[3]. In less than half of the patients, CMV manifested as tissue-invasive disease, and frequently affected the gastrointestinal tract[3]. Factors such as age[3], female gender[3,89], renal dysfunction[77], and allograft rejection[113] predisposed to the development of late-onset primary CMV disease. Late-onset CMV disease appears to be clinically less severe, although it is associated with significant mortality after liver transplantation[35].

Because of the negative effect of late-onset CMV disease on overall outcome, a better method for CMV prevention is needed among CMV D+/R- liver transplant recipients. The recently updated AST and TTS guidelines suggest that the duration of antiviral prophylaxis may be prolonged from the standard 3 mo to 6 mo in CMV D+/R- liver transplant recipients[41,81]. This recommendation is based on the trial that investigated the approach in CMV high-risk D+/R- "kidney" transplant recipients. In the Improved Protection Against Cytomegalovirus in Transplantation study, the incidence of CMV disease was significantly lower in the 200 d *vs* 100 d of prophylaxis at the end of 1 year (16.1% *vs* 36.8%, $P < 0.0001$) and the result was persistent up to 2 years after transplantation (21.3% *vs* 38.7%, $P < 0.001$)[90,91]. In a retrospective study on 203 liver transplant recipients who received valganciclovir 900 mg daily for 3 to 6 mo, the overall incidence of CMV disease was 14%. The incidence was highest in D+/R- (26%) compared to 16% in D+/R+ group and 7% in D-/R+ group[92]. However, it is emphasized that 6 mo of antiviral prophylaxis has not yet been studied prospectively in the liver transplant recipients, and that valganciclovir is not FDA-approved for the prevention of CMV disease after liver transplantation. In addition, there are theoretical concerns about ganciclovir resistance and drug toxicity particularly with leukopenia with prolonged prophylaxis, although these were not demonstrated in the clinical trial. The cost of prophylaxis will need to be evaluated with the use of prolonged prophylaxis.

In summary, the duration of prophylaxis in D+/R- liver transplant recipients should generally be between

3 and 6 mo. For seropositive patients with either donor seropositive or seronegative, a majority of the experts suggested that 3 mo of prophylaxis is sufficient[55].

Hybrid approach

A new strategy has been utilized in some transplant centers to prevent late-onset CMV disease is hybrid strategy in which preemptive monitoring is initiated after completing prophylaxis. A retrospective study of 199 liver transplant recipients [including 23 (11%) high-risk D+/R- patients] who received 3 mo of valganciclovir prophylaxis and were monitored by CMV antigenemia after prophylaxis (twice a month up to month 6, and monthly until one year). The results were modest at best[93], possibly due to difficult and non-standardized logistics of this approach[94].

TREATMENT OF CMV DISEASE AFTER LIVER TRANSPLANTATION

The first line treatment of CMV disease after liver transplantation is IV ganciclovir or valganciclovir[62,76,93]. In contrast, oral ganciclovir should not be used for the treatment of CMV disease because of its poor bioavailability[20]. In addition, the degree of pharmacologic immunosuppression should be reduced if possible[20].

In a multi-center non-inferiority trial, 321 solid organ (including liver) transplant recipients with non-severe CMV disease were randomized to valganciclovir (900 mg twice daily) or IV ganciclovir (5 mg/kg twice daily) for a fixed 21-d course, followed by valganciclovir (900 mg once daily) maintenance treatment for 4 wk; the proportion of patients with viral eradication at 21 and 49 d were comparable in the IV ganciclovir and valganciclovir groups[93]. The overall time to viral eradication was 21 d with valganciclovir and 19 d with IV ganciclovir. The calculated viral decay was 11.5 d with valganciclovir and 10.4 d with IV ganciclovir. Likewise, clinical resolution was not different between the two groups. It was noted that patients enrolled into this trial were mostly CMV-seropositive, the majority were kidney recipients (although there were good number of liver transplant recipients), and patients with severe CMV disease were excluded. Despite these limitations, this pivotal trial now supports the use of valganciclovir for oral treatment of CMV disease, at least in selected transplant patients[93]. IV ganciclovir is preferable to valganciclovir in patients with severe or life-threatening disease, or in patients who may have a problem with gastrointestinal absorption of oral drug. In many instances, valganciclovir is used as a step-down treatment when the clinical symptoms have resolved after an initial induction treatment with IV ganciclovir.

The duration of treatment of CMV disease should be individualized[62,77]. The persistence of the virus at the end of therapy (by PCR or pp65 antigenemia) is associated with a higher risk of clinical relapse[78]. In the recent study that evaluated the role of viral load using a WHO standard calibrated assay, the degree of viral load at the time of CMV disease diagnosis and the presence or absence of viral load at the end of treatment were significantly associated with CMV disease resolution. It is now generally accepted that multiple (at least two) weekly negative CMV PCR results should be obtained before antiviral therapy is discontinued. Although this may be true for non-tissue invasive CMV syndromes, the utility of such an approach may not necessarily apply to some tissue-invasive disease, which may manifest as "compartmentalized disease"[20].

Treatment of compartmentalized CMV disease

Compartmentalized CMV disease refers to clinical syndromes wherein the virus is detected in the affected tissues but is minimally detectable or undetectable in the blood[20]. In the current era, gastrointestinal CMV disease constitutes the vast majority of tissue-invasive cases[3,8,20], and in a number of cases, especially in CMV R+ patients, this type of CMV disease is "compartmentalized". In a retrospective study, the sensitivity of pp65 antigenemia assay (defined as detection of $\geqslant$ 1 positive cells/2 $\times 10^5$ leukocytes) for diagnosis of CMV gastrointestinal disease was only 54%[79]. Such a clinical presentation is reminiscent of CMV retinitis, a very rare manifestation of tissue-invasive CMV disease after transplantation, that is often not accompanied by viremia[75,80]. This dilemma brings to the forefront the limitation of viral load monitoring in assessing duration of treatment. In our clinical practice, it is not uncommon to have negative blood PCR assay even when there remains histologic evidence of tissue invasion. Accordingly, it has been suggested to perform colonoscopy or upper endoscopy to document clearance of gastrointestinal CMV disease prior to discontinuation of therapy. However, our retrospective review of this practice suggests that this should not be generalized to all patients with gastrointestinal CMV disease. We observed that relapse of gastrointestinal CMV disease was significantly associated with extensive involvement of gastrointestinal tract at the time of diagnosis[81]. In contrast, CMV serologic conversion, degree of viral load, treatment duration, maintenance therapy, and endoscopic findings at the end of therapy were not significantly predictive of CMV relapse. Our experience indicates that endoscopic evidence of resolution of gastrointestinal disease may not be necessary in mild to moderate disease as long as sufficient therapy is provided[81].

Treatment of ganciclovir-resistant CMV disease

Ganciclovir-resistant CMV is now emerging as an important complication of prolonged antiviral drug use after transplantation[2,20,44]. Currently, ganciclovir-resistant CMV is very rarely seen in liver transplant recipients (while it is relatively more common after kidney-pancreas and lung transplantation). The estimated incidence of ganciclovir-resistant CMV after liver transplantation is < 0.5%[95,96]. Several studies have identified risk factors for ganciclovir-resistant CMV[2,20,44], including CMV D+/R- status, high levels of viral replication, potent

immunosuppressive therapy, and suboptimal ganciclovir levels. The vast majority of drug-resistant cases involve the selection of viral strains with UL97 (kinase) mutation[2,20,44,83,84]. UL97 mutation generally confers resistance to ganciclovir, although in some cases, a concomitant UL54 mutation (CMV DNA polymerase) is also observed, in which case, cross-resistance with cidofovir and/or foscarnet is likely.

Drug-resistant CMV is associated with significant morbidity and mortality, and there is a very limited number of antiviral drugs (which are often toxic) available for treatment[82]. Drug-resistant CMV should be suspected when viral load or antigenemia rises or does not decline to undetectable levels despite IV ganciclovir treatment. In our retrospective study of 225 CMV D+/R- solid organ transplant recipients who received 3 mo of valganciclovir prophylaxis, CMV disease occurred in 65 patients (29%), including four (8%) caused by drug-resistant CMV, judged by the failure of the viral load to decline to undetectable levels while on IV ganciclovir treatment. The diagnosis is confirmed by genetic analysis to demonstrate mutational changes in UL97 and UL54 genes encoding for kinase and polymerase, respectively[40,82]. In patients where foscarnet or cidofovir was used, nephrotoxicity was a major and common adverse effect[85].

Other potential drugs for treatment of multi-drug resistant CMV include the off-label use of CMV Immunoglobulin (Cytogam®), adoptive infusions of CMV-specific T cells, leflunomide (an immunosuppressive drug), and artesunate (anti-malaria drug), although data supporting their use are only anecdotal[20,86]. Leflunomide acts at the stage of viral capsid assembly, not DNA replication, and therefore there is a potential use against ganciclovir-resistant strains. A single center retrospective study including 15 solid organ transplant recipients (but not including liver recipients) with drug-resistant[20,86], CMV infection treated with leflunomide monotherapy or in combination with other drugs showed some potential utility. At least half of patients (53%) had long-term responses in terms of control of CMV viremia and recurrences. The common side effects from this medication included diarrhea, anemia, and hepatic dysfunction[97].

Maribavir has also been used for treatment of drug-resistant CMV[98]. Anecdotal use in a small case series of 9 solid organ transplant recipients infected with resistant CMV showed the individual changes varied from a rapid decrease in viral load (n = 4) to no response (n = 3) with some late response slowly decreasing CMV viremia (n = 3)[99]. It has been used as salvage therapy at a higher dose (400 mg twice daily) for drug-resistant CMV infection, with mixed results including success in treating lower initial viral loads[97]. A new phase Ⅱ trial of maribavir for salvage treatment of refractory and resistant CMV infection was launched in 2012 (ClinicalTrials.gov ID: NCT01611974).

Other investigational drugs being developed for CMV management are CMX001 and AIC246 (Letermovir). CMX001 is an orally bioavailable derivative of cidofovir with lipid acyclic nucleotide converted intracellularly to the active antiviral to avoid the high renal concentrations and nephrotoxicity[100]. It has demonstrated *in vitro* activity against CMV. It has successfully completed phase Ⅱ clinical development for the prevention of CMV infection. There is an ongoing open-label, expanded-access study, CMX001-350 (ClinicalTrials.gov ID: NCT01143181), to provide access to CMX001 for patients who had no other treatment options[101]. Optimal dosing has yet to be determined, and diarrhea is a dose-limiting adverse effect. Letermovir (AIC246) is a small-molecular-weight compound with both *in vitro* and *in vivo* anti-CMV activity. It has distinct mechanism which acts late in the CMV replication cycle *via* a mechanism by not involving polymerase. Due to a lack of a human counterpart of the viral terminase complex, target-related toxicities are not expected. It also does not affect human blood precursor cells, and thus may allow the generation and expansion of CMV specific immunity during treatment. Theoretically, this may result in a lower rate of relapse after treatment of CMV infection or disease. Antiviral efficacy of letermovir was reported in phase Ⅱ prophylaxis studies in HSCT recipients[102]. The successful use of letermovir in decreasing viral load has been reported in one case report of lung transplant recipient with drug-resistant CMV disease[103].

CONCLUSION

Remarkable advances in molecular diagnostics and therapeutics led to marked reduction in the incidence and severity of CMV disease after liver transplantation, and a parallel decline in associated morbidity and mortality. However, despite these improvements, CMV remains a common infectious complication and continues to negatively influence the outcome of liver transplantation. In addition to viral factors and pharmacologic immunosuppression, the role of innate and adaptive immune deficiencies is being recognized in the pathogenesis of CMV disease after liver transplantation. Such novel findings should provide additional avenues and opportunities for improving our management strategies. Indeed, there have been increasing evidence to support the use of immunodiagnostics, by measuring CMV-specific T cells, as a tool to predict the risk of CMV disease. Prevention of CMV with antiviral prophylaxis and preemptive therapy is effective, and a clinical trial assessing and comparing these two strategies in a head-to-head comparison in liver transplant recipients is currently being performed in the United States. The international standard for CMV viral load testing has allowed for standardization of viral load reporting, hence permitting the derivation of thresholds for preemptive and diagnostic protocols. Currently, valganciclovir prophylaxis is the most common approach for the prevention of CMV disease in CMV D+/R- and R+ liver transplant recipients. Hybrid approach of prevention (antiviral prophylaxis followed by preemptive therapy) has been utilized in some institutions among high-risk D+/R- liver transplant patients, but the efficacy is debatable due to inconsistency in the monitoring lo-

gistics. The practice of prolonging antiviral prophylaxis in D+/R- liver transplant recipients from 3 to 6 mo has been extrapolated from studies in kidney transplant recipients. IV ganciclovir and oral valganciclovir are the standard drugs for treatment of established CMV disease, although valganciclovir should be limited to patients with mild to moderate CMV disease. Oral valganciclovir should be avoided as initial therapy for patients with severe CMV disease and those with questionable gastrointestinal absorption. The duration of treatment should be individualized, depending upon clinical and laboratory parameters such as the decline of CMV load in the blood as measured by rapid and sensitive molecular standardized testing. In this context, it is generally recommended that treatment be continued until all evidence of active infection, such as positive CMV viral load, has resolved. Ganciclovir-resistant CMV and compartmentalized tissue-invasive disease (most commonly with gastrointestinal CMV disease) are emerging challenges to the management of CMV after liver transplantation. These, together with the common occurrence of late-onset CMV disease in high-risk patients, should serve as catalysts to the ongoing search for the optimal management strategy for CMV disease after liver transplantation.

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REVIEW

Clinical impact of occult hepatitis B virus infection in immunosuppressed patients

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Abstract

Occult hepatitis B infection (OBI), is characterized by low level hepatitis B virus (HBV) DNA in circulating blood and/or liver tissue. In clinical practice the presence of antibody to hepatitis B core antigen in hepatitis B surface antigen (HBsAg)-/anti-HBs-negative subjects is considered indicative of OBI. OBI is mostly observed in the window period of acute HBV infection in blood donors and in recipients of blood and blood products, in hepatitis C virus chronic carriers, in patients under pharmacological immunosuppression, and in those with immunodepression due to HIV infection or cancer. Reactivation of OBI mostly occurs in anti-HIV-positive subjects, in patients treated with immunosuppressive therapy in onco-hematological settings, in patients who undergo hematopoietic stem cell transplantation, in those treated with anti-CD20 or anti-CD52 monoclonal antibody, or anti-tumor necrosis factors antibody for rheumatological diseases, or chemotherapy for solid tumors. Under these conditions the mortality rate for hepatic failure or progression of the underlying disease due to discontinuation of specific treatment can reach 20%. For patients with OBI, prophylaxis with nucleot(s)ide analogues should be based on the HBV serological markers, the underlying diseases and the type of immunosuppressive treatment. Lamivudine prophylaxis is indicated in hemopoietic stem cell transplantation and in onco-hematological diseases when high dose corticosteroids and rituximab are used; monitoring may be indicated when rituximab-sparing schedules are used, but early treatment should be applied as soon as HBsAg becomes detectable. This review article presents an up-to-date evaluation of the current knowledge on OBI.

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Key words: Occult hepatitis B virus infection; Silent hepatitis B virus infection; Hepatitis C virus infection; Liver fibrosis

Core tip: In occult Hepatitis B infection (OBI), hepatitis B virus reactivation is more common in anti-HIV-positive subjects, in those in onco-hematological settings, in patients who undergo hemopoietic stem cell transplantation and in those treated with anti-CD20 or anti-CD52 monoclonal antibody. Reactivation may be severe and in nearly 20% of cases it may take a life-threatening course. The use of nucleot(s)ide analogues to prevent this reactivation is mandatory in hepatitis B surface antigen-negative/anti-hepatitis B core-positive patients in all conditions of strong and/or prolonged immunosuppression. We describe the characteristics of OBI in onco-hematological and rheumatological diseases, in solid cancers and in HIV infection.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem in most countries, with approximately 2 billion people worldwide with serological evidence of previous exposure to the virus, of whom nearly 300 million have HBV chronic infection and over 1 million deaths per year are due to HBV-related cirrhosis and/or hepatocellular carcinoma (HCC)[1-6].

HBV infection is identified in most cases by the presence of circulating hepatitis B surface antigen (HBsAg), but an HBsAg-negative HBV infection has also been described [Occult B infection (OBI)][7], characterized by low levels of HBV DNA in circulating blood[8,9] and/or in liver tissue[10]. OBI has also been described as a serological condition characterized by the presence of hepatitis B core antigen (anti-HBc) in the absence of HBsAg and anti-HBs (isolated anti-HBc)[7,11-15]. OBI may be observed in the window period of acute HBV infection[16] in blood donors and in recipients of blood and blood products[9,17,18], in patients with HCV chronic infection[7,19], in cryptogenic chronic hepatitis, in patients under pharmacological suppression of the immune system[20,21] and in those with immunodepression due to HIV infection; it has also been associated to the development of hepatocellular carcinoma[22-30].

It has been shown that the hepatitis B virus maintains its pro-oncogenic properties in OBI[31] and that its presence in patients with chronic hepatitis C is associated with a higher risk of disease progression and HCC development[32-36] and with a reduced response to alfa interferon treatment[37-39]. The clinical importance of OBI is also underscored by the need for nucleot(s)ide treatment to prevent the recurrence of HBV infection in HBsAg-negative/anti-HBc-positive patients in various immunosuppressive settings[40-42].

This review article presents an up-to-date evaluation of the current knowledge on OBI, focusing in particular on the clinical approach in onco-hematological and rheumatological diseases, solid cancers and HIV infection.

OCCULT HBV INFECTION AND IMMUNOSUPPRESSION

Reactivation of HBV infection in patients under immunosuppressive treatment is a well-known, life-threatening event described in HBsAg-positive patients (overt HBV infection) and in subjects with OBI[20,21,42-50]. The reactivation of HBV infection, overt or occult, is characterized by a marked enhancement of viral replication during immunosuppressive therapy, with a wide spread of HBV to uninfected hepatocytes and a substantial increase in the HBV DNA serum level followed by the restoration of the immune function after treatment withdrawal and consequent cytotoxic-T-cell-mediated necrosis of HBV-infected hepatocytes usually responsible for a hepatic flare and in some instances for liver failure and even death[42]. A schematic representation of the dynamics of serum HBV DNA and alanine aminotransferase (ALT) before and during the reactivation of OBI is shown in Figure 1.

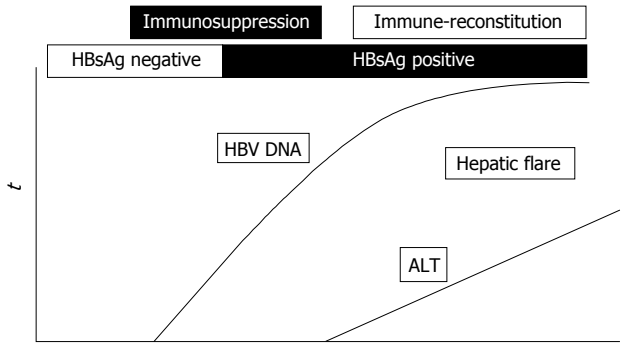

Figure 1 Virological and biochemical dynamics of reactivation of occult hepatitis B infection. HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HBsAg: Hepatitis B surface antigen.

Both in overt and occult infection the risk of HBV reactivation is estimated as high when immunosuppression is marked, particularly in onco-hematological patients (21%-67%), in those receiving hematopoietic stem cell transplantation and in those treated with the anti-CD20 monoclonal antibody rituximab or with the monoclonal anti-CD52 antibody alemtuzumab, both responsible for profound, long-lasting immunosuppression[20,21,43,51-59]. Under these conditions HBV reactivation has a mortality rate close to 20%, due to a hepatic failure or to the progression of the underlying disease due to the discontinuation of specific treatment[51,60,61]. Besides host factors, also some virological characteristics have been described as possibly associated with HBV reactivation. In 7 of 84 HBsAg-negative/anti-HBc-positive patients treated for hematological diseases or solid cancer, HBV reactivation was due to non-A HBV genotypes, core promoter and/or precore HBV mutants. In these 7 patients mutations known to impair HBsAg antigenicity were also detected[62]. A precore stop mutation (A1896) was detected in one patient with genotype Bj who developed fulminant liver failure[63]. Also sub-genotype D1 has been described as possibly associated with HBV reactivation in two studies, one from Egypt and one from Italy[21,64].

There is general agreement for the use of nucleos(t)ide analogues to prevent HBV reactivation in HBsAg-positive immunosuppressed patients, whereas it is still a matter of debate whether subjects with occult HBV infection should be treated or closely monitored for early treatment once HBsAg positivity has developed.

PHARMACOLOGICAL PROPHYLAXIS OF OCCULT HBV INFECTION IN DIFFERENT CLINICAL SETTINGS

Hematological diseases

A crucial role in the reactivation of OBI is played by the

severity and duration of immunosuppression, which in turn reflects the extent of immunodepression due to the hematological disease and of the degree of immunosuppression induced by chemotherapy. The drugs commonly responsible for HBV reactivation are those used in hematological malignancy, such as fludarabine, anthracyclines, high dose corticosteroids[51,52] and, more recently, rituximab (anti-CD20) and alemtuzumab (anti-CD52)[53].

Evidence has become available in hematological malignancy that the reactivation of occult HBV infection is frequent in patients treated with rituximab or fludarabine in the absence of lamivudine prophylaxis[21,60,61]. However, due to the retrospective nature of most studies published, the geographical differences in HBV epidemiology and the genetic differences in HBV and the host have not been investigated, and the prevalence of HBV reactivation varies widely (from 3% to 45%)[21,48,52,65-69]. The first prospective study[65] on 244 occult HBV carriers with malignant lymphoma showed a reactivation in 8 (3.3%) cases, with a higher risk of reactivation in patients receiving rituximab plus corticosteroids than in those under a rituximab-sparing schedule. In a prospective study on patients with diffuse large B-cell lymphoma (DLBCL), Yeo *et al*[20] reported reactivation of HBV infection in 5 of 21 (23.8%) patients treated with rituximab plus cyclophosphamide, adriamycin, vincristine and prednisone (R-CHOP) and in none of the 25 patients receiving only CHOP. Recently Fukushima *et al*[48] observed reactivation in 2 (4.1%) of 48 HBsAg-negative/anti-HBc-positive patients. In addition, in 150 patients with lymphoma and a resolved HBV infection who received rituximab-based chemotherapy, Hsu *et al*[70] described an incidence of HBV reactivation and of HBV hepatic flares of 10.4 and 6.4, respectively, per person per year. Matsui *et al*[71] followed up for a median period of 20.5 mo 59 patients with isolated anti-HBc and lymphoma treated with rituximab-based chemotherapy and observed HBV reactivation in 4 (6.8%).

Lower prevalences of HBV reactivation in HBsAg-negative patients after rituximab-based therapy have been reported in two studies from eastern Asia, 1.5% and 4.2%, respectively[46,72]. In another Asian study only one (2.3%) of 43 DLBCL patients treated with an R-CHOP regimen showed reactivation of HBV replication[73], for which a remission was obtained with antiviral therapy with no need to discontinue chemotherapy. Koo *et al*[74] described HBV reactivation in two (3%) of 62 HBsAg-negative/anti-HBc-positive patients treated with rituximab-based chemotherapy who did not undergo anti-HBV prophylaxis. More recently, the Asia Lymphoma Study Group investigated for HBV reactivation HBsAg-positive patients and HBsAg-negative/HBcAb-positive patients who received rituximab-based chemotherapy; the study was retrospective and performed on 340 patients, with a reactivation rate of 2.4% in subjects with OBI and 27.8% in HBsAg-positive patients[75].

The different frequency of cases with reactivation of occult HBV infection in different countries may explain, at least in part, the discordance in different national guidelines on lamivudine prophylaxis, some of which indicate the use of this nucleoside analogue for a pharmacological prophylaxis of HBsAg-negative/anti-HBc-positive patients undergoing highly immunosuppressive treatment for onco-hematological diseases[76], and others conclude that the information available does not allow any routine prophylaxis to be recommended for these patients[77].

The data of a recent meta-analysis, however, suggest that rituximab-based chemotherapy increases the risk of HBV reactivation in HBsAg-negative/anti-HBc-positive patients with non-Hodgkin lymphoma[78], an observation of clinical importance to be taken into consideration for future guidelines.

Rheumatological disease

Biological therapies targeting tumor necrosis factor-alpha (TNF-α) have been used increasingly over the last decade to treat various immune-mediated inflammatory diseases such as rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease[42]. Studies carried out over this period showed that monoclonal antibodies against TNF-α (anti-TNF-α) and high doses of steroid treatment may induce HBV reactivation in patients with overt HBV infection[79-82], thus suggesting the need for anti-HBV pharmacological prophylaxis for inactive HBsAg carriers[83] and treatment for patients with HBsAg-positive chronic hepatitis.

The reactivation of OBI during anti-TNF therapy has not been extensively investigated and the data available are anecdotal and mostly from case reports. In a recent evaluation of the literature data, HBV reactivation was found in only 8 (1.7%) of 468 HBsAg-negative/anti-HBc-positive patients with rheumatological diseases treated with anti-TNF[82]. In addition, none of 20 HBsAg-negative/HBV DNA-negative/anti-HBc-positive patients receiving anti-TNF-α for rheumatoid arthritis and spondyloarthropathy experienced reactivation of OBI during a 4-year follow up[84].

Solid cancers

The literature data give evidence of HBV reactivation in HBsAg-positive patients treated with chemotherapy for solid tumors[85-87], and, consequently, pharmacological prophylaxis for inactive HBsAg carriers and therapy for patients with HBsAg-positive chronic hepatitis is recommended. Instead, the studies on HBV reactivation in patients with OBI are not conclusive, but so far no evidence of reactivation of OBI in these patients has emerged. The longitudinal study by Saitta *et al*[88] on 44 HBsAg-negative patients with solid tumors undergoing chemotherapy did not find cases with HBV reactivation. Further prospective studies are needed to improve our knowledge of the clinical importance of OBI in patients with solid cancers.

STRATEGIES TO PREVENT REACTIVATION OF OCCULT HBV INFECTION

In patients with OBI, pharmacological prophylaxis with

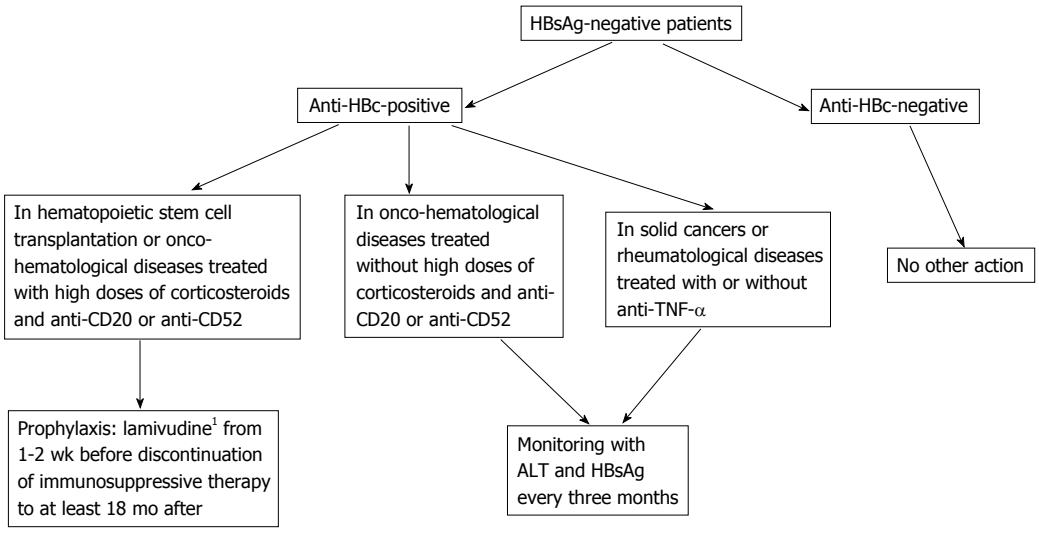

Figure 2 Management of occult hepatitis B infection in hematological and rheumatological diseases and in solid cancers. [1]Entecavir instead of Lamivudine, when appropriate. HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase; Anti-HBc: Hepatitis B core antigen; TNF-α: Tumor necrosis factors-alpha.

nucleot(s)ide analogues should be based on the HBV serological status (anti-HBc-positive or -negative), the underlying diseases (onco-hematological diseases, hematopoietic stem cell transplantation or others) and the type of immunosuppressive treatment (rituximab, high doses of corticosteroids, anthracyclines, or others). In anti-HBc-positive patients, the prophylaxis with anti-HBV nucleos(t)ide analogues is indicated in hematopoietic stem cell transplantation and in onco-hematological diseases when high doses of corticosteroids and rituximab are used, whereas monitoring is indicated in all other clinical conditions or when rituximab-sparing schedules are used (Figure 2). The literature data have shown the efficacy of lamivudine in preventing HBV reactivation in these subsets of patients[17,43,61]. Also entecavir has been proposed in the prophylaxis of reactivation of OBI. In a randomized controlled trial[89] 80 patients with CD20+ lymphoma and resolved hepatitis B were randomly assigned to a prophylactic schedule with entecavir, started before rituximab-based chemotherapy and stopped 3 mo after its discontinuation, or to be treated with entecavir once HBV reactivation and reversion to HBsAg positivity had occurred (control group). During an 18-mo follow up, HBV reactivation occurred in 2.4% of patients who underwent entecavir prophylaxis and in 17.9% of cases in the control group ($P < 0.05$).

Although the efficacy of lamivudine and entecavir in preventing the reactivation of OBI has never been compared in published studies, we can conclude, in agreement with current international guidelines[2,76], that lamivudine, despite of its low genetic barrier, remains the nucleos(t)ide analogue of choice for the prophylaxis of reactivation of OBI because of its low cost and of the low or absent HBV viremia in OBI. Instead, entecavir should replace lamivudine for patients with advanced liver diseases for whom reactivation of OBI might be life threatening.

Monitoring of pharmacological prophylaxis is not standardized and the widespread habit of determining HBsAg at three-monthly intervals is not the optimal strategy in all clinical conditions. In addition, it is not fully understood how long the pharmacological prophylaxis should last in order to prevent the reactivation of HBV infection. Observational studies suggest extending the prophylaxis to the 12th month after the discontinuation of immunosuppressive treatment, but in some case reports HBV reactivation occurred later, especially in patients treated with rituximab[39,90]. Recently, Tonziello *et al*[39] described a reactivation of OBI in an HBsAg-negative/anti-HBc-positive woman with non-Hodgkin lymphoma occurring 20 mo after rituximab discontinuation despite lamivudine prophylaxis covering the 4 mo of rituximab administration and the 12 mo after its discontinuation. Concluding on this point, prospective studies are needed to ascertain whether the pharmacological prophylaxis should be extended to the 18th month after the discontinuation of immunosuppressive treatment in patients receiving rituximab-based chemotherapy.

MANAGEMENT OF REACTIVATION OF OCCULT HBV INFECTION

Once reactivation has occurred, effective antiviral treatment should be immediately administered. Lamivudine monotherapy has been demonstrated to be ineffective in reducing mortality[21]. Consequently, patients should be treated with drugs of high potency and high genetic barrier such as entecavir or tenofovir.

OCCULT HBV INFECTION IN HIV-POSITIVE SUBJECTS

As a consequence of the availability of highly active antiretroviral therapy (HAART), which has determined a substantial improvement in the patients' survival, vi-

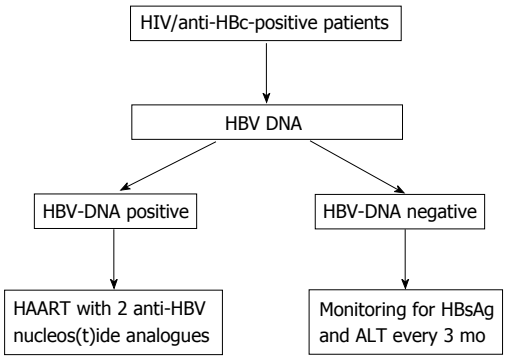

Figure 3 Management of occult hepatitis B infection in anti-human immunodeficiency virus-positive subjects. HCV: Hepatitis C virus; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; HAART: Highly active antiretroviral therapy; HBsAg: Hepatitis B surface antigen.

ral hepatitis has become the leading cause of morbidity and mortality in HIV-infected subjects. In these patients particular attention should be paid to OBI since it may have a strong clinical impact because of damage to the immune system and its frequent occurrence in HIV-HCV coinfected patients.

EPIDEMIOLOGY OF OBI IN HIV-POSITIVE SUBJECTS

The prevalence of OBI in HIV-infected patients is controversial, and the associated risk factors and the effect of HAART undefined. Also controversial is the role of the immune system in the genesis of OBI in HIV-positive patients. Some investigators never observed OBI in patients with CD4 counts > 500 cells/μL and concluded for a significant association of OBI with lower CD4 counts[91]. Other investigators, however, described no association of OBI with the CD4 count[92].

The prevalence of OBI in HIV-HCV coinfected patients varies in different studies from less than 1% to 40%[22,93-102].

OBI may also be observed in anti-HIV-positive patients with chronic HBV/HCV coinfection, due to an HBsAg serum clearance consequent to a strong inhibitory effect of the HCV genome on HBV replication[103].

In HIV subjects a strong association between OBI and HCV infection has been observed in several studies[28,101,104-106]. In contrast, Jardim *et al*[107] reported no significant difference in the rate of OBI in HIV-positive patients with or without HCV coinfection.

The discrepancies in the rate of OBI in the different studies most probably reflect differences in HBV, HCV and HIV epidemiology in different countries, a variation in the sensitivity of the assays used to detect HBV DNA and the retrospective nature of some of the studies.

Cassini *et al*[108] proposed a new approach to the detection of HBV DNA. By the genomic amplification of the partial S, X and precore/core regions, these Authors analyzed for the presence of HBV DNA the circulating blood, liver tissue and peripheral blood mononuclear cells (PBMC). HBV DNA was never found in serum samples of the 24 HBsAg-negative patients investigated, but was detected in the liver tissue in 7 (29%) and in PBMC in 6 (86%) of these 7. The clinical value of these data should be confirmed in larger studies, but they suggest that the detection of HBV DNA in PBMC offers a useful tool to identify OBI. Morsica *et al*[104] analyzed 1593 anti-HIV-positive patients enrolled in the Italian Cohort of Antiretroviral Naïve patients and found 175 (11%) HBsAg-negative/anti-HBc-positive patients: 27of these 175 (15%) patients had detectable HBV DNA in plasma. This prevalence was significantly higher (21%) in the 101 anti-HCV-positive than in the 74 (8%) anti-HCV-negative, regardless of the immune status, HIV load, or ART regimen.

CLINICAL SIGNIFICANCE OF OBI IN HIV-POSITIVE SUBJECTS

The impact of OBI on the prognosis of HIV-positive patients is still unclear. In our previous study[22] on the clinical and virological impact of OBI in HIV-positive patients, we analyzed 115 HBsAg-negative patients, 86 of whom were observed in a long-term follow-up. A hepatic flare occurred more frequently in the 17 patients with occult HBV infection than in the 69 without (64.7% *vs* 24.6%; $P < 0.005$). These preliminary data still await confirmation in larger studies.

Lamivudine-based HAART is effective in suppressing HBV replication even in anti-HIV-positive patients with OBI, as most of these cases clear HBV DNA during treatment. However, in approximately half of the lamivudine-treated patients, occult HBV replication became detectable again after 12-40 mo of lamivudine treatment, always associated with a hepatic flare. Although the presence of *YMDD* mutants in patients who became HBV-DNA-positive under lamivudine was not detected, most probably because of the low levels of plasma HBV DNA, the hypothesis that lamivudine induced the selection of *YMDD* mutants in these anti-HIV-positive subjects with OBI cannot be ruled out. In another study the ALT and aspartate aminotransferase levels showed a tendency to increase more frequently in patients with OBI than in those without[104].

Concluding on this point, OBI seems relatively frequent in anti-HIV-positive patients, particularly in cases with HIV/HCV co-infection. This makes the clinical condition of HIV/HCV co-infection more complex since OBI may unfavorably affect the outcome of the liver disease. Lamivudine seems inadequate for a long-term prevention of hepatic flares in anti-HIV-positive patients with OBI and possibly in reducing the risk of HBV oncogenicity. Therefore, for these patients a high potency, high genetic barrier nucleos(t)ide analogue should be preferred (Figure 3).

CONCLUSION

Clinicians should pay careful attention to OBI since it has

been demonstrated that it occurs with some frequency and may have clinical consequences.

Further studies are needed to better define the biological and clinical role of OBI and to identify new measures to prevent or limit its unfavorable clinical action. It would be of particular benefit to investigate the oncogenicity of OBI, particularly in anti-HIV-positive subjects, in order to devise new strategies for the prevention of HCC.

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REVIEW

Challenge of liver disease in systemic lupus erythematosus: Clues for diagnosis and hints for pathogenesis

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Abstract

Systemic lupus erythematosus (SLE) encompass a broad spectrum of liver diseases. We propose here to classify them as follows: (1) immunological comorbilities (overlap syndromes); (2) non-immunological comorbilities associated to SLE; and (3) a putative liver damage induced by SLE itself, referred to as "lupus hepatitis". In the first group, liver injury can be ascribed to overlapping hepatopathies triggered by autoimmune mechanisms other than SLE occurring with higher incidence in the context of lupus (*e.g.*, autoimmune hepatitis, primary biliary cirrhosis). The second group includes non-autoimmune liver diseases, such as esteatosis, hepatitis C, hypercoagulation state-related liver lesions, hyperplasic parenchymal and vascular lesions, porphyria cutanea tarda, and drug-induced hepatotoxicity. Finally, the data in the literature to support the existence of a hepatic disease produced by SLE itself, or the occurrence of a SLE-associated prone condition that increases susceptibility to acquire other liver diseases, is critically discussed. The pathological mechanisms underlying each of these liver disorders are also reviewed. Despite the high heterogeneity in the literature regarding the prevalence of SLE-associated liver diseases and, in most cases, lack of histopathological evidence or clinical studies large enough to support their existence, it is becoming increasingly apparent that liver is an important target of SLE. Consequently, biochemical liver tests should be routinely carried out in SLE patients to discard liver disorders, particularly in those patients chronically exposed to potentially hepatotoxic drugs. Diagnosing liver disease in SLE patients is always challenging, and the systematization of the current information carried out in this review is expected to be of help both to attain a better understanding of pathogenesis and to build an appropriate work-up for diagnosis.

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Key words: Systemic lupus erythematosus; Lupus hepatitis; Esteatosis; Regenerative nodular hyperplasia; Hepatitis C; Autoimmune hepatitis; Hepatotoxicity; Nonsteroidal anti-inflammatory drugs; Methotrexate

Core tip: The existence of liver disease associated with lupus itself, or increased susceptibility to concomitant liver diseases, either autoimmune or non-autoimmune ones, is still somewhat controversial, and difficult to diagnose. Data in the literature are scarce, and often based on case reports or clinical studies with limited patient size or histological evidence. The pros and cons to support the existence of such pathological entities, and the still preliminary studies on the mechanisms involved, are critically discussed here. We concluded that liver is often a target of systemic lupus erythematosus, and biochemical liver tests should be systematically carried out in these patients.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with variable clinical presentation, usually characterized by several immunological signs and symptoms[1-3]. It primarily affects women under 50 years of age, and is diagnosed on the basis of presence of at least 4 out of 11 criteria identified by the American College of Rheumatology (ACR), either sequentially or simultaneously, namely malar rash, discoid rash, photosensitivity, oral ulcers, nonerosive arthritis, pleuritis or pericarditis, renal disorders (proteinuria or cellular casts), neurologic disorder (seizures or psychosis), hematologic disorder (hemolytic anemia, leukopenia or thrombocytopenia) and immunologic disorders (anti-DNA, anti-Sm or antiphospholipid antibodies)[4-6].

The most common symptoms are fever, weight loss, and a general lack of wellbeing and athralgia, while the most frequent signs are skin rashes. Biochemical exams typically present anemia, and increased rates of erythrosedimentation. Treatment includes nonsteroidal anti-inflammatory drugs (NSAIDs), corticoids, and immunomodulators. Death is generally caused by progressive renal insufficiency, severe impairment of the central nervous system, or multi-organic failure after systemic infection[4].

Even though, as above mentioned, alterations of skin, joints and kidney, as well as of the cardiovascular, hematological and central nervous systems, are part of the criteria indicating morbidity, the liver can also be affected[1-5]. Although a true liver disease triggered by SLE itself is a controversial issue, 25% to 50% of patients may present alterations in the liver function tests (LFTs)[7]. The for and against data in the literature to support the existence of the multiple associations of SLE with liver disease will be discussed in detail in this review. Our literature inclusion criteria limited the citation of clinical cohort studies to those written in English language and published in peer reviewed journals; only very exceptional studies in other languages were included, when dealing with topics with extremely scarce information. The quotation studies in abstract form, when equivalent full papers were unavailable, was also very exceptional, and limited to peer reviewed, highly prestigious meetings.

PREVALENCE OF BIOCHEMICAL AND HISTOLOGICAL HEPATIC ALTERATIONS IN PATIENTS WITH SLE

Subclinical liver disease is common in SLE, and 25%-50% of patients with lupus may develop abnormal liver function at some point[8,9]. The more common laboratory abnormalities associated with the different kinds of liver disease related to lupus are summarized in Table 1. In addition, an overview of the main biochemical and histological findings reported in the literature is depicted in Table 2.

Hepatomegalia is detected in 12%-55% of SLE patients, depending on the analyzed series[10]. In an original article by Mackay *et al*[11], the authors observed hepatomegalia and/or alterations in LFTs in 19 SLE patients, normal liver biopsies in 6 cases, and minimal histological changes in another 11 ones (fatty liver, portal fibrosis, and mild to moderate portal infiltrate). Histological changes compatible with chronic hepatitis with progression to cirrhosis were confirmed in the remaining 2 patients. Similar findings were obtained by Polish researchers in a study of 18 SLE patients; whereas 5 of them showed normal liver histologies, the other 13 ones showed only minimal hepatocellular changes[12]. These results do not agree with those observed by Runyon *et al*[13] who, in a retrospective review of 238 patients with SLE, observed hepatomegalia in 39% of patients, splenomegaly in 6% and jaundice in 24%. Twenty one percent of patients were defined as carriers of liver disease based on abnormal liver histologies or, in some cases, elevation of liver enzymes 2 times over the upper limit of normal (ULN).

In the same study, liver histology of 33 patients showed steatosis (36%), cirrhosis and chronic active hepatitis (12%), hepatic granulomatosis, centrilobular necrosis (9%), and chronic hepatitis and microabscesses (6%). These findings were very challenging for the common view at the beginning of 80 s, and prompted other researchers to replicate these results. However, only one year after this report, Gibson *et al*[14] failed to reproduce such a high rate of severe liver disease associated with SLE. They reported 55% of patients with increase in transaminase levels among 81 patients with SLE, and identified SLE as the only explanation for this abnormality in 29% of the cases. Histological analysis of 7 of these patients revealed portal inflammation in 5, fatty liver in 1, and active chronic hepatitis in the remaining one. They also reported a 23% increase in the levels of alanine aminotransferase (ALT)/aspartate aminotransferase (AST) and alkaline phosphatase (ALP) ($\leqslant$ 2 times ULN), with a notable predominance among patients that presented active clinical signs of SLE. All of these abnormalities normalized with steroid treatment.

A prospective analysis by Miller *et al*[15] recruited 260 patients with SLE that were followed up for a 12-mo period. In the follow-up examinations, liver enzymes levels were high in 23% of them. Clinical liver disease was observed in only 2% of the cases, while causes for liver compromise unrelated to SLE were verified in only 15% of the cases. No specific cause for liver disease other than SLE could be identified in 8% of the patients. The histological analysis carried out on 14 patients found only minimal and non-specific changes. It is noteworthy that the increase in transaminase levels in 12 out of 15 patients appeared concomitantly with lupus activity.

Table 1 Biochemical and histological liver abnormalities in systemic lupus erythematosus patients according to different reports in the literature

Ref.	Study type	Patients with SLE	NO. of patients with biochemical alterations and alteration types	Liver histological findings
Mackay *et al*[11]	Retrospective	19	(*n* = 19) ↑ AST, ALT	Minimal changes, portal fibrosis, steatosis, inflammation (*n* = 11) Normal (*n* = 6) Chronic hepatitis (*n* = 2)
Chwalińska-Sadowska *et al*[12]	Retrospective	18	NA	Minimal changes (*n* = 13) Normal (*n* =5)
Runyon *et al*[13]	Retrospective	238	(*n* = 124) ↑ AST, ALT, total bilirubin, ALP, GGT, LDH (≥ 2 × ULN)	(*n* = 33) Steatosis (*n* = 12) Others: cirrhosis, chronic hepatitis, granulomatosis, chronic hepatitis, steatosis, cholestasis, centrilobular necrosis
Gibson *et al*[14]	Retrospective	81	(*n* = 64) ↑ AST, ALT, ALP	(*n* = 7) Portal inflammation (*n* = 5) Steatosis (*n* = 1) Chronic hepatitis (*n* = 1)
Miller *et al*[15]	Prospective	260	(*n* = 84) ↑ AST, ALT, ALP	Minimal changes (*n* = 14)
Matsumoto *et al*[17]	Retrospective	73	NA	Hepatic arteritis (*n* = 11) Steatosis (*n* = 53) RNH (*n* = 5) Viral hepatitis (*n* = 2) SLE-PBC overlap syndrome (*n* = 1) SLE-AIH overlap syndrome (*n* = 1)
Luangjaru *et al*[9]	Retrospective	225	(*n* = 80) ↑ AST, ALT (≤ 4 × ULN)	NA
Chowdhary *et al*[7]	Retrospective	192	(*n* = 40) ↑ AST, ALT	HCV (*n* = 3) Steatosis (*n* =5) SLE-AIH overlap syndrome (*n* = 4) SLE-PBC overlap syndrome (*n* = 3) Cryptogenic cirrhosis (*n* = 1)
Piga *et al*[3]	Retrospective	242	(*n* = 59) ↑ AST, ALT (≥ 2 × ULN)	NA
Her *et al*[138]	Retrospective	141	(*n* = 46) ↑ Total bilirubin, AST, ALT, LDH, ALP (≥ 2 × ULN)	NA
Huang *et al*[90]	Retrospective	1533	(*n* = 134) ↑ AST, ALT (≥ 2 × ULN during 2 yr)	Chronic Hepatitis (*n* = 6) Minimal changes (*n* = 4) Normal (*n* = 3)
Zheng *et al*[2]	Retrospective	504	(*n* = 47) ↑ Total bilirubin (13%), ALT (98%), ALP (42%), GGT (49%)	(*n* = 10) Portal blood cell infiltration (*n* = 8) Hydropic degeneration (*n* = 8) Steatosis (*n* = 2) Mild cholestasis (*n* = 2) Focal necrosis (*n*= 1) Nodular cirrhosis (*n* = 1)
Takahashi *et al*[18]	Prospective	206	(*n* = 123) ↑ AST, ALT (99%) ↑ ALP and GGT (81%)	(*n* = 25) Lupus hepatitis (*n* = 16): Unspecific reactive hepatitis (88%) Active hepatitis (12%) SLE-AIH overlap syndrome (*n* = 6): Interface hepatitis (100%) Cirrhosis (33%) SLE-PBC overlap syndrome (*n* = 3)

SLE: Systemic lupus erythematosus; ULN: Upper limit of normal; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDH: Lacto dehydrogenase; ALP: Alkaline phosphatase; GGT: Gamma glutamil transferase; HCV: Hepatitis C virus; PBC: Primary biliary cirrhosis; AIH: Autoimmune hepatitis.

A much lower frequency of liver abnormalities was reported by Fox *et al*[16] in a retrospective cohort of 200 patients, where an increase of liver enzymes was documented in only 2.5% of the cases. These biochemical changes were associated with liver clinic manifestations only in few cases, and had no relationship with plasmatic ribosomal-P antibodies.

Very interesting findings were published by Matsu-

Table 2 Laboratory abnormalities in the different hepatic manifestations associated with systemic lupus erythematosus

Hepatic alteration	Laboratory abnormalities
Hepatic steatosis	GGT, ALT/AST
Viral hepatitis	ALT, AST, HCV, cryoglobulinemia
Toxic hepatitis	ALP, GGT, AST/ALT, bilirubin
Nodular regenerative hyperplasia	ALT, AST, thrombocytopenia
Primary biliary cirrhosis	ALP, GGT, AMA
Autoimmune hepatitis	ANA, ASMA, gammaglobulin
Hepatic venous thrombosis	Antiphospolipidic antibodies
Lupus hepatitis	Anti-ribosomal P autoantibodies

AMA: Antimitocondrial antibody; ANA: Antinuclear antibody; ASMA: Antismooth muscle antibody; GGT: Gamma glutamil transferase; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

moto *et al*[17], who analyzed liver histology of 73 patients with SLE. They identified fatty liver as the major feature in 72% of the cases, while nodular regenerative hyperplasia, viral hepatitis, primary biliary cirrhosis (PBC), and autoimmune hepatitis (AIH) were identified as the main cause of liver disease only in few cases (6.8%, 4.1%, 2.7%, and 2.7%, respectively).

Finally, Takahashi *et al*[18] reported recently that liver dysfunction was apparent in 123 (59.7%) out of 206 patients. They identified different causes of liver dysfunction as follows: induced by drug (30.9%), caused by SLE itself (28.5%), fatty liver (17.9%), AIH (4.9%), PBC (2.4%), cholangitis (1.6%), alcohol (1.6%), and viral hepatitis (0.8%). The liver dysfunction tends to be mild, except when caused by AIH.

From the studies reported above, it is readily apparent that the published data linking liver diseases with SLE during the last four decades are highly heterogeneous, and that a high number of cases lack adequate histological documentation.

LIVER DISEASES IN THE SLE CONTEXT

The frequent association between SLE and LFT alterations may be accounted for by three possibilities, namely: (1) the existence of some kind of liver parenchymal injury associated with SLE alone, often referred to as "lupus hepatitis"; (2) the occurrence of an overlap syndrome by which SLE shows additional features of another autoimmune liver disease; and (3) the concurrency of comorbility of SLE with a non-autoimmune hepatopathy, *e.g.*, drug-induced liver damage, viral hepatitis or thrombotic liver disease, among others.

Lupus hepatitis

Although it is still a controversial issue, there is compiling evidence in the literature that lupus itself is not associated with a specific, severe and progressive liver injury. However, several authors have pointed a role for SLE in triggering an often subclinical hepatopathy, referred to as "lupus hepatitis". They described this disease as an asymptomatic hypertransaminasemia frequently associated with exacerbations of the lupus disease, which returns to normal values after corticosteroid therapy[2,14,15].

May be a part the confusion begun in the early 50's, when AIH was wrongly referred to as "lupoid hepatitis"[11]. Subsequent studies added more confusion when no serology was available to rule out overlapping chronic viral diseases [hepatitis C virus (HCV), hepatitis B virus (HBV), cytomegalovirus, *etc.*] in SLE patients with hypertransaminasemia.

In the early 80's, Runyon *et al*[13] reactivated the debate publishing a very controversial study describing both a "canalicular cholestasis" profile and SLE-related cirrhosis as diseases triggered by lupus itself. As mentioned before, the sample analyzed in this study consisted of 33 lupus patients presenting different types of liver damage that were documented by liver biopsy, namely steatosis, chronic hepatitis, hemochromatosis, granulomatose hepatitis, cholestasis and cirrhosis. Serological and virological markers to rule out hepatitis C did not exist at this time.

As was also stressed above, another condition that is needed to rule out among SLE patients with hypertransaminasemia is an overlap with AIH, which represents a separate disease from lupus, both because of its distinct pathogenic mechanism (specific organ) and its distinctive biochemical, serological, and histological characteristics that allow for a clear differentiation.

Hypergammaglobulinemia, autoantibodies [antinuclear antibody (ANA), antismooth muscle antibody (ASMA), anti-liver-kidney microsome antibodies], a histological profile characterized by piecemeal necrosis (interface hepatitis), and a rich plama cells infiltrate are highly distinctive aspects of AIH. On the other hand, if a lupus patient presents evidence of progressive non-autoimmune chronic hepatitis characterized by persistent severe inflammatory damage, we need to consider first other probable diagnosis of chronic liver injury, such as hepatitis B or C, or other autoimmune diseases overlapping with lupus. The discrimination is further complicated by the fact that liver histopathological features in patients with lupus hepatitis are miscellaneous and non-specific, similar to those in other liver diseases. It is therefore important, before diagnosing lupus hepatitis, to rigorously rule out other liver diseases, including drug-induced liver injury, alcohol liver disease, viral hepatitis (hepatitis A, B, C, D, E, Epstein-Barr virus or cytomegalovirus), and other autoimmune-associated liver diseases [AIH, PBC, primary sclerosing cholangitis (PSC)].

A recent study by Zheng *et al*[2] based on this strict discrimination criteria reported a 9.3% lupus hepatitis incidence among 504 SLE patients evaluated. However, the prevalence reported in the literature is rather variable, with both lower[4,8,17,19] and higher[14,18,20] rate values.

Zheng *et al*[2] also reported that the prevalence of lupus hepatitis in patients with active SLE was higher than those with inactive SLE (11.8% *vs* 3.2%). The patients with lupus hepatitis mostly showed mild to moderate elevations of serum transaminase levels, though 6 patients had jaundice as the predominant feature. ALP and Gamma

glutamil transferase elevations were far less frequent. Only 12.8% had liver injury-related clinical manifestations. Lupus hepatitis responds well to moderate to high doses of corticosteroids[3].

In patients suspected to have lupus hepatitis, it has been often reported a correlation between hepatic enzymes abnormalities and autoantibodies to ribosomal P proteins (anti-ribosomal P), a highly specific marker for SLE[19,21,22]. Indeed, several reports suggest that SLE-related hepatitis may be associated with, or even caused by this autoantibody.

Anti-ribosomal P occurs in 12%-16% of patients with lupus[21-24], although this proportion increased to 30% when more sensitive methods were employed [enzyme-linked immunosorbent assay (ELISA) based upon the combination of different ribosomal-P antigens], with Caucasian ethnicity having lower values[25]. The proportion of serum anti-ribosomal P occurrence raised to 44% among SLE patients with liver dysfunction and, from them, 70% had SLE-associated hepatitis, a far higher value as compared with SLE patients suffering from other hepatic alterations, such as fatty liver (29%), drug-induced hepatitis (17%), or SLE-AIH overlap syndrome (20%)[26]. Furthermore, Koren *et al*[27] reported the development of chronic active hepatitis in a patient with SLE followed several months later by the appearance of high serum levels of anti-ribosomal P antibodies, and suggested a possible causal relationship. As for the mechanism explaining this causal relationship, anti-ribosomal P positive sera from SLE patients were found to react strongly "*in vitro*" with a polypeptide antigenically related to a 38 kD ribosomal P_0 protein present on the plasma membrane of hepatoma cells[28], thus further strengthening the possibility that anti-ribosomal P antibodies could be directly detrimental in lupus patients by inducing hepatocellular lysis, and further transaminase release. Finally, anti-ribosomal P antibodies up-regulate the expression of proinflammatory cytokines by peripheral monocytes in SLE, which may be a contributing factor for hepatitis development[29].

Given that auto-antibodies directed against eukaryotic P proteins are highly specific to SLE, they can be used as diagnostic markers of the disease. However, there is no standard methodology for its detection and titration in clinical practice. The plasma titers of this antibody often fluctuate in relation to lupus activity, and were formerly associated with neuropsychiatric kidney and liver failure[22,26].

Several isolated cases have been reported of association of anti-ribosomal P antibody occurrence with hepatitis, and also with kidney failure[27,30]. However, it was Arnett *et al*[19] the first to report this association in a cohort study in 1995. They found lupus-related hepatitis in 3% of 131 lupus patients in a retrospective study that analyzed the hepatic manifestations of SLE. The clinical outcome for these patients was variable, from a minimum, subclinical increase of transaminases to acute hepatitis and overt liver failure. Unfortunately, histological studies were not carried out in this study to correlate the degree of liver injury associated with lupus hepatitis and the levels of anti-ribosomal P antibodies.

Although these lines of evidence link anti-ribosomal P antibodies to liver damage in SLE patients, the association is still highly controversial. For example, lack of a clear association between lupus hepatitis and anti-ribosomal P levels was reported in a recently published retrospective study of 73 patients with SLE, where 12 of them (16%) were reported to have lupus hepatitis. In this group, 6 patients had a concurrent liver involvement with the diagnosis of SLE, and it occurred later during an exacerbation of the disease in the remaining 5 patients[19]. Clinical manifestations were as follows: hepatomegaly (n = 4), jaundice (n = 4), abdominal pain (n = 3), ascitis (n = 2), portal hypertension (n = 1), and hepatic failure with encephalopathy (n = 1). Despite elevated liver enzymes were noted in 11 cases and cholestasis in 8 ones, the presence of anti-ribosomal P antibodies was observed only in one case, and therefore an association between lupus hepatitis and any kind of specific antibody could not be documented. Liver biopsy in 5 patients showed chronic active hepatitis in 3 cases, chronic hepatic granulomas in 1 case, and nonspecific inflammation in another one. Although the authors showed clear evidence of immunosuppressive therapy response in most patients, liver biopsy was performed in less than half of them, and their description was not detailed enough to clearly differentiate lupus hepatitis from AIH.

In part, disagreements on the association between anti-ribosomal P antibody and lupus hepatitis can be explained by different features of the studied populations (*e.g.*, ethnicity), environmental factors affecting autoantigen expression, and distinct degrees of sensitivity/specificity of the methods used to detect anti-ribosomal P antibodies. Usually, associations between anti-ribosomal P antibody levels and hepatitis were investigated by using not well-standardized, or even "in-house" immunological methods[19,26]. Unfortunately, large cohort studies where lupus hepatitis or other SLE hepatic manifestations have been reliably documented, and where well-standardized, high sensitivity/specificity immunological methods are employed to detect anti-ribosomal P antibodies (*e.g.*, those using a mixture the ribosomal P antigens P_0, P_1, and P_2), are lacking, and we eagerly await them to confirm or deny the existence of this association.

To complicate the picture further, Calich *et al*[31] reported recently the presence of anti-ribosomal P antibodies in patients having AIH not associated with lupus (9.7%; 9/93), and suggested that this antibody predicts worse prognosis of the disease, with follow-up data showing higher prevalence of cirrhosis in anti-ribosomal P antibody-positive AIH patients (100%, 7/7). This finding suggests that anti-ribosomal P antibodies can be involved in the pathogenesis of other hepatic autoimmune diseases, apart from lupus hepatitis. The debate is still open, and it is apparent that we need more data to support the role and impact of anti-ribosomal P antibodies in both SLE and AIH pathogenesis.

Overlap of SLE with autoimmune liver diseases (overlap syndromes)

The existence of overlap syndromes linking SLE with other autoimmune liver diseases is matter of controversies since, again, the data in the literature are scarce.

According to the so called "theory of the mosaic of autoimmunity"[32], each of these associations may represent a particular variant of a major underlying autoimmune disease, which can shows up under the form of multiple autoimmune liver diseases coexisting in the same patient. Other good examples of such variants are more typical hepatic overlap syndromes, such as AIH-PBC and AIH-PSC[33].

Although AIH or PBC are rare among SLE patients taken as a whole[34], the co-existence of SLE with either of these liver diseases is not uncommon among the subgroup of SLE patients with liver enzyme abnormalities. Chowdhary *et al*[7] reported a strong association between SLE and autoimmune liver disease. They found that 8 out of 40 SLE patients (20%) were AIH carriers, while 6 (15%) showed evidence of PBC.

In another study by Efe *et al*[35], 36 SLE patients out of 147 (25%) had liver enzyme abnormalities, and 7 of them (4.7%) had SLE associated with another autoimmune liver disease. The rate rose to 19.4% when the subset of SLE patients having HLTs altered was considered and, from them, 72.3% fulfilled the criteria for AIH proposed by the International Autoimmune Hepatitis Group. The therapy with ursodeoxycholic acid, prednisone, immunosuppressive thiopurine analogs, or a combination of them, was successful in these patients.

SLE-AIH overlap syndrome

There have been very few reported cases of AIH associated with SLE. It is therefore apparent that AIH and SLE overlap syndrome is a rare condition, although its exact incidence is unclear.

Oka *et al*[36] reported 5 (3%) patients with AIH in an analysis of 162 cases of SLE meeting the ACR criteria. Similar findings were documented by Tamai *et al*[37], who found 10% of AIH in a series of 21 SLE cases.

There is evidence in the literature suggesting that SLE and AIH are different diseases, even when clinical, biochemical and serological characteristics may show overlapping features, such as the presence of polyarthralgia, hypergammaglobulinemia, and positive ANA, ASMA and anti-ribonucleoprotein[38]. In these cases, liver histology is the decisive tool to define diagnosis. The presence of cirrhosis or periportal hepatitis associated with lymphocytes and plasma cell infiltration, as well as rosette formation of liver cells, tips the scales towards AIH. On the other hand, the presence of mainly lobular and occasionally portal inflammation with a paucity of lymphoid infiltrates is more compatible with SLE. Finally, a mixed histological pattern is expected in SLE-AIH syndrome, displaying chronic hepatitis with severe inflammatory activity characterized by focal necrosis of hepatic cells, erosion of the lobular limiting plate, periportal hepatitis, infiltration by lymphocytes and plasma cells, presence of fibrosis in the portal areas and, eventually, cirrhosis[39,40]. In this context, positivity for anti-Sm antibodies, which are highly specific though relatively insensitive to SLE, helps to confirm SLE-AIH overlapping. In addition, presence of antibodies to double-stranded (ds) DNA, another hallmark of SLE, were found to be associated with poorer immediate response to corticosteroid treatment in AIH[41].

SLE-PBC overlap syndrome

PBC is also an autoimmune liver disease, and overlapping with PBC is likely to some extent. However, the co-existence of PBC and SLE is the subject of few reports in the literature, mostly based upon single case reports[42,43]. A large-scale study reported that, among 1032 PBC patients, 27 (0.03%) had also SLE[44]. Interestingly, anti-dsDNA and anti-ribosomal-P antibodies, two serological markers of SLE, were detected in 22% and 5%, respectively, of "pure" PBC patients[45].

SLE-PBC association has been documented mainly in patients with arthritis, polyserositis, and high titers of anti-native DNA and anti-mitochondrial antibodies (AMAs), two pathognomonic signs of SLE and PBC, respectively. Again, PBC can appear in a pre-existing lupus as an expression of an immunological disorder that has not been totally clarified. Osteopontin, a soluble ligand with pleomorphic immunologic activities that plays an important role in inflammation and immunity, may be a link. Osteopontin was reported to be highly expressed in the murphy roths large/lpr mouse[46], a well recognized models of SLE, and it is involved as a chemoattractant cytokine in the recruitment of macrophages and T lymphocytes in the liver granulomas in PBC[47]. Interestingly, Han *et al*[48], in a large cohort of 1141 SLE patients, confirmed the association between osteopontin and SLE.

Finally, AIH-PBC overlap syndrome has been reported to occur in 2.8% of SLE patients, suggesting the association of not only two but even three autoimmune diseases (SLE-AIH-PBC overlap syndrome)[49]. Furthermore, anti-dsDNA antibodies, which are known to be strongly associated with SLE, were detected in 60%[50] or 56%[51] of patients with AIH-PBC overlap syndrome.

SLE-PSC overlap syndrome?

Evidence for SLE-PSC overlap syndrome is limited at best, and only based upon few case reports[52-55]. Whether this clinical association indicates that some immune disorders are common to the two autoimmune diseases or whether they were casual associations remains to be ascertained.

Association of SLE with non-autoimmune liver diseases (comorbidity)

SLE patients often present comorbidity with a number of non-autoimmune liver diseases. In many cases, the prevalence of the concomitant hepatopathy is higher when associated with SLE than alone, indicating either increased susceptibility to the concomitant disease trig-

gered by SLE or *vice versa*.

Association of SLE with hepatitis C

Autoimmunity and viral infections are closely associated fields, and viruses have been proposed as a likely etiological, contributing or even triggering factor of systemic autoimmune diseases[56]. This holds true also for SLE, since some hypotheses have identified viruses as potential agents that trigger SLE, with a close relationship to the pathogenic mechanism of damage[57].

Very little association has been found between SLE and patients infected with HCV. Most reports linking the two diseases refer to the presence in these patients of skin lesions, anti-DNA antibodies, hypocomplementemia and cryoglobulinemia[57].

In a study of 134 patients carrying SLE, the presence of anti-HCV antibodies (ELISA) was observed in 18 patients (13%), while the prevalence among voluntary blood donors in a large number of countries ranges from 0.5% to 2%, only. Active infection by HCV was confirmed in 15 (11%) of the patients with positive ELISA HCV[57]. Similar results were obtained in other study where HCV was detected in 4 out of 40 SLE patients (10%), whereas prevalence among voluntary blood donors was only of 0.13%[58]. Steroid therapy in these patients did not seem to alter the HCV course[59]. Whether this reflects a true higher HCV prevalence associated to SLE or it is a mere consequence of the multiple admissions and blood transfusions that these patients are subjected remains to be defined. Large-scale studies avoiding these potential bias are awaited.

It should be on the other hand acknowledged that HCV chronic infection is associated with different biochemical and histological manifestations of autoimmunity that, in certain cases, can mimic SLE[60]. Different types of non-organ-specific autoantibodies can be detected in chronic hepatitis C (*e.g.*, anti-soluble liver antigen, ANA, AMT, rheumatoid factor) and, less frequently, it is associated with low anti-DNA titers; for example, about 20% with hepatitis C patients are ANA positive[61]. In addition, chronic hepatitis C can occur with cryoglobulinemia, which can lead to a wrong SLE diagnosis, due to the simultaneous occurrence of ANA, dermathological and renal lesions and plaquetopenia; this is why, in patients suspected to have SLE, HCV infection must be excluded using routine anti-HCV serology and, HCV-RNA tests. Several factors lead to the production of autoantibodies in HCV patients, including leakage of intracellular components due to the persistent destruction of infected cells[61], the molecular mimicry between HCV and autoantigens[62], and the functional abnormalities of infected B lymphocytes, with production of excessive autoantibodies and cryoglobulins[63].

Fukuyama *et al*[64] reported for the first time in the literature the development of an SLE profile after interferon α-2 therapy. There are over 10 currently published cases that link the use of interferon to treat hepatitis C with the appearance of SLE associated with different levels of severity, including one patient with a serious lupus cardiomyopathy that threatened his/her life.

Although chronic infection with HCV can induce clinical and serological changes that can be confused with an autoimmune disease (arthritis, nephropathy, and cytopenias), the appearance of malar rash, discoid lesion, photosensitivity, neurological damage, high titers of ANA or anti-DNA antibodies, and anti-Sm antibody occurrence usually constitute sufficient evidence to diagnose SLE[7].

The clinician must consider three situations in the context of a HCV antibody in a patient with SLE, namely: (1) it may be a false positive HCV ELISA test due to the high levels of autoantibodies that are frequently presented in SLE patients; (2) could be true association between SLE and hepatitis C; and (3) HCV can trigger the occurrence of low levels of ANA and/or anti-DNA, associated with cryoglobulinemia, without typical skin changes[19].

One common complication of SLE patients is the so called "lupus nephritis", and HCV may play a role. Few cases of lupus nephritis coexisting with HCV infection have been described[65,66]. Although speculative, it is likely that the altered immune response in SLE facilitates HCV infection, and *vice versa,* that different autoantibodies associated with HCV infection facilitate the development of lupus nephritis due to formation of immune complex deposits in the kidneys. The increase in serum B-lymphocyte activating factor levels in chronic HCV patients with infection and SLE may be a contributing factor, by reinforcing B-cell activation and autoantibody production[67].

Association of SLE with hypercoagulation state-related liver lesions

SLE patients have a high potential to develop thromboembolic disorders that can impact on hepatic circulation[68]. The frequent presence of anti-phospholipid antibodies among these patients can include thrombotic manifestations in different territories of the splachnic vasculature, both in arterial and venous areas (thrombosis of the hepatic artery, portal thrombosis, and Budd-Chiari syndrome)[69]. Portal hypertension profiles and esophageal varices have also been reported in several cases as secondary events linked to thrombosis of the portal vein, triggered by the presence of anti-cardiolipin antibodies[10].

Regenerative nodular hyperplasia (RNH), which follows hepatic vein thrombosis and hepatic circulation disorders, has also been reported in association with SLE (Figure 1)[70]. The pathogenesis of RNH complicating SLE is believed to be related to vasculitis of intrahepatic arteries, leading to secondary portal venous obliteration and thrombosis of the adjacent portal veins[1]. Alternatively, occlusion of intrahepatic small vessels may result from coagulopathy in patients with associated antiphospholipid syndrome[9]. It has been suggested that antiphospholipid antibodies play a pathogenic veno-occlusive role in the pathogenesis of RNH[71].

One of the most attractive theories regarding RNH

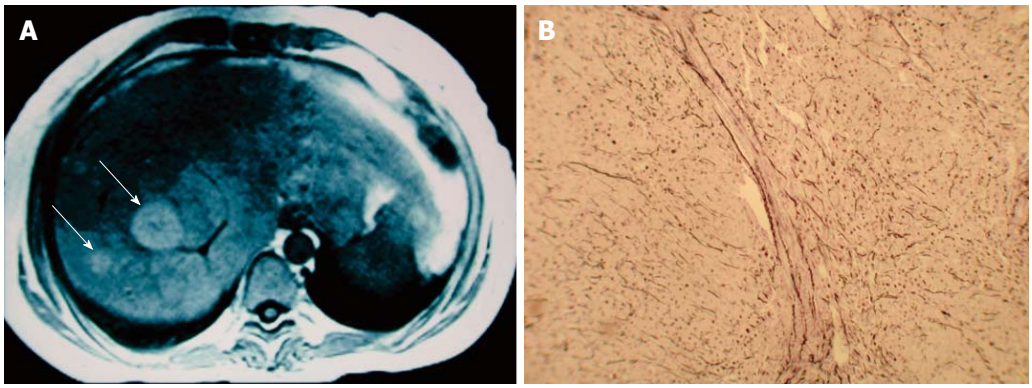

Figure 1 Regenerative nodular hyperplasia. A: Magnetic resonance image of RNH (axial T1 FSE). Note the two hyperintense, solid nodules localized in the right hepatic lobe (arrows); B: Typical findings of a RNH lesion revealed by reticulin staining to highlight the sinusoidal architecture of the liver. Note the liver sinusoidal shrinking, mimicking a pseudonodule. RNH: Regenerative nodular hyperplasia.

origin involves the storage of immune complexes in small caliber intrahepatic vessels, and the further appearance of obliterative venopathy[70]. The liver histology pattern is characterized by the presence of multiple hepatic nodes that do not have their own walls and that, in the absence of fibrosis, are circumscribed by thin bands formed by the flattening of hepatocyte columns emulating thin fibrous membranes. This condition is another component of a long list of diseases linked to non-cirrhotic portal hypertension. It is often associated with hematological diseases and various conditions that typically present systemic impairment (rheumatoid arthritis, CREST syndrome, Felty's syndrome)[72]. Another theory suggests that the association between RNH and anti-phospholipid antibodies is due to the cellular regeneration process that begins in the liver to maintain its functional capacity after the ischemic injury induced by these antibodies in the hepatic microcirculation[68].

RNH should be suspected in any patient with both SLE and portal hypertension in the absence of cirrhosis. The diagnosis can be established after a liver biopsy. Due to the large size of the regenerative nodes, there is a chance for the needle to be positioned in an area with no histological damage, which accounts for sampling error. When RNH is to be diagnosed, laparoscopic wedge biopsy is a safe and efficient way to obtain enough tissue to preserve the hepatic architecture required for analysis, avoiding in turn the morbidity associated with an unnecessary open resection[73].

Hepatic imaging of RNH shows several additional findings, including focal nodular hyperplasia (FNH), hepatocellular adenoma, regenerative nodules, and liver metastatic disease. Computed tomography can show normal liver, numerous small nodules, or larger coalesced nodules spanning several centimeters. On nuclear medicine imaging, these lesions may take up sulfur colloid, but will remain iso- or hypodense in both arterial and portal venous phases; this helps to distinguish RNH from FNH[74]. The use of magnetic resonance imaging (MRI) to enhance diagnostic accuracy is still controversial. RNH lesions appear hyperintense on T1-weighted imaging and iso- or hypointense on T2 images (Figure 1). However, the sensitivity and specificity are variable, according to a recent report[75].

RNH may be differentiated from large regenerative nodules (LRN) by either tomography or MRI. LRN can have a distinct presentation, and very often results in enhancing liver nodules, whereas RNH usually does not[76].

The spontaneous rupture of the liver has also been reported in patients with SLE as a serious consequence related to the occurrence of a large area of infarction, due to a thrombotic phenomena of the hepatic artery[77].

Focal disturbance of the hepatic blood supply associated with lupus might also facilitates the hyperplastic development of benign lesions in the liver, such as FNH and hemangiomas[78]. In a recent study analyzing a cohort of 35 SLE patients, FNH was observed at higher rates (5.7%) than in the normal population (0.6%-3.0%), and the same holds true for hemangiomas (54.2% *vs* 0.4%-20% in the general adult population)[79]. Whereas FNH is thought to be part of an abnormal adaptive regenerative response of the liver parenchyma to local hemodynamic disturbances[80], hemangioma formation may be also favored by an increase of angiogenic factors whose circulating levels are increased in SLE patients, such as estrogens[81], vascular endothelial growth factor, and interleukin-18[82,83]. Confirmation of an increased incidence of these kinds of hepatic benign lesions in SLE patients awaits large-scale studies.

Association of SLE with porphyria cutanea tarda

The association of SLE with porphyria cutanea tarda (PCT), the most frequent type of porphyria, is rare, and data defining whether this concomitance is pure coincidence or true association are still lacking[84-86].

Common features in both diseases may be a confusing factor. SLE is similar to PCT regarding photosensitivity, but the presence of blisters involving crusts and miliae in sun-exposed areas of PCT patients, which is characteristic of PCT but rare in SLE (< 5% of the cases)[87], can help to differentiate both diseases.

Co-existence of PCT is usually associated with an-

Table 3 Hepatotoxicity induced by drugs used in lupus treatment

Drug	Liver injury and clinical significance
Corticosteroids	Hepatomegalia
	Fatty liver
NSAIDs	Asymptomatic ALT increase
	Hepatocellular, cholestatic, or mixed injury
ASA	Acute and chronic hepatocellular injury (resolve with withdrawal)
Methotrexate	Asymptomatic ALT increase at high doses
	Esteatosis, fibrosis, or cirrhosis
Anti-malarial drugs[1]	Rare hepatotoxic effects
	Porphyria cutanea tarda
Azatioprine	Cholestasis, peliosis, SOS, RNH
Thioguanine	SOS, RNH, portal hypertension
Ciclophosphamide	Rare case reports at conventional doses
	SOS at high doses (resolve with dose reduction)
Mycophenolate mofetil	Asymptomatic ALT increase (resolve with dose reduction)
Rituximab	No liver reactions have been reported
Belimumab	No liver reactions have been reported

[1]Anti-malarial drugs: chloroquine, hydroxychloroquine. ALT: Alanine aminotransferase; NSAIDs: Non-steroidal antiinflamatory drugs; ASA: Acetylsalicilic acid; RNH: Nodular regenerative hyperplasia; SOS: Sinusoidal obstruction syndrome.

timalarial drugs for treating lupus (*e.g.*, chloroquine, hydroxychloroquine), and the regular use of these drugs in SLE patients should be considered a risk for PCT. This usually represents a diagnostic problem, given the frequent association of PCT with a long list of drugs apart from antimalarial agents, which makes the diagnosis of the cause even more complicated[88,89]. The risk associated with antimalarial drugs is dose-dependent; this is why several authors have contraindicated the daily intake of these drugs for SLE due to the risk of massive porphyrinuria, which is often associated with fever, nausea and hepatocelular injury, leading eventually to hepatic necrosis[78-81].

Association of SLE with drug-induced hepatotoxicity

Patients with SLE seem to have a relatively high rate of drug-induced hepatotoxicity (Table 3). For example, Huang *et al*[90] reported 35 cases of drug-induced hepatotoxicity among 1533 SLE patients reviewed. In another study by Takahashi *et al*[18], liver damage could be ascribed to drug-induced liver injury in 31% from a total of 123 SLE patients with overt liver dysfunction.

At the moment, it is impossible to know with certainty whether this high incidence is due to the chronic use, at relatively high doses, of different drugs commonly prescribed to treat this disease, or whether there is any kind of particular susceptibility that makes these patients prone to drug-induced hepatotoxicity. Of note, SLE patients have been shown to have elevated levels of systemic oxidative stress, which well correlated with liver enzyme elevations[91]. This relationship can be tentatively explained by drug-induced oxidative stress in the liver of these patients, with consequent liver injury. The elevated pro-oxidant liver status associated with a pro-inflammatory conditions like SLE may also make the organ prone to develop hepatotoxicity by drugs exerting detrimental effects *via* oxidative mechanisms. Indeed, several drugs used in autoimmune disease may themselves be converted into free radicals "*in vivo*", thus aggravating oxidative damage[92,93]. Controlled, comparative studies on differential susceptibility to the same drug in patients with SLE and other autoimmune disease (*e.g.*, rheumatoid arthritis) are lacking, but they would be useful to establish whether SLE is indeed a peculiar prone condition for drug-induced liver injury.

Around 80% of SLE patients are treated with analgesic and NSAIDs, prescribed for febrile syndrome, athralgia/arthritis, serositis and/or cephale[94]. Hepatitis , fulminant hepatic failure, cholestasis, and mixed damage were reported to be caused by these compounds[95-98].

Lupus patients usually present a higher rate of NSAID-related complications than SLE-negative subjects. The most common complications are increased transaminase levels, skin rashes triggered by sun, increased retention of body fluids with arterial hypertension, gastric ulcers, and aseptic meningitis. NSAIDs should not be indicated over the counter in SLE, and prescription must always be accompanied by recommendations related to strict clinical and laboratory vigilance[94].

For many years, aspirin was the most common drug associated with SLE-related liver damage. Increments of ALT, AST and ALP have been reported in up to 25% of the SLE patients consuming high doses of aspirin (> 2 g/d)[94].

In the early 70's, the first publications appeared identifying aspirin as responsible for the hepatic damage in SLE patients[99,100]. It was not however until 1981 that Zimmerman, in a review focused on this issue, showed with certainty that aspirin generates both acute and chronic dose-dependent liver damage[101].

The onset of aspirin-induced liver disease is marked by the appearance of anorexia, nausea and non-specific pain in the upper abdomen. The patient usually does not present jaundice, and ALT and AST values are usually not more than 10 times ULN values. It is very common that AST levels are higher than ALT, and that these alterations are associated with normal ALP levels[102].

Although hepatotoxicity can occur with low levels of plasma salicylate, the mechanism is often dose-dependent, and the biochemical abnormalities revert when the drug is discontinued. In 3% of the cases, the lesion can be severe enough to lead to fatal hepatic failure. Chronic liver damage observed in the hepatic histology as a chronic active hepatitis pattern is much less common, and also returns to normality when the drug is withdrawn[103].

There is also controversial evidence that rheumatic patients usually have underlying conditions that increase the risk of aspirin-induced hepatic failure. However, SLE-related hypoalbuminemia and juvenile rheumatoid arthritis are well documented risk factors as well[104-106].

Thiopurine analogues, such as azathriopine (AZA) and 5-mercaptopurine, are immunosuppressive drugs often employed in autoimmune diseases, including their

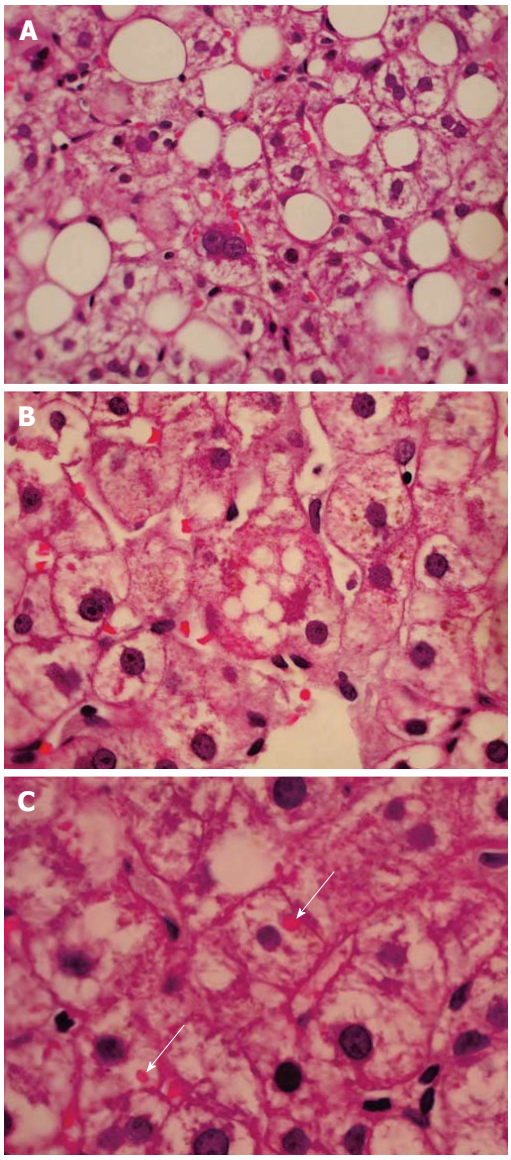

Figure 2 (A) Macrosteatosis, (B) microsteatosis and (C) megamitochondria (arrows), in a non-alcoholic 27-year-old patient with active systemic lupus erythematosus, treated with steroids and methotrexate (H and E staining).

use to gain or maintain remission in SLE. Hepatotoxicity induced by thiopurine analogues occurs very often with increase in serum transaminase levels. It is associated generally with not severe liver injury, which responds to dose reduction in most patients. RNH is also a very rare but potentially severe complication of thiopurine-based therapies. It is often asymptomatic, and neither biochemical nor molecular markers are indicative of RNH. The suspicion should arise when there are clinical symptoms of portal hypertension, increments of transaminase levels, or thrombocytopenia. A liver biopsy is essential in this case to confirm diagnosis[107].

A recent review by Musumba[108] reports that inflammatory bowel disease patients treated with AZA have a cumulative incidence of RNH at 5 and 10 years of 0.6% and 1.3%, respectively, whereas those treated with high TG doses (> 40 mg/d) have an incidence of RNH of up to 62%; this rate is even higher in patients with elevated liver enzymes and/or thrombocytopenia, as compared with those lacking these abnormalities (76% *vs* 33%).

Methotrexate (MTX) is currently the first-line therapy for early and chronic rheumatic and psoriasic arthritis, but it is also indicated to symptomatic patients with SLE[109]. The recognition of risk of chronic liver damage with MTX has prompted the need for intensive biochemical monitoring from several decades ago onwards. The frequency of hepatotoxicity varies widely according to differences in sampling, definitions of damage, dose regimens, and presence of other risk factors[110]. Although one study showed transaminase elevations higher than twice the upper limit of normal in 13% of patients[111], another report assessing 6000 patients receiving MTX, transaminase elevation was described in only 0.6% of patients[112]. Despite this wide difference, most studies concluded that prolonged use of low-dose MTX monotherapy (10 mg/wk for 2-15 years) has favorable long-term safety, and that the development of significant liver fibrosis and cirrhosis is very low[113]; rather, steatosis was the main finding when biopsies were carried out for surveillance dictated by cumulative MTX dose (Figure 2)[114]. Due to this disparity, Society's guidelines differ on how patients on MTX should be monitored to prevent MTX-induced liver fibrosis[115,116].

Although liver biopsy is still suggested in these patients in case of persistent elevation of transaminase after drug discontinuation, and for ruling out other potential cause of chronic liver disease, there is robust evidence that Fibroscan Elastography may become in a near future the gold standard for fibrosis investigation in patients treated with MTX[117,118]. Most studies concluded that MTX therapy is safe, and that Fibroscan is useful for monitoring liver fibrosis in patients treated with this drug. Conclusions drawn from several studies indicate that severe liver fibrosis is a rare event in patients treated with MTX, and that it is probably unrelated to the dose. A recent work also studied the accuracy and feasibility of Fibroscan and Fibrotest to detect MTX-induced liver fibrosis in 24 psoriasis patients[119]. The results obtained using Fibroscan and Fibrotest were compared with those obtained by liver histology. In this cohort, Fibrotest accurately predicted the presence of liver fibrosis, while Fibroscan accurately predicted the absence of liver fibrosis in MTX users. These findings suggest that a combination of approaches should prospectively be evaluated in monitoring and detecting significant MTX-induced liver fibrosis.

An association between MTX-induced toxicity and genetic polimorfism was suggested. Fisher *et al*[120] conducted a meta-analysis of published studies including 1400 patients for association of the C677T polymorphism of the gene encoding methylene tetrahydrofolate reductase (MTHFR), and over 660 patients for the A1298C variant. They observed that the former but not the latter *MTHFR* gene variant was significantly related to MTX toxicity, including hepatotoxicity (OR = 1.71; CI: 1.32-2.21, $P < 0.001$). Despite results for MTHFR

A1298C are not conclusive, C677T polymorphism appears to be a promising risk factor for the development of low-dose-MTX-induced hepatotoxicity. Only few studies reported variants in genes that are predictive for MTX-induced hepatotoxicity[121].

Recent results showed that the administration of metformin in rats receiving MTX normalized altered liver function tests and improved liver histopathological findings. Therefore, this result suggests that this drug confers hepatoprotection against MTX-induced hepatotoxicity[122].

Minor abnormalities of liver enzymes are relatively common when using anti-tumor necrosis factor (TNF) agents, such as infliximab, etanercept, and adalimumab, as anti-inflammatory and immunosuppressive compounds for the treatment of autoimmune diseases[123,124]. Severe hepatic reactions are much less common, and include jaundice, hepatitis, cholestasis, and acute liver failure[125-127]. AIH is a rare, but increasingly recognized adverse event linked to treatments with anti-TNF agents[122]. In addition, lupus-like syndrome and anti-TNF-α-induced SLE were the most common disorders listed in a registry of autoimmune diseases associated with anti-TNF-α agents[128,129]. Finally, rituximab is listed as able to reactivate HBV, even in patients with HBsAg negative and anti-HBsAg positive. This concept was recently reinforced by Seto *et al*[130], who reported a HBV reactivation rate of 24% in HBs Ag-negative, anti-HBc-positive patients undergoing rituximab-based chemotherapy for hematologic malignancies, with most of reactivations occurring during the first 6 mo of therapy. The Food and Drug Administration recently announced the requirement of a Boxed Warning for the anti-cancer immunosuppressive drugs Rituxan (rituximab). The Boxed Warning is specific for the risk of HBV reactivation in patients who were previously infected with the virus. Use of these drugs in patients with previous HBV infection can result in severe liver damage if the virus is reactivated[131].

Minocycline, a drug used in the treatment of rheumatoid arthritis and acne, can induce a lupus-like syndrome[132]. In addition, statins, which inhibit hydroxymethylglutaryl-coenzyme A reductase, are widely used nowadays in SLE patients due to their immunomodulator and antiatherogenic effect. Several reports have suggested that this drugs may also induce acute hepatitis and a lupus-like syndrome[133]. Finally, cyclophosphamide, an immunosuppressive and potent alkylating agent that improves the outcome of major organ disease when administered at high doses to SLE patients unresponsive to conventional therapy[134], was reported to induce hepatotoxicity associated with liver inflammation in isolated cases[135,136]. There is a report of one case in the literature showing that this effect may occur even when the drug is administered at low doses[137].

CONCLUSION

Liver abnormalities is very common among patients with SLE, especially if they are assessed from the biochemical point of view. It is generally asymptomatic, and frequently associated with steatosis, reactive unspecific changes and drug-related hepatotoxicity. Severe and progressive liver injury may occur, and even more often in the context of a coexisting primary liver disease or during pharmacotherapy.

SLE by itself is not usually associated with aggressive liver disease, but with an often asymptomatic entity referred to as "lupus hepatitis", which is characterized by a mild increase in serum transaminase levels. However, there are overlapping profiles with other autoimmune disease, such as AIH and PBC, related to chronic and aggressive damage, sometimes accompanied by changes in immunological liver tests that help to establish an accurate diagnosis. These overlap syndromes are thought to be variants of an underlying general autoimmune disease, which shows up in a variable arrangement of autoimmune disorders. An etiological role for anti-ribosomal P antibodies in triggering both lupus hepatitis and AIH has been proposed, but it remains uncertain and controversial.

SLE patients often present comorbility with non-autoimmune liver diseases. They includes HCV, thrombotic events in the splachnic vasculature, PCT, and drug-induced hepatotoxicity, among others.

Hepatic circulation disorders may lead to adaptive parenchymal regenerative processes (*e.g.*, RNH, FNH) or formation of hemangiomas. RNH must be ruled out in all lupus patients who present evidence of portal non-cirrhotic hypertension associated with hepatic pseudonodular images.

Drug-induced liver toxicity is also a common event in SLE, and may be ascribed to the chronic use, at high doses, of medicines used to control the autoimmune disorder (*e.g.*, thiopurine analogues, anti-TNF-α agents, statins, minocycline, cyclophosphamide) or to mitigate SLE symptoms (*e.g.*, NSAIDs, MTX). SLE is an oxidative-stress-prone condition, and the pro-oxidant effects of many of these drugs may be a causal factor.

Due to the relatively frequent multifaceted manifestations of liver diseases in SLE, with an often difficult differential diagnosis each others, an assessment of immunological, serological and virological markers should be systematically carried out in patients with elevated levels of liver enzymes. Testing for AMA, ASMA, and HCV may be particularly helpful. In addition, an analysis of the patient's medical history so as to have an accurate record of the drugs taken by the patient should be carefully done. Finally, histology is in some cases the only reliable method of diagnosis, and should be carried out accordingly. We hope the information provided by this review helps to systematize the knowledge of the field, so as to make the challenge of identifying liver diseases associated with SLE more approachable to the clinician.

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MINIREVIEWS

Management of autoimmune hepatitis: Focus on pharmacologic treatments beyond corticosteroids

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Abstract

In autoimmune hepatitis, patients who are intolerant or with toxicity experience, non-responders, relapsers or refractory are challenging. Non-standard drugs are being tried to preemptively avoid corticosteroid-related side effects. Prognosis and quality of life of life rely on treatment optimization. Recently, emergence of powerful immunosuppressive agents, mainly from liver transplantation, challenged the supremacy of the corticosteroid regime and promise greater immunosuppression than conventional medications, offer site-specific actions and satisfactory patient tolerance. Successes in experimental models of related diseases have primed these molecular interventions. We performed a literature review on alternative treatments. Azatioprine intolerance is the principal indication for mycophenolate use but it can be used as a front-line therapy. Cyclosporine A and tacrolimus have been tested for non-responders or relapsers. Rituximab may be used as salvage therapy. Anti-tumor necrosis factor-alpha agents may be used for incomplete responses or non-responders. Methotrexate is possibly an alternative for induction of remission and maintenance in refractory patients. Cyclophosphamide has been included in the induction regimen with corticosteroids. Ursodeoxycholic acid action is mainly immunomodulatory. Non-standard treatments are coming slowly to the attention, but its use should be cautious performed by experienced centers.

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Key words: Autoimmune hepatitis; Pharmacologic non-standard treatment; Immunosuppression; Azathioprine intolerance; Difficult-to-treat patients; Salvage therapy

Core tip: With our review we pretend to describe the non-standard pharmacologic treatments available for autoimmune hepatitis, the indications for its use and the main applications. Also, we pretend to enhance that those alternatives are only available guided by the experience in liver transplant patients and should be only used by experienced centers. The difficult-to-treat patients lead to the application of those therapies mainly as salvage treatments.

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INTRODUCTION

Liver chronic inflammation, interface hepatitis (on histol-

ogy), hypergammaglobulinemia, and autoantibodies presence are landmarks of autoimmune hepatitis (AIH)[1,2]. AIH is an immune-mediated liver disease, its etiology is unknown[3]. A loss of tolerance seems to be the principal immunologic explanation[3,4]. Women are more affected than men and it occurs across all ages. Men are diagnosed at 40 years of age and women at 50 years of age in median[5]. Prevalence and incidence data on AIH are still limited. In Western Europe and North America Caucasian people the estimated prevalence ranges from 50 to 200 cases per million[6]; the annual incidence in Northern Europeans is 1.9 cases per 100000 persons per year[1,2,7]. An acute presentation occurs in 25% of patients, fulminant presentation is rare but AIH should be considered as etiology in the study of acute liver failure[4]. In addition, the prognosis of disease is influenced by age (young patients having an increased risk), presence of cirrhosis, treatment response (as opposed to activity) and relapses.

The clinical manifestations are heterogeneous. Unspecific symptoms like fatigue, lethargy, jaundice and right upper quadrant pain, are the most frequent clinical presentation. The complications of portal hypertension, *i.e.,* ascites, esophageal varices, hypersplenism and encephalopathy, may ensue on the natural course of the disease. About 25% of patients present extrahepatic immune-mediated symptoms and diseases, arthralgia is the most frequent[4]. Clinical criteria were developed in 1993 and they help to establish the diagnosis when there isn't a single clinical or biochemical test to affirm it[8,9]. These diagnostic criteria include hypergammaglobulinaemia; positivity for autoantibodies: anti-nuclear antibody (ANA), smooth muscle antibody (SMA) or anti-LKM1; typical histology; other causes of hepatitis (viral or toxic) should be excluded as well as other diseases with similar presentation of AIH[8,10]. The autoantibody profile helps to classify AIH: in type 1, SMA and ANA are present; on type 2 anti-LKM1 antibodies are present. Type 1 AIH affects adults and children, while type 2 AIH is mainly a disease of children and adolescents[11]. The scoring system for AIH has a sensitivity of 97% to 100%. In the presence of chronic hepatitis C, the specificity for excluding AIH relies between 66% to 92%[7,10].

Inflammatory activity at the onset of disease and cirrhosis are the main determinants of natural history and prognosis of AIH. Without treatment, mortality of 90% in 10 years is expected when a 5- to 10-times elevation of aspartate aminotransferase and a twofold increase of γ-globulins are present. Cirrhosis occurs in 17% within 5 years of the diagnosis in patients periportal hepatitis and in 82% of patients with bridging necrosis or necrosis of multiple lobules[2]. At diagnosis, 58% of mortality is expected within 5 years of diagnosis[2,4].

The diagnostic criteria may be too strict when applied to diverse ethnic groups because heterogeneous clinical phenotypes and outcomes may be present[12-14] which may be determined by antigenic exposure, variations in immune response, genetic predisposition and cultural, social and economic factors[14]. The diagnosis may be delayed as the institution of corticosteroid treatment[14].

The human leukocyte antigen (HLA) profile also determines the clinical outcome of AIH: HLA DR3 is associated with more severe disease; HLA DR4 is associated with onset at a later age and a more benign outcome of AIH[4]. The HLA-DRB1 locus, specially the alleles HLA-DR3 (DRB1*0301) and DR4 (DRB1*0401) are related to AIH type 1 susceptibility in European populations and North Americans and the strongest genetic associations contributing to the diagnosis of AIH and are included in the IAIHG revised diagnostic scoring system[15]. Genetic profile determines the response to treatment: those who do not respond to corticosteroid treatment have usually DRB1*0301 alleles[15]. Also, clinical manifestations and prognosis may be determined by genetic profile.

AIH, if left untreated, may lead to cirrhosis, liver failure and even death[16]. Survival is increased when immunosuppressive therapy is used. Initially, induction of remission is the main goal[4]. Corticosteroid regimens are effective[2,17]. Prednisolone, alone or in association to azathioprine leads to symptom improvement, laboratory and histologic manifestations of liver inflammation within 6-12 mo in the majority of patients[4,18]. Standard therapy leads to complete biochemical response in 77% in 6 mo[19], improves hepatic fibrosis[18] and 20-year life expectancy is increased in 80%[20].

Early recognition and treatment of the disease, treatment until complete resolution of inflammation, prevention of complications of treatment and early identification and treatment of problematic patients may improve the outcomes of current therapy[21]. Main prognostic determinant is the response to corticosteroid therapy: rapid disease progression is expected when the treatment is delayed or deferred[17].

Between those 23% that do not respond, 5% are intolerant or present toxicity, 7% are non-responders or have refractory disease and the remain 10% have incomplete responses[2]. The relapses after drug withdrawal are frequent (50%-86%)[2,22]. Other efficient treatments are needed. Complete biochemical remission determines the outcome[23] and, therefore, optimization of treatment has implications on prognosis and quality of life[2]. Liver transplantation supersedes empirical drug therapy in decompensated patients[21].

PHARMACOLOGIC TREATMENT OF AIH

The recognition of the response of AIH to immunosuppression changed its prognosis[24,25]. Immunosuppressive treatments should be established immediately, especially in the presence of severe disease[24].

The goal of AIH treatment includes: induction of remission; maintenance of remission; prevention of the establishment of cirrhosis and complications using the lowest possible dose of medication[11,15]. AIH has a good response to immunosuppressive treatment with 80% of remission rate[15,26].

According to the American Association for Study of Liver Diseases guidelines treatment is indicated for patients with established diagnosis of AIH, elevation

of aminotransferase activities [⩾ 5 times upper normal limit-(ULN)], rises of immunoglobulin G (⩾ 2 times upper normal value) and presence of interface hepatitis or necroinflammatory activity (Ishak score 4-6)[2,16]. If untreated, high mortality of 60% at 6 mo is expected when serum aspartate aminotransferase (AST) levels of 10 times the ULN or more than 5 times the ULN especially if associated with serum γ-globulin level more than twice the ULN. Also, in 82% there is progression from bridging necrosis or multilobular necrosis at presentation to cirrhosis, associated with 45% mortality within 5 years[2,16]. Corticosteroid treatment is indicated in the presence of these findings[2,16]. Treatment should also be started in the presence of incapacitating, such as fatigue and arthralgia[2].

Standard therapy may be not an option if coticosteroids, azathioprine or other immunosuppressive therapies are contraindicated by itself or by patient risk factors. The treatment doesn't alter the outcome in patients with decompensated liver cirrhosis on waiting list for liver transplantation or in those with cirrhosis without inflammatory activity[6].

The outcomes of therapy include: remission, relapse, treatment failure, and stabilization[11]. Normal inflammatory parameters and histology is necessary to assume remission. Histological remission should be differentiated from biochemical remission (complete normalization of aminotransferase levels including IgG). Treatment should definitely be considered in any patient with proven AIH, histological activity and a more than marginal elevation of aminotransferase levels, not only in patients with levels greater 5 × ULN. In 65% to 75% of patients after 24 mo on standard therapy remission is achieved[11,27]. Relapse is defined as a flare in aminotransferase levels with symptoms under treatment, following the minimum dose of maintenance therapy, or after withdrawal. Relapse occurs in about 50%; loss of remission in 42% within 6 mo of treatment withdrawal and in 80% after 3 years; progression to cirrhosis occurs in 38% and liver failure in 14%[11,17]. Retrospective analysis indicates that loss of remission or relapse occurs in virtually all patients with AIH in long-term remission when immunosuppressive therapy is discontinued[28]. Treatment failure should be assumed when there is progression of symptoms, non-improvement of histological parameters and deterioration of serologic features during standard therapy. In case of treatment failure, diagnosis should be reconsidered to exclude an overlap syndrome with primary sclerosing colangitis or primary biliar cirrhosis or different etiologies[11]. Partial remission corresponds to stabilization of the disease[11].

STANDARD PHARMACOLOGIC TREATMENT

The standard initial treatment of AIH includes the corticosteroids only or combined with azathioprine. Combination therapy is the first choice and low-dose of prednisolone (30 mg/d) with 1 mg/kg azathioprine are used in the induction phase[11]. In the United States, 50 mg is used for azathioprine, but in Europe a dose of 1-2 mg/kg bodyweight is used[2]. Alternatively, monotherapy may be used, with 60 mg of steroid and reductions of 10 mg/wk to maintenance dose of 20 mg for at least 6 mo, and further reduction until lowest dose in 2.5 mg decrements. Maybe the initial prednisolone dose in combination therapy should be considered since the percentage of response is higher. There are no differences in the remission induction. Combined treatment is preferred because it allows to decrease the dose of the prednisone dose to below 10 mg and reduces the steroid side effects[6].

Standard therapy is the best option unless contraindicated and may be especially useful by reducing corticosteroids side-effects in older patients, in patients with osteoporosis, metabolic syndrome or psychiatric lability[6]. Monotherapy with steroids is the best treatment option in patients with hematological abnormalities or a proven homozygous deficiency of thiopurine methyltransferase because azathioprine causes hematological side effects such as leukopenia or anemia[6]. Thiopurine methyltransferase (TPMT) is an enzyme responsible for the conversion in one of azathioprine to 6-mercaptopurine (active metabolite) and in 6-methyl mercaptopurine or 6-thiouric acid (inactive metabolites)[12]. In patients with azathioprine intolerance, lower TPMT activity is documented but measurements of TPMT activity cannot be used to identify those patients[29]. Pre-treatment TPMT testing provides some certain of the presence of risk for azathioprine toxicity and strengths physician confidence in the treatment regimens[12]. Allopurinol may safely and effectively optimize thiopurine therapy in patients with intolerance and/or nonresponse due to an unfavourable thiopurine metabolism and this is another option in order to maintain the standard treatment[30].

Corticosteroids are the first option of treatment in all populations, but its use should be individualized in the presence of cholestatic features[14].

However, as the combination treatment fails, other drugs have been tried although its use requires further validation[24].

In fulminant hepatic failure and in en-stage liver disease, transplantation is the treatment of choice. Post-transplantation AIH recurrence may occur[24].

ALTERNATIVE CORTICOSTEROID REGIMEN-BUDESONIDE

Budesonide is glucocorticoid from the next-generation, more than 90% has first pass hepatic clearance and metabolites don't have glucocorticoid activity[31]. These pharmacological properties seem to predict less secondary effects. In non-cirrhotic patients, treatment combination between budesonide and azathioprine may be an alternative in uncomplicated AIH with mild disease[32,33] or with conditions that may be worsened by prednisone treatment like hypertension, osteopenia, diabetes and obesity[34].

In corticosteroids refractory or dependent AIH patients may not be used as a rescue treatment. The budesonide

regimen normalized serum AST and alanine aminotransferase (ALT). Budesonide histological resolution and persistent response is unknown[34].

NON-STANDARD PHARMACOLOGIC TREATMENT

There are some difficult-to-treat patients for whom newer immunosuppressive agents, usually employed as anti-rejection drugs, have been tried with variable success. Immunosuppression with non-standard drugs is being tried to avoid corticosteroid side effects (13%) but are being used specially as superior regimens to corticosteroid treatment[21]. The use of such regimens has to be weighed and data available comes only from few small studies or case reports[15]. Other treatments considered are: mycophenolate mofetil (MMF)[35-43], cyclosporine A (CyA)[44,45], tacrolimus (FK506)[46-49], ritximab[50], anti-tumor necrosis factor-alpha (TNF-α) agents[51], methotrexate[52], cyclophosphamide and ursodeoxycholic acid (UDCA)[53] (Table 1). These non-standard treatments application is not widespread and they are not included into any standard management algorithm[15].

Only recently has the emergence of powerful immunosuppressive agents, mainly from liver transplantation, challenged the supremacy of the corticosteroid regimens[22,54]. Drugs outside of the standard repertoire now promise greater immune suppression than conventional medications, offer site-specific actions and satisfactory patient tolerance[22,54]. Site-specific molecular treatments are also possible because of improved understanding of the central pathogenic disease pathways and technological advances that now enable modulation of these pathways[22,54]. Furthermore, successes in experimental models and in other autoimmune diseases have primed these molecular interventions for study in AIH[22,54].

Importantly, publication bias may be considered since there is, probably, underreport of studies with negative results. Also, target populations, dosing schedules, safety profiles and monitoring strategies are not yet clear; adjunctive therapy with corticosteroids is still required; the standard algorithms do not include already the risks and expense of these drugs[55]. Newer agents are much more expensive than the standard treatment irrespectively of the generic use (as recently available generic MMF may attenuate this problem) and this may be a limitation to the accessibility to these treatments.

MMF

MMF, is most frequently used in patients with refractory AIH or azathioprine intolerance but it may be used as a first choice treatment[16,22,35,38,40,41]. It acts as a purine antagonist.

MMF is hydrolyzed by to mycophenolic acid by liver esterases and acts as reversible noncompetitive inhibitor of inosine monophosphate dehydrogenase: it selectively impairs the synthesis of nucleotides based on purines, inhibits the new synthesis of DNA, impairing proliferation of activated lymphocytes. The thiopurine methyltransferase pathway does not interfere on the activation or elimination of lymphocytes[16,22].

De novo synthesis of purines, in contrast with other cells, is essential for B and T cell proliferation: this is why MMF exerts its cytotoxicity specially on these cell populations[4].

According to eleven small single-centre experiences, MMF is effective in difficult-to-treat patients in doses ranging from 0.5 g/d to 3 g/d[22,35]; 2 g/d in divided doses was the most used regimen, initially with corticosteroids[16].

Recent studies[43,56-58] showed that 47% of the patients had positive response and 53% showed no response or drug intolerance[22]. From 11 studies, 40% of the patients included achieved complete corticosteroid withdrawal and 15% experimented treatment-ending side effects[22]. MMF treatment was more efficient in patients where it was used because of azathioprine intolerance than in patients who where treated for refractory liver disease (58% *vs* 12%)[57,58]. Nonresponders were mainly children with AIH and sclerosing cholangitis[56].

MMF has been used as first choice therapy in naive patients. MMF was used in 59 previously untreated AIH patients for up to 92 mo: 88% showed normal aminotransferase and gamma-globulin serum levels (within three months) and 12% showed partial response[59]. Corticosteroids withdrawn occurred within eight months in 58% and 3% presented serious side effects. MMF can be administered effectively and safely as a front-line treatment, but the reasons for preferring this treatment as a front-line strategy are unclear[22].

The most common side effects of treatment with MMF in AIH patients have been gastrointestinal discomfort (nausea, diarrhea and abdominal pain) (11%), rash (including skin cancers) (7%), fatigue (7%) and leukopenia (1%)[57]. The frequency of side effects has ranged from 3% to 33%[57,59] and the frequency of treatment-ending complications has been as high as 13%[57].

The differences between the costs of MMF and azathioprine may be important[60]; treatment ending side effects occur in 3% to 13%[57,59]; most patients require continuous corticosteroid therapy; the duration of treatment is indefinite; and is more efficient as a salvage therapy in patients with azathioprine intolerance than in patients with steroid-refractory liver disease[57,59]. MMF has a limited and evolving off-label role in AIH, and its use as a salvage therapy for azathioprine intolerance is currently its most effective application[22].

Data about histological remission are poor and further studies are needed before recommend MMF as a first-line treatment for AIH[16]. MMF is contraindicated in pregnancy[16,22].

Calcineurin inhibitors

CyA and FK506 are calcineurin inhibitors that alter phosphatase activity, interfere with lymphocyte T proliferation blunting cell-mediated immune responses. Cyclosporine and FK506 have each been used in AIH patients, primarily as salvage therapies for steroid-refractory disease[22,54].

Table 1 Non-standard immunosuppressive drugs used in autoimmune hepatitis

Non-standard pharmacologic treatments	Studies	Indications	Contra-indications	Outcomes
Mycophenolate mofetil, 0.5 to 3.0 g/d Purine antagonist (inhibits inosine monophosphate dehydrogenase, limits purine nucleotides, impairs lymphocyte proliferation)	[1]46 mo, 7 patients[35] [1]19 mo, 8 patients[39] [1]41 mo, 15 patients[40] [1]61.5 mo, 26 patients[56] [1]26 mo, 59 naïve-patients[59]	Azatioprine Intolerance Refractory AIH Front-line therapy	Pregnancy Hypersensitivity to mycophenolate mofetil, mycophenolic acid or mycophenolate sodium	Salvage[22,35-43,56]: 47% overall improvement 58% azathioprine intolerance 12% refractory disease 53% failure or side effects 40% steroid withdrawal 3%-33% Serious side effects Front-line[59]: 88% complete response 12% partial response 58% steroid withdrawal 3% serious side effects
Cyclosporin, 2 to 5 mg/kg per day Calcineurin inhibitor (impairs NF-κB, reduces IL-2 and lymphocyte proliferation)	6 mo, 19 patients[44] 3 mo, 5 patients[45]	Refractory AIH Relapsing AIH Non-responding AIH	Rheumatoid arthritis and psoriasis: abnormal renal function, uncontolled hypertension, malignancies Psoriasis: under PUVA, UVB therapy, methotrexate Hypersensitivity to cyclosporin or to polyoxyethylated castor oil Pregnancy	Composite results[22,44,45]: 93% improvement 7% failure/side effects
Tacrolimus, 0.075 to 4 mg/kg twice a day Calcineurin inhibitor (impairs NF-κB, reduces IL-2 and lymphocyte proliferation)	12 mo, 21 patients[46] 25 mo, 11 patients[48] 18 mo, 9 patients[49]	Refractory AIH Relapsing AIH Non-responding AIH	Hypersensitivity to tacrolimus Pregnancy	Composite results[22,46,49]: 98% improvement 2% failure/side effects
Rituximab, 1.0 g, two doses 15 d apart[1] Anti-CD20 (B-cell depletion, impairs type 2 cytokine pathway, interferes with antibody-dependent cell-mediated cytotoxicities)	5 mo, 6 patients[66] case reports; data from studies for hematological malignancies, rheumatoid arthritis	Refractory AIH Relapsing AIH Non-responding AIH	Type 1 hypersensitivity or anaphylatic reaction to murine proteins Progressive multifocal leukoencephalopathy	Biochemical improvement
Infliximab, dose of 5 mg/kg at weeks 0, 2 and 6, and then every 4 to 8 wk[1] Anti-TNF-α (neutralizing soluble transmembrane forms of TNF-α impairing cytotoxic type 1 cytokine pathway)	Case reports	Refractory AIH Relapsing AIH Non-responding AIH	Heart failure NYHA class Ⅲ/Ⅳ Hypersensitivity to infliximab or murine proteins	Biochemical improvement
Cyclophosphamide, 1 to 1.5 mg/kg per day Alkylating agents (covalent binding and crosslinking to deoxyribonucleic acid-DNA, ribonucleic acid-RNA and proteins)	95 mo, 94 patients with long-term auto-immune hepatitis[71]	Refractory AIH Relapsing AIH Non-responding AIH	Hypersensitivity to cyclophosphamide, urinary outflow obstructions, severe myelosuppression, severe renal or hepatic impairment, severe immunossupression Pregnancy	91% complete remission
Methotrexate, 7.5 mg/wk Purine antagonist (inhibits the binding of dihydrofolic acid)	Case reports[52]	Refractory AIH	Hypersensitivity Breast-feeding Pregnancy	Biochemical and histologic improvement
Ursodeoxycholic acid, 13 to 15 mg/kg per day Immunomodulation (epimer of chenodeoxycholic acid)	6 mo, 37 patients[53]	In addition to other immunosuppressive strategies	Hypersensitivity Unremitting acute cholecystitis, cholangitis, biliary obstruction, gallstone pancreatitis, biliary-gastrointestinal fistula, allergy to bile acids	Biochemical improvement Corticosteroid dose reduction

[1]Careful is needed in women of childbearing age since those treatments have uncertain effects on reproduction and are presumable teratogenic. AIH: Autoimmune hepatitis; NF-κB: Nuclear factor kappa B; TNF-α: Tumor necrosis factor-alpha; IL-2: Interleukin-2; UVB: Ultra-violet B; PUVA: Psoralen and ultra-violet A.

Calcineurin activates nuclear factor-κB *via* a pathway dependent on phosphatase activity. The activated nuclear factor binds to promoter regions of interleukin-2 (*IL-2*) gene increasing transcription of IL-2. In turn, IL-2 stimulates the cell cycle by binding to IL-2 receptor, and lymphocytes proliferate by a type 1 cytokine pathway[22,54]. In difficult-to-treat AIH patients calcineurin inhibitors have been used as a rescue treatment[15].

CyA: CyA is a calcineurin inhibitor extracted from the *tolypocladium inflatum* and *cylindrocarpum lucidum*[11]. It has been used, since 1985, mainly as a rescue therapy but also in relapsing or non-responsive AIH[22]. There are no long-term reports on safety but results in these situations seem promising[16]. Ten studies[22,44,61] showed that 93% of the 133 patients included within 26 years had a positive response, and 7% showed no response or drug intolerance[22].

Serum aminotransferases and histological activity index scores decreased over 6 mo in an open label trial of 19 patients[16,44].

In a multicenter study, 32 children were included and CyA was administered as monotherapy for 6 mo (200-250 ng/mL levels). Then, prednisolone and azathioprine were given in low doses for 1 mo and stopped after[62]. Alanine aminotransferase activity levels normalized in 25 patients by 6 mo and in all patients by 1 year of treatment. There was a trend to improvement of *Z*-scores for height during treatment[62].

Between 1994 and 2000, 84 children where recruited from five centers, CyA was administered during 6 mo in doses similar to that previously described; after 6 mo, patients with AST/ALT levels lower than 2-ULN started standard therapy. Aminotransferase levels were normal in 94% of patients, 72% within the first 6 mo of treatment[16].

In all studies, CyA adverse effects seem to be mild and transient and standard therapy is not related with relapse during follow-up[16,44,62].

The data are encouraging and CyA might be considered an alternative therapy to steroids in patients who do not achieve a complete remission. However, side effects are a serious problem and include: dyslipidemia, hypertension, renal failure, infection, hirsutism and malignancy[16].

FK506: FK506, macrolide lactone antibiotic, acts as a potent immunosuppressive agent on CD4+ T-helper cells[11]. FK506 and CyA have similar mechanisms of action however, FK506 binds to a different immunophilin (FK-binding protein) leading to the inhibition of lymphokine synthesis (IL-2, IL-3 and IFN-α), IL-2 receptor expression and the generation of cytotoxic T cells[6]. FK506 has been used as a rescue therapy since 1995[22]. Experience with this drug is reported in three studies, 41 patients were included within 16 years: 98% presented a positive response; 2% presented no response or treatment-ending drug intolerance[22,46,49]. There are no controlled trials on the use of FK506 in AIH[11]. In a preliminary trial, 21 patients were treated with FK506 (drug levels of 0.6-1.0 ng/mL): biochemical improvement was documented after 3 mo[46]. Although the reported results are encouraging, more extensive studies are warranted before FK506 can be recommended as a safe and useful agent in AIH[11]. Remission can be achieved with FK506 for most patients, only or combined with corticosteroids. All series are limited by a short time of follow-up[16].

The success of the calcineurin inhibitors as a salvage therapy for AIH has been impressive, but the overall reported clinical experience with these agents has been lacking. Calcineurin inhibitors still lack a uniform dosing schedule, an acceptable safety profile and an established monitoring protocol for AIH despite their longstanding empirical use in this disease. Efforts to launch large, multicentre, clinical trials have been frustrated by low patient recruitment. Calcineurin inhibitors remain empirical, off-label treatments reserved for steroid-refractory disease and even in these cases should be used with caution and only in experienced centres[22].

Rituximab

Rituximab is an anti-CD20 chimeric monoclonal antibody, a surface marker expressed on B cells, from early pre-B to memory B lymphocytes. Treatment with rituximab leads to B cell depletion through both complement- and antibody-dependent cellular cytotoxicity[63]. Initially developed for the treatment of B-cell lymphoma, rituximab has since proven effective for the treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus or autoimmune haemolytic anemia[64], suggesting it might also be effective in patients with AIH.

Treatment with rituximab has been reported as effective in patients with Epstein Barr virus infection associated with lymphoproliferative disease secondary to azathioprine[64], in a patient with concurrent diagnoses of B cell lymphoma[65] and steroid resistant AIH/primary biliar cirrhosis overlap syndrome, in patients with concomitant idiopatic thrombocytopenic purpura, cryoglobulinemic glomerulonephritis, or Evans syndrome. Isolated AIH refractory to standard treatment in 6 patients was studied in a phase 1 study: they were treated with rituximab (1000 mg at days 1 and 15)[66]. All patients were maintained on stable doses of prednisolone plus azathioprine for at least 1 mo before and 3 mo after rituximab infusions, after which steroids were tapered. Biochemical remission was achieved by all patients by week 12, with good tolerance to treatment with no serious adverse event being reported during the 72-wk follow-up[66]. Although these results are promising and the toxicity profile is favourable, controlled clinical trials are needed before rituximab can be recommended as an alternative treatment in AIH[11].

Anti-TNF-α agents

TNF-α is a pro-inflammatory cytokine known to be implicated in the pathogenesis of AIH[67]. Additionally, genetic polymorphisms in the TNF promoter region have been identified in patients with AIH type 1, associated with a poorer response to corticosteroid therapy

and higher incidence of cirrhosis[68,69]. Infliximab, etanercept and adalimumab are anti-TNF-α agents commonly used for treatment of immunemediated diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel disease[11]. Soluble and transmembrane forms of TNF-α are neutralized by anti-TNF-α agents. It also seems to have pro-apoptotic effect on activated lymphocytes. Its effect in AIH is explained by the impairment of activated lymphocytes activity[51].

Weiler-Normann *et al*[51] reported the first series of AIH patients treated with infliximab in a single centre. This retrospective study included 11 AIH patients who did not achieve remission with a standard immunosuppressive regimen upon diagnosis, and who also failed to respond to other alternative treatments, including cyclosporine, FK506 and cyclophosphamide. Patients were given infusions of infliximab at a dose of 5 mg/kg at weeks 0, 2 and 6, and then every 4 to 8 wk depending on response. After 3 infusions of infliximab, all patients showed a decrease in the levels of transaminases and of IgG; normalisation of transaminases and IgG levels was observed in 8 and 6 patients respectively. Of the 5 patients in whom a liver biopsy was performed after treatment, all showed reduction of inflammation, as expressed by a modified histological activity index. Some cautions in the use of this agents in AIH must be present since treatment with infliximab has been associated with the induction of severe de novo AIH in some patients treated for other diseases[51,70].

For all the above reasons, while more studies are warranted to evaluate the efficacy and tolerability of infliximab in AIH, this type of treatment should be considered in defined cases and administered only in specialised centres[11,70].

Cyclophosphamide

For the induction of remission in combination with steroids cyclophosphamide was used in the dose of 1-1.5 mg/kg per day[71]. Cyclophosphamide use is highly experimental because of the potential severe hematological side effects[6].

Methotrexate

Methotrexate is an antagonist of folate metabolism, it has anti-inflammatory and immunomodulating properties. Bone marrow suppression and mucosal ulceration at higher doses are the principal side-effects but it is generally well tolerated[52].

A once-weekly dose is reported as induction and maintenance regimen in two case reports. Fibrogenic effect might enable its long-term use[72].

UDCA

UDCA may have immunomodulatory functions. It is a hydrophilic bile acid that changes HLA-1 antigen expression on cellular surfaces and suppresses the production of immunoglobulin. Non-controlled studies show improvement in histology features, in clinical presentation and biochemical parameters. A reduction of fibrosis wasn't established in four AIH type 1 patients. Its role in AIH treatment is not yet established[6,53]. UDCA monotherapy is effective for some Japanese AIH patients, may have a role during the taper of corticosteroids for prevention of early relapse but is not recommended on patients with high-grade inflammatory activity or poor residual capacity of liver[73].

CONCLUSION

In AIH, identification of efficient salvage treatment options is urgently needed for the difficult-to-treat patients: those who experience intolerance or toxicity, non-responders, relapsers or with refractory disease. Also, non-standard drugs are being tried as superior drugs to corticosteroid regimens and to minimize its side effects. Optimization of treatment plays a major role in long-term prognosis and quality of life for patients with AIH. Recently, the emergence of powerful immunosuppressive agents, mainly from liver transplantation, challenged the supremacy of the corticosteroid regime and promise greater immunosuppression than conventional medications, offer site-specific actions and satisfactory patient tolerance. Successes in experimental models and in other autoimmune diseases have pointed these molecular interventions for study in AIH. Some encouraging results were described, but the establishment of these non-standard drugs as alternative treatments has evolved slowly and they weren't already included into a standard management algorithm. Therefore, those treatments should be used with caution and only in experienced centers.

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MINIREVIEWS

Management of hepatitis C virus infection in hemodialysis patients

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Abstract

The prevalence of hepatitis C virus (HCV) infection in patients on maintenance hemodialysis (MHD) is relatively higher than those without MHD. Chronic HCV infection detrimentally affects the life quality and expectancy, leads to renal transplant rejection, and increases the mortality of MHD patients. With the application of erythropoietin to improve uremic anemia and avoid blood transfusion, the new HCV infections during MHD in recent years are mainly caused by the lack of stringent universal precautions. Strict implementation of universal precautions for HCV transmission has led to markedly decreased HCV infections in many hemodialysis units, but physicians still should be alert for the anti-HCV negative HCV infection and occult HCV infection in MHD patients. Standard interferon alpha and pegylated interferon alpha monotherapies at a reduced dose are currently the main treatment strategies for MHD patients with active HCV replication, but how to increase the sustained virological response and decrease the side effects is the key problem. IFNα-free treatments with two or three direct-acting antivirals without ribavirin in MHD patients are waiting for future investigations.

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Key words: Hemodialysis; Hepatitis C virus; Epidemiology; Risk factors; Prophylaxis; Treatment

Core tip: The new hepatitis C virus (HCV) infections during maintenance hemodialysis (MHD) in recent years are mainly caused by the lack of stringent universal precautions. Strict implementation of universal precautions for HCV transmission has led to markedly decreased HCV infections in many hemodialysis units, but the anti-HCV negative HCV infection and occult HCV infection in MHD patients still should be noted. How to increase the sustained virological response and decrease the side effects is the key problem for the currently recommended interferon alpha-based antiviral therapy in MHD patients. Interferon alpha-free treatments with two or three direct-acting antivirals without ribavirin in MHD patients are waiting for future investigations.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem worldwide which can lead to chronic hepatitis

C, liver cirrhosis and hepatocellular carcinoma (HCC)[1,2]. Prevalence of HCV infection is markedly higher in patients on maintenance hemodialysis (MHD)[3-7]. Chronic HCV infection detrimentally affects the life quality, decreases life expectancy, leads to renal transplant rejection, and increases the mortality of MHD patients suffering from chronic kidney failure[1,5,6]. Moreover, HCV infection has been shown to increase the prevalence of renal insufficiency, defined by serum creatinine $\geqslant$ 1.5 mg/dL; the mechanisms may include the direct HCV-related renal injury and HCV-related cirrhosis with subsequent renal impairment[7], and this will be harmful for patients who receive renal transplantation. The rates of HCV infection in MHD patients vary markedly among different countries and hospitals. Multiple factors are associated with the high risk of HCV transmission in MHD patients[3]. Standard interferon alpha (ST-IFNα) and pegylated IFNα (PEG-IFNα) are currently the main treatment strategies for HCV infection in MHD patients, and the key problems are how to increase the sustained virological response (SVR), control the side effects and minimize the dropout rates[1,2,8-10]. This review summarizes the advancement in understanding the prevalence, risk factors, monitoring strategy, and more importantly, prophylaxis and treatment of HCV infection in MHD patients.

EPIDEMIOLOGY

HCV infection in hemodialysis patients varies by patients' behavioral and cultural differences, geographic location, socioeconomic aspects, community exposure factors, number of patients per hemodialyzer and rigorous use of the strictest biosafety standards[4,11], with the reported prevalence ranging from 1.9% to 90% (Table 1)[3]. Generally, new cases of HCV infection related to hemodialysis are more frequent in regions that have a higher prevalence of serum anti-HCV, and HCV genotypes in hemodialysis patients are usually in accordance with those found in non-hemodialysis patients; but some HCV genotypes that are rare in the general population may be more prevalent in hemodialysis patients because of the nosocomial person-to-person transmission in the hemodialysis unit[4]. For instance, a higher prevalence of HCV genotype 2b has been found in hemodialysis populations in southern Brazil, a genotype rarely occurring in Brazil, where the 1a, 1b, or 3a are more common[4].

Table 1 Prevalence of anti-hepatitis C virus seropositivity in hemodialysis patients

Country/region	Prevalence	Investigators and year of publication
Slovenia	1.9%	Buturović-Ponikvar[12], 2001
Netherlands	3.4%	Schneeberger *et al*[13], 1999
Puerto Rico	3.5%	López-Navedo *et al*[14], 1999
United Kingdom	4.0%	Wreghitt[15], 1999
Germany	6.1%	Hinrichsen *et al*[16], 2002
Mexico	6.7%	Méndez-Sánchez *et al*[17], 2004
Belgium	6.8%	Jadoul *et al*[18], 2004
United States	7%-23.3%	Kalantar-Zadeh *et al*[5], 2007 Kalantar-Zadeh *et al*[19], 2005 Sivapalasingam *et al*[20], 2002 Kelley *et al*[21], 2002 Saab *et al*[22], 2001
Brazil	6%-90%	da Silva *et al*[4], 2013 Mello Lde *et al*[23], 2007 Lopes *et al*[24], 2006 Albuquerque *et al*[25], 2005 Carneiro *et al*[26], 2001
China Maintain	7.01%-37.34%	Ren *et al*[27], 2011 Qi *et al*[28], 2003
Greece	10%-29%	Garinis *et al*[29], 1999 Rigopoulou *et al*[30], 2005 Sypsa *et al*[31], 2005
Sweden	11.0%	Almroth *et al*[32], 2002
Iran	13.2%	Alavian *et al*[33], 2003
France	16.3%	Salama *et al*[34], 2000
Tunisia	19%-41.7%	Bouzgarrou *et al*[35], 2005 Ayed *et al*[36], 2003
Libya	20.5%	Daw *et al*[37], 2002
Italy	22.5%-32.1%	Petrosillo *et al*[38], 2001 Lombardi *et al*[39], 1999
Sudan	23.7%	El-Amin *et al*[40], 2007
Vietnam	26.6%	Dunford *et al*[7], 2012
Bosnia and Herzegovina	59.0%	Ahmetagić *et al*[41], 2006
Peru	59.3%	Sanchez *et al*[42], 2000
Kuwait	71.0%	Wreghitt[15], 1999
Moldavia	75.0%	Covic *et al*[43], 1999
Senegal	80.0%	Diouf *et al*[44], 2000

RISK FACTORS

Currently, it is still unclear how MHD patients become HCV-infected. Nevertheless, both intradialysis (number of blood transfusions, duration and mode of dialysis, prevalence of HCV in the hemodialysis unit, breakdown of standard infection control practices) and extra dialysis (high risk of lifestyle behaviour) variables have been identified.

First, many of these patients had severe uremic anemia needing blood transfusion, which is the most important route of HCV transmission[3,7]. Thus, it was highly possible that some of the hemodialysis patients got HCV infection through this way, especially in regions with poor socioeconomic conditions, where the qualified medical staff and equipments available to treat MHD patients were very limited. In the past two decades, the sensitivity and specificity of laboratory tests for detection of HCV have improved greatly, leading to the more stringent screening of blood donors and the marked decline of new HCV infections[6,45,46]. On the other hand, the availability of erythropoietin has reduced the need of blood transfusion in hemodialysis patients. Accordingly, the risk of HCV infection through blood transfusion in hemodialysis patients has decreased significantly in many countries[45].

Second, new HCV infections can occur in patients who lack the risk factors of blood transfusion, intravenous drug use, high-risk sexual activity, or exposure to known HCV-positive persons. It is believed that these patients were infected by HCV during the course of hemodialysis[47]. Phylogenetic analysis of HCV isolates implies that many HCV infections during hemodialysis

are surely the result of nosocomial patient-to-patient transmission[45,47-50]. The infection risk usually increases with the prevalence of HCV, and the number and length of hemodialysis exposure in corresponding hemodialysis units[4,5,31,40]. Recently, da Silva *et al*[4] reported that HCV-infected patients had been on hemodialysis for 91.9 mo, more prolonged than HCV-negative patients ($P = 0.001$). Another investigation showed that the prevalence of HCV infection at admission in a New York City hemodialysis unit was 18%, far higher than the 1.6% in the United States population overall. During 2001-2008, nine patients treated in this unit were found to have seroconversion from anti-HCV negative to positive. Of them the sources for four HCV infections were identified phylogenetically and epidemiologically as four other patients in the unit. The epidemiologic and site investigations showed that the hemodialysis unit had inadequate HCV infection surveillance and patient follow-up, inadequate cleaning and disinfection practices, failing to wear or change gloves or perform hand hygiene between contacted patients, lack of a separate clean area for medication storage and preparation, and short turnover periods between patient treatments[47]. Accordingly, it is suspected that the way for HCV transmission in these patients may be direct percutaneous exposure to infectious blood because of inadequate infection control[1]. On the contrary, the use of dedicated hemodialyzer specially prepared for each patient and the strict implementation of hygienic precautions against HCV transmission could markedly decrease the incidence of nosocomial HCV infection in hemodialysis patients[45].

MONITORING

Monitoring serum anti-HCV by enzyme-linked immunosorbent assay or enzyme immunoassay every three to six months is essential to identify HCV seroconversion[45,47]. Sometimes the recombinant immunoblot assay for anti-HCV should be added to confirm the positivity of anti-HCV[47]. Of note is that the anti-HCV tests may fail to detect HCV infection in 1.66%[45] to 7.2%[46] of MHD patients, because the immunocompromised status of these patients prevents them from having detectable anti-HCV antibodies[1]. So it is necessary to detect HCV core antigen by chemiluminescent assay or HCV RNA by polymerase chain reaction (PCR) in anti-HCV negative patients who are at high risk of HCV transmission[6,46]. If HCV RNA is positive, it is necessary to quantitate and genotype the HCV RNA further to provide important information for phylogenetic analysis of HCV isolates and selection of treatment strategy in MHD patients[45,50]. In addition, serum alanine aminotransferase (ALT) and other liver-associated biochemical tests, alpha fetoprotein and ultrasonic scan of the liver should also be conducted regularly.

Occult HCV infection, defined as detectable HCV RNA in the liver or peripheral blood mononuclear cells (PBMCs) in the absence of both serum HCV RNA and anti-HCV[51], is a serious fact that might be ignored in hemodialysis patients. Barril *et al*[51] reported that occult HCV infection, determined by the presence of genomic HCV RNA in PBMCs, was found in 45% of the 109 MHD patients, and 53% of these patients had ongoing HCV replication indicated by the presence of antigenomic HCV RNA. Patients with occult HCV infection had spent a significantly longer time on hemodialysis and had significantly higher mean ALT levels during the 6 mo before study entry. Accordingly, for patients with long time of hemodialysis and a relatively higher serum ALT level, the PBMCs or liver biopsy samples should be collected to detect HCV RNA to rule out occult HCV infection[51,52].

PROPHYLAXIS

There is no active vaccine to prevent MHD patients from HCV infection. It has been adopted by many medical centers to assign HCV-infected patients to dedicated hemodialysis machines in a dedicated room in order to separate HCV positive patients from the negative patients, and this has been considered to be able to decrease the risk of HCV transmission[53]. In those hemodialysis units with high HCV prevalence but without fulltime medical staff on HCV-infection control, this strategy may help decrease the risk of HCV transmission among patients[3]; but for hemodialysis units with strict universal precautions against HCV transmission, some specialists consider that the dedicated hemodialysis machine in a dedicated room for HCV-infected patients is somewhat unjustified and unnecessary[49,54].

Universal precautions, especially stringent adherence of all necessary biosafety measures during hemodialysis, are considered to be the keystones to minimize HCV transmission related to hemodialysis and have maximized ideal prophylactic effects[45,47,53]. These measures include: (1) applying a disposable hemodialyzer to avoid sharing of a hemodialyzer; (2) systematic decontamination of the equipment and circuits after each patient's treatment; (3) avoiding sharing of medications, such as multiuse vials of heparin among patients; (4) avoiding sharing of instruments such as tourniquets; (5) preparing any medications in a separate area; (6) disinfecting hemodialysis station surfaces timely; (7) cleaning hands and changing gloves before contacting different patients; (8) periodic testing of all patients for anti-HCV and HCV RNA; and (9) systematic training of health workers in hemodialysis units.

TREATMENT

HCV infection has a significant adverse effect on the health of persons with chronic kidney disease, leads to a higher mortality in MHD patients than non-infected MHD patients, and reduces the survival rates of patients who undergo kidney transplantation, as do their grafts. Moreover, HCV infection renders the patients at high risk of developing diabetes mellitus, membranous glomerulonephritis as well as fibrosing cholestatic hepatitis after

Table 2 Current recommendations for antiviral treatment of hepatitis C virus infection in maintenance hemodialysis patients with kidney failure[1,54,56,57]

Drug	Dosage	Notes
ST-IFNα-2a	3 million units, three times a week	Usually 48 wk for HCV genotypes 1 and 4, and 24 wk for HCV genotypes 2 and 3, or receiving response-guided treatment
ST-IFNα-2b	3 million units, three times a week	
PEG-IFNα-2a	135 μg, once a week	A more reduced dose, a longer interval between two injections, or temporary cessation of IFNα should be considered in patients with severe side effects such as dangerous bone marrow suppression
PEG-IFNα-2b	1 μg/kg, once a week	
Ribavirin	200 mg, once a day, every other day, or thrice weekly after hemodialysis	Ribavirin is applied in combination with interferon, and should be prohibited if severe anemia or other adverse effects occurs

HCV: Hepatitis C virus; ST-IFNα: Standard interferon alpha; PEG-IFNα: Pegylated interferon alpha.

kidney transplantation[1]. Accordingly, patients with MHD who are infected with HCV should be treated if conditions permit, no matter whether they will receive kidney transplantation or not. On the other hand, occult HCV infection is usually persistent and can not be eradicated spontaneously. Though it seems to be less aggressive than chronic hepatitis C, occult HCV infection may also lead to liver cirrhosis and even HCC[52,55]. Accordingly, if occult HCV infection could be confirmed in MHD patients, the antiviral therapy should be given too[40]. Recommendations for the treatment of HCV infection in MHD patients with kidney failure are summarized in Table 2.

ST-IFNα and PEG-IFNα monotherapies are currently the main treatment strategies for MHD patients with active HCV RNA replication. For adult patients, ST-IFNα-2a or ST-IFNα-2b should be given at a reduced dose of 3 million units three times a week, and PEG-IFNα-2a or PEG-IFNα-2b should be given at a reduced dose of 135 μg and 1 μg/kg once a week, respectively[1]. If the patients still cannot endure the side effects even in the use of erythropoietin, granulocyte-macrophage colony stimulating factor, interleukin-11 or other symptomatic and supporting treatments, a more reduced dose of IFNα should be given, and/or the intervals between two injections should be prolonged, or the IFNα should be stopped temporarily. Generally, the recommended treatment duration of IFNα is based on the HCV genotypes, *i.e.*, 48 wk for HCV genotypes 1 and 4, and 24 wk for HCV genotypes 2 and 3[54]; but the response-guided treatment strategy should also be emphasized, *e.g.*, shorter treatment duration for patients achieving rapid virological response (defined as seronegativity of HCV RNA at week 4 of treatment) than those with early virological response (EVR, defined as a seronegative or at least a 2 $\log_{10}$ decrease from baseline in the serum HCV RNA at week 12 of treatment), and early termination in those without an EVR[56]. Moreover, a shorter treatment duration of IFNα might be considered in patients with interleukin-28B (IL-28B) genotype rs12979860 CC or rs8099917 TT, but a longer treatment duration should be given in those with IL-28B genotype rs12979860 CT/TT or rs8099917 TG/GG[56].

Though PEG-IFNα can be used and may be associated with improved SVR rates in MHD patients[57], a group of experts in both kidney and liver disease recommended ST-IFNα in preference to PEG-IFNα for the treatment of MHD patients with HCV infection[1]. The rationale for this recommendation is that ST-IFNα has appeared as effective as PEG-IFNα in MHD persons because its excretion is reduced in these patients, its adverse effects may be lower, and management of adverse effects is relatively easier than PEG-IFNα[1].

Because ribavirin has the high risk of inducing or aggravating hemolytic anemia in uremic patients and can not be removed by hemodialysis, it should be prohibited or used at a markedly reduced daily dose with careful monitoring of anemia and other adverse effects in MHD patients[1,8-10]. If RBV is to be applied, it should be given at an individualized dosing of 200 mg once a day, or 200 mg every other day, or 200 mg thrice weekly after hemodialysis, and substantial hematopoietic support is essential[57].

In a meta-analysis made by Gordon *et al*[9] in 2009, which included 428 patients from 20 prospective studies from 1966 to February 2009, IFNα treatment for at least six months against chronic HCV infection in MHD patients was shown to result in a high overall SVR of 45%. Both univariate and multivariate regression analyses demonstrated that the higher SVR was related to the following factors: (1) three million units or higher dosage of IFNα, three times weekly; (2) completion of treatment for at least six months; (3) lower baseline HCV RNA; (4) female gender; and (5) early virological negativity[9]. In a later meta-analysis by Alavian *et al*[8] published in 2010, 491 MHD patients from 21 studies of ST-IFNα and 279 MHD patients from 12 studies of PEG-IFNα were meta-analyzed. The pooled SVR for ST-IFNα and ST-IFNα monotherapy in random effects model were 39.1% and 39.3%, respectively. Pooled dropout rates were 22.6% and 29.7%, respectively. Only age less than 40 years was significantly associated with SVR. HCV RNA level, HCV genotype, ALT pattern, female gender, duration of infection, liver fibrosis stage, and treatment duration were not associated with SVR[8]. These conclusions are conflicting with that of Gordon *et al*[9]. Accordingly, the factors associated with the SVR are worthy of further investigations.

Tolerance to initial IFNα monotherapy was lower in MHD than in non uremic patients with chronic HCV infection. The most frequent side effects requiring interruption of treatment were severe flu-like symptoms, bone marrow suppression, neurological and gastrointestinal

discomfort. However, about 40% of MHD patients with HCV infection have been successfully treated with IFNα monotherapy. Further studies are warranted to define whether longer duration of IFNα monotherapy will have a better SVR on IFNα for chronic hepatitis C in MHD population[10].

Telaprevir and boceprevir are HCV protease inhibitors (PIs) developed in recent years. No significant impact of renal dysfunction on telaprevir or boceprevir exposure was found in patients with end-stage renal disease[58], suggested that both drugs might be used to treat HCV infection in this setting[57]. A recent study that included 36 treatment-naive HCV genotype 1 infected MHD patients showed that telaprevir-containing triple therapy had superior efficacy than PEG-IFNα/RBV dual therapy, but was accompanied with anemia more frequently and severely[57]. Generally speaking, in consideration of added severe side effects and drug-drug interactions, triple or quadruple combinations based on IFNα/RBV therapy with one or two PIs are believed not very suitable for MHD patients with HCV infection. On the other hand, several IFNα-free clinical studies combining two or three new direct antiviral agents without RBV are now under investigation in HCV-infected patients without renal dysfunction[2]. This will bring new hopes to increase SVR with decreased side effects not only for HCV-infected patients without MHD, but also for patients with MHD.

CONCLUSION

MHD patients without initial HCV infection may be infected by HCV through blood transfusion or negligence of universal precautions during hemodialysis. The application of erythropoietin has decreased the necessity of blood transfusion for uremic anemia greatly, and the improved detection tests of anti-HCV, HCV core antigen and HCV RNA have minimized the risk of HCV transmission through blood transfusion. Accordingly, the new HCV infections during MHD in recent years are mainly caused by the lack of standard universal precautions. Construction of detailed surveillance systems and implementation of stringent universal precautions for HCV transmission have led to a markedly decreased prevalence of HCV infection in many hemodialysis units[16], and the effectiveness of different preventive strategies for HCV infection in hemodialysis units should be further investigated and clarified. The occult HCV infection in MHD patients should be paid more attention, and detection of HCV RNA by PCR from PBMCs or liver biopsy is necessary for MHD patients with unexplainable elevated serum ALT or liver cirrhosis. Currently, ST-IFNα and PEG-IFNα monotherapies at a reduced dose are the main treatment strategies for MHD patients with active HCV replication, and the SVRs are up to 40% or so. The emphases of future study for the treatment of HCV infection in MHD patients include how to increase the SVR, how the genetic factors such as polymorphisms of *IL-28B* gene will affect the SVR, how to optimize the treatment duration, how to conquer the side effects of IFNα, and whether IFNα-free treatments with two or three DAAs without RBV are effective and practical for HCV eradication in MHD patients[2].

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ORIGINAL ARTICLE

Hepatitis E virus in patients with acute severe liver injury

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Abstract

AIM: To examine the incidence of hepatitis E (HepE) in individuals with acute liver injury severe enough to warrant treatment at a transplant unit.

METHODS: Hepatitis E virus (HEV) is an emerging pathogen in developed countries causing severe illness, particularly in immunocompromised patients or those with underlying chronic liver disease. HepE infection is often under diagnosed, as clinicians can be reluctant to test patients who have not travelled to regions traditionally considered hyperendemic for HepE. There are few data regarding the significance of HEV in patients with very severe acute liver injury in developed countries. Eighty patients with acute severe liver injury attending the Scottish Liver Transplant unit were tested for HEV and anti-HEV IgG and IgM. Severe acute liver injury was defined as a sudden deterioration in liver function confirmed by abnormal liver function tests and coagulopathy or presence of hepatic encephalopathy. Eighty percent of these patients were diagnosed with paracetomol overdose. No patients had a history of chronic or decompensated chronic liver disease at time of sampling. IgG positive samples were quantified against the World Health Organization anti-HEV IgG standard. Samples were screened for HEV viral RNA by quantitative reverse transcription polymerase chain reaction.

RESULTS: Four cases of hepatitis E were identified. Three of the four cases were only diagnosed on retrospective testing and were initially erroneously ascribed to drug-induced liver injury and decompensated chronic liver disease, with the cause of the decompensation uncertain. One case was caused by HEV genotype 1 in a traveller returning from Asia, the other three were autochthonous and diagnosed on retrospective testing. In two of these cases (where RNA was detected) HEV was found to be genotype 3, the most prevalent genotype in developed countries. Three patients survived, two of whom had been misdiagnosed as having drug induced liver injury. The fourth patient died from sepsis and liver failure precipitated as a result of hepatitis E infection and previously undiagnosed cirrhosis. Histopathology data to date is limited to mainly that seen for endemic HepE. All patients, with the exception of patient 1, demonstrated characteristics of HepE infection, as seen in previously described locally acquired cases.

CONCLUSION: In patients with acute severe liver injury, HEV testing should be part of the initial diagnostic investigation algorithm irrespective of suspected initial

diagnosis, age or travel history.

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Key words: Virology; Infection; Acute liver injury; Hepatitis E virus

Core tip: Misdiagnosis of hepatitis E infection in drug induced liver injury has been noted in patients previously in South East England (13%) and the United States (3%). However, hepatitis E virus is still not given precedence when diagnosing these individuals. In our study, 5% of individuals tested were misdiagnosed and viraemic. It is an important clinical point that the diagnosis of drug induced liver injury is not secure without first excluding hepatitis E, irrespective of travel history, particularly in patients with elevated transaminases.

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INTRODUCTION

Hepatitis E has previously been considered a disease of developing countries. In these hyperendemic settings hepatitis E often occurs in large outbreaks involving hundreds or thousands of cases, such as the recent epidemic in south Sudan[1,2]. In such geographical settings hepatitis E virus (HEV) is spread oro-faecally *via* infected water supplies. Most patients recover, but the mortality rate is high in pregnant females and patients with underlying chronic liver disease[2]. Over recent years, locally acquired hepatitis E has been reported from many developed countries, where it is considered to be a porcine zoonosis[3]. Locally acquired acute hepatitis E is more common in middle aged and elderly males, and in most patients causes a self-limiting hepatitis which last 4-6 wk[4].

In some European countries such as England, France and Germany, a large number of sporadic cases of locally acquired hepatitis E have been documented. For example, in 2011, 454 cases of laboratory-confirmed cases were documented in England and Wales[5] (http://www.hpa.org.uk/hpr/archives/2012/news3212.htm#hev), and these were mostly locally acquired. In contrast, in the United States only a handful of cases have been documented[6] despite an anti-HEV seroprevalence of 21%[7]. This suggests that sub-clinical and/or unrecognised infection is common.

In developed countries, there have been very few previous studies of HEV in patients with acute liver injury severe enough to warrant assessment and treatment at a liver transplant unit. The aim of this study was to retrospectively determine the role and contribution of HEV infection in patients presenting with acute severe liver injury to the Scottish Liver Transplantation Unit (SLTU), Edinburgh, Scotland.

MATERIALS AND METHODS

The SLTU admits patients from hospitals in Scotland with severe acute liver injury for assessment and treatment, and covers a population of 5254800 (http://www.gro-scotland.gov.uk/files2/stats/annual-review-2011/rgar 2011.pdf). The cohort studied included 80 patients with severe acute liver injury admitted to the SLTU between December 2008 and May 2012 (Tables 1 and 2). Severe acute liver injury was defined as a sudden deterioration in liver function confirmed by abnormal liver function tests and coagulopathy or presence of hepatic encephalopathy[8]. No patients had a history of chronic or decompensated chronic liver disease. The majority (80%) of cohort patients were referred to SLTU in the context of paracetamol overdose (POD). The other aetiologies are reflective of the diversity of patients referred to the unit. Despite the large proportion of POD cases within the cohort we felt HEV testing was justified given that infection with hepatitis A, hepatitis C andthere are several publications implicating HEV in the misdiagnosis of POD and DILI.[9-14]. Also, paracetamol ingestion is common in patients with viral hepatitis and may lead to confusion as to the cause liver injury[15].

Serum samples from all 80 patients, taken at presentation and stored at -80 ℃[16], were tested for the presence of anti-HEV IgM and IgG antibodies and HEV RNA. Antibody screening was carried out using commercial assays for anti-HEV IgM and IgG (Wantai, Beijing, PR China) according to manufacturer's instructions. IgG positive samples were quantified against the World Health Organization (WHO) anti-HEV IgG standard. HEV RNA screening was carried out using a HEV pan-genotype quantitative reverse transcription polymerase chain reaction assay with taqman probe and primer sequences targeting the open reading frame (ORF)2/3 region of the HEV genome described by Jothikumar *et al*[17]. RNA positive samples were quantified against the WHO HEV RNA standard and the limit of detection of the assay was determined to be 250 WHO IU/mL. Positive samples underwent conventional PCR using primer sequences targeting the ORF2 region previously described by Erker *et al*[18]. Cloning of the ORF2 amplicons was performed using the pGem-T-Easy vector (Promega, Southampton, United Kingdom) and sequenced (GATC, Konstanz, Germany). Sequence data was aligned against known HEV genotype sequences using alignment software ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The Health Protection Agency guidelines for HEV diagnosis were followed when assigning diagnoses in this study. Briefly, the criteria for diagnosing an acute HEV infection is defined as; clinical and/or biochemical findings consistent with acute viral hepatitis together with virology laboratory markers consistent with acute infection; this must include the de-

Table 1 Patient cohort

n	Gender		Age	ALT	Bilirubin	Creatinine	PT	WBC	Liver	Outcome
	Male	Female	mean ± SD	(10-50 IU/L)	(< 17 μmol/L)	(45-110 μmol/L)	(8-12 s)	(4.3-10.8 ×10^9 cells/L)	failure[1]	
80	36 (45%)	44 (55%)	38.7 ± 14.1	5112 ± 3492	118 ± 96	181 ± 136	52 ± 34	9.96 ± 6.51	47 (58.8%)	SWOTX 54 (67.5%) SWTX 11 (18.3%) Died 15 (13.8%)

[1]Defined as loss of hepatic cellular function and subsequent development of coagulopathy, jaundice and encephalopathy. Normal range indicated in brackets. ALT: Alanine aminotransferase; PT: Prothrombin time; WBC: White blood cell; SWOTX: Survived without transplant; SWTX: Survived with transplant.

Table 2 Prevalence of patient

Diagnosis	POD	Acute viral hepatitis	Autoimmune hepatitis	Post LTX graft nonfunction	Fatty liver of pregnancy	Malignancy	DILI	Acute porphyria	Ischaemic hepatitis
Prevalence	64 (80%)	6[1] (7.5%)	3 (3.75%)	2 (2.5%)	1 (1.25%)	1 (1.25%)	1 (1.25%)	1 (1.25%)	1 (1.25%)

[1]The causes of acute viral hepatitis prior to retrospective testing were acute hepatitis B (n = 3), acute hepatitis C (n = 1), and acute hepatitis E (n = 1, case 4 who had travelled to India and was tested for hepatitis E virus at presentation). The diagnoses described are the diagnoses before retrospective testing for hepatitis E virus was undertaken. POD: Paracetamol overdose; LTX: Liver transplant; DILI: Drug induced liver injury.

tection of HEV RNA. Laboratory markers of probable cases must include the detection of anti-HEV IgG and IgM antibodies but allows for the absence or non-testing of HEV RNA (http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1287146735973).

RESULTS

From the 80 patients cohort; 72 (90%) patients tested anti-HEV IgG negative, anti-HEV IgM negative and HEV RNA negative; 4 (5%) patients tested anti-HEV IgG positive, anti-HEV IgM negative and HEV RNA negative; 3 (3.75%) patients tested anti-HEV IgG positive, ant-HEV IgM positive and HEV RNA positive; 1 (1.25%) patient tested anti-HEV IgG positive, anti-HEV IgM positive and HEV RNA negative (Table 3). No patient diagnosed with hepatitis B, hepatitis C, autoimmune hepatitis, post liver transplant graft non-function, fatty liver of pregnancy, ischaemic hepatitis, malignancy or acute porphyria tested positive for hepatitis E (Table 1). The 4 patients with corresponding anti-HEV IgM positive results, suggestive of active infection at time of testing, are described in further detail below.

Patient 1

A 58-year-old female presented with a 24 h history of jaundice and itch. She complained of generalised malaise/fatigue for 1 wk and dark tea-coloured urine for 3 d, but no abdominal pain, nausea or vomiting. The patient had travelled to Ibiza (Spain) 1 mo and Cornwall (England) 2 wk previously. After returning with a dry cough, she visited her General Practitioner, who prescribed a short course of clarithromycin. The patient had also recently begun taking simvastatin and diclofenac and was a regular user of aspirin and nifedipine. The patient had no history of liver problems and only light alcohol consumption (< 48 g/wk, maximum recommended limit for females = 112 g/wk). Liver function tests showed highly elevated transaminases (Table 3). Tests for hepatitis A, B, C and autoantibodies were negative, alpha-1-antitrypsin and ceruloplasmin were normal, serum ferritin 1871 ug/L (normal range 14-150), iron 15 μmol/L (normal range 10-28), transferrin 2.1 g/L (normal range 2-4), transferrin saturation 27%. An ultrasound scan showed no focal abnormality, contracted gall bladder, no biliary dilatation, normal kidneys/spleen and no free intra-abdominal fluid.

After withdrawal from all her medication, the patient's liver function tests improved. The patients liver dysfunction was therefore attributed to drug induced liver injury (DILI). However, in retrospect a diagnosis of acute hepatitis E was made, as she was anti-HEV IgM and IgG positive, and her serum contained a high titre of HEV RNA (Table 3). Sequencing of the patient's viral RNA showed it to be of genotype 3 (Figure 1).

Patient 2

A 67-year-old female presented to the referring hospital with acute hepatitis after returning from Spain 4 wk earlier. She had significant comorbidity; non-insulin dependent diabetes, hypertension, chronic kidney disease and alcohol excess (224-258 g/wk). Because of developing hepatic encephalopathy, increasing fluid overload and renal injury the patient was transferred to SLTU. The patient underwent a transjugular liver biopsy to clarify the diagnosis. The biopsy showed cirrhosis with sparse steatohepatitis and a low grade cholestatic hepatitis (Figure 2). She developed sepsis complicated by multi-organ failure and died despite supportive care, including dialysis and norepinepherine. This patient was anti-HEV IgM and IgG positive. Retrospective RNA screening and sequencing showed this patient demonstrated HEV genotype 3 in her stored blood sample (Figure 1 and Table 3).

Patient 3

A 27-year-old male Polish immigrant living in London

Table 3 Hepatitis E virus immunoglobulin M positive patients

Patient ID	Gender	Age	Liver function tests (normal range)						HEV				Travel history	Initial/ retrospective diagnosis	Outcome
			ALT (10-50 IU/mL)	Billirubin (< 17 μmol/L)	ALP (40-125 IU/L)	Gamma GTP (5-35 IU/mL)	Albumin (30-50 g/L)	PT (8-12 s)	IgG (IU/mL)	IgM	RNA (IU/mL)	Genotype			
1	Female	58	3648	92	540	480	35	16	48.4	+	6.4×10^4	3	Spain, Cornwall	DILI/hepatitis E	Survived
2	Female	67	98	516	256	Icteric[1]	19	14	36.49	+	1.4×10^3	3	Spain	Alcohol, T2Diabetes, obesity, CLD/hepatitis E	Died
3	Male	27	5288	140	186	368	27	35	24.7	+	-	-	London	POD/hepatitis E	Survived
4	Male	27	4044	269	253	Icteric[1]	38	25	42.05	+	1.9×10^4	1	India	Hepatitis E	Survived

[1]GTP could not be calculated due to high serum bilirubin levels. Values obtained from serum samples taken on admission to SLTU. ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GTP: Glutamyl transpeptidase; PT: Prothrombin time; HEV: Hepatitis E virus; Ig: Immunoglobulin; RNA: Ribonucleic acid; DILI: Drug induced liver injury; CLD: Chronic liver disease; POD: Paracetamol overdose.

travelled to Scotland to stay with a relative. Without suicidal intent, and in conjunction with excessive alcohol, he ingested 8 g of liquid paracetamol. He was transferred to SLTU because of deranged liver function tests (Table 3) and confusion. He developed hepatic encephalopathy and acute kidney injury with oliguria and peak creatinine 600 μmol/L which resolved spontaneously, without renal replacement therapy. Twelve days after admission he was discharged to the referring hospital with the following blood tests (normal range indicated in brackets); prothrombin time 35 s (8-12 s), bilirubin 343 μmol/L (< 17 μmol/L), alanine aminotransferase 97 IU/L (10-50 IU/L), alkaline phosphatase 160 IU/L (40-150 IU/L), gamma glutamyl transpeptidase 190 IU/L (5-35 IU/L), Albumin 19 g/L (30-50 g/L). In retrospect, this patient tested positive for anti-HEV IgM and IgG antibodies but no viral RNA could be detected in his serum.

Patient 4

A 27-year-old male experienced a short self-limiting episode of nausea and diarrhoea just before returning to Scotland from working at a sanitation project in Northern India. He rapidly became jaundiced and fatigued. He was febrile, had no stigmata of chronic liver disease, but was deeply jaundiced with a palpable non-tender liver. Upper abdominal ultrasound was normal and other liver diseases excluded by serology, biochemistry and immunology. A transjugular liver biopsy was performed, revealing a severe acute lobular hepatitis (Figure 3). Due to the patient's travel history he was contemporaneously tested for HEV, and was IgM, IgG and PCR positive. Sequencing performed in retrospect showed the patient to be infected with HEV genotype 1 (Figure 1).

DISCUSSION

In developed countries there have been few previous studies of HEV in patients with acute liver injury severe enough to warrant assessment and treatment at a liver transplant unit[13]. The current study shows that in four of 80 (5%) of patients with acute severe liver injury the cause was hepatitis E, thus making HEV the commonest cause of viral hepatitis in this cohort. Three of the four cases were only diagnosed on retrospective testing and were initially erroneously ascribed to drug-induced liver injury (n = 2) and decompensated chronic liver disease, with the cause of the decompensation uncertain (n = 1). These findings suggest that in patients with acute severe liver injury HEV testing should be part of the initial diagnostic investigation algorithm, irrespective of suspected initial diagnosis, age or travel history.

In the case of patient 1, we initially misdiagnosed the case as DILI because, at the time, we did not consider hepatitis E as a diagnostic possibility. A study from England showed that 6/47 (13%) of patients with criterion-referenced drug-induced liver injury had been misdiagnosed, as they had locally-acquired hepatitis E infection[12]. A similar study from the United States showed that 9/318 (3%) had been similarly misdiagnosed[13]. More recently, Chen *et al*[14] described another case of hepatitis E which had been erroneously diagnosed as "DILI". An accurate diagnosis of drug-induced liver injury depends on excluding of all other possible cases of hepatocellular injury[21]. Patient 1 case illustrates the important clinical point that the diagnosis of drug-induced liver injury is not secure without first excluding hepatitis E, irrespective of travel history, particularly in patients with highly elevated transaminases.

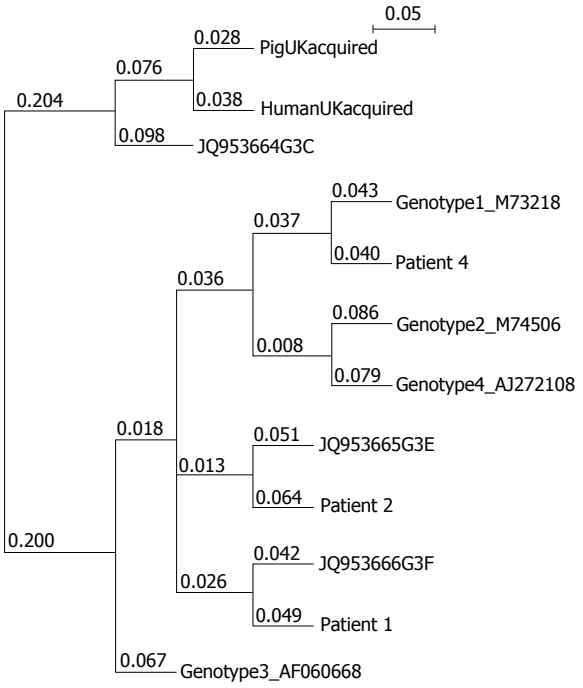

Figure 1 Phylogenetic relationship between the 4 hepatitis E virus genotype reference sequences and those isolated from a United Kingdom swine (AF503512), a United Kingdom patient with locally acquired hepatitis E virus (AY362357), genotype 3 subtypes described in[19] and the patients discussed in this paper. Sequences were assembled using ClustalW and the phylogenetic tree expressed in the Newick format using NJ plot[20]. The 121 bp sequences correspond to nucleotides 6332-6476 of hepatitis E virus genotype 3 reference strain AF060668.

Patient 2, although presenting with severe acute liver injury, had previously undiagnosed cirrhosis, likely due to a combination of alcoholic and fatty liver disease, and died from multi-organ failure precipitated by hepatitis E infection. Studies from Europe and Southeast Asia show, that hepatitis E infection in patients with underlying chronic liver disease, have a poor prognosis, with a 12-mo mortality rate from subacute liver failure of up to 70%[22,23]. The diagnosis of acute hepatitis E infection in such patients is easily overlooked, and may commonly be ascribed to other causes such as alcoholic hepatitis[24]. Patient 2 illustrates this diagnostic difficulty, as there were no specific clinical or laboratory clues which prompted consideration of hepatitis E as a diagnostic possibility, and the liver biopsy appearances could easily have been ascribed simply to decompensated alcoholic cirrhosis in a previously undiagnosed patient. Often the only clue in such hepatitis E cases is the elevation of transaminases at presentation[25]. Within a week, the alanine aminotransferase declines to the range seen in alcoholic hepatitis[24,25], as was the case in patient 2 (98I U/mL) (Table 3). Despite the difficulties in identifying cases, it is important to establish an early diagnosis of hepatitis E infection in patients with underlying chronic liver disease, as the prognosis may be improved by early anti-viral therapy with ribavirin[26,27]. Indeed, this case highlights the poor prognosis in older patients with underlying liver disease and acute hepatitis E infection.

In the case of patient 3 we were unable to detect HEV RNA, despite the patient being anti-HEV IgM positive. However, the absence of detectable HEV RNA does not exclude recent infection as peak viremia occurs during incubation and the window of detectable RNA is narrow (approximately 2-3 wk)[28,29]. Also, studies show patients with a history of alcohol abuse who are exposed to HEV are significantly more likely to develop clinically apparent hepatitis[30], as this patient did. Interestingly, this patient was originally considered to have paracetamol induced liver necrosis, developing significant coagulopathy and an acute kidney injury as is commonly observed in such cases. Although the stated dose of paracetamol was relatively low and more recent studies have reported the validity of patient history in the context of hepatotoxicity, the reported dose of paracetamol is not related to eventual outcome[31,32]. It is possible that HEV, like other viral infections, can augment the hepatotoxicity of paracetamol[9-11]. However, coexisting acute hepatitis E in patients with paracetamol hepatotoxicity in this cohort was an uncommon finding (1.6%) and it is not possible to make comparisons with other cases of paracetamol hepatotoxicity based on a single case.

Of the four cases of hepatitis E identified in this study, only 1 patient (patient 4) had travelled to a region considered hyperendemic for HEV (India). Phylogenetic analysis revealed this patient to be infected with HEV genotype 1 (Figure 1 and Table 2), supporting the hypothesis that this was an imported case of HEV infection. This was the only case that was diagnosed contemporaneously, as HEV testing was prompted by the travel history. The remaining 3 patients had travelled outside of Scotland, although only to regions previously considered non-endemic for HEV (England and Spain). Phylogenetic analysis of patient's 1 and 2 samples revealed these patients were infected with HEV genotype 3, the most prevalent genotype in cases of autochthonous hepatitis E infection in developed countries[2]. It is not possible to determine for certain whether these patients contracted their HEV infection in Scotland or the other "non-endemic" regions they visited prior to onset of symptoms. However, given the absence of cases of hepatitis E in patients in this series who had not recently travelled outside of Scotland, it suggests that locally acquired hepatitis E infection in Scotland is uncommon. This notion is supported by a low IgG seroprevalence rate of 4.5% in blood donors from southeast Scotland and, at least compared to other European countries, a modest rate of asymptomatic viraemia at the time of donation (1 in 14500)[33].

Patient 4, aligned with Genotype 1 to confirm the acquisition of HepE during travel. However, HEV3 infection in developed countries is commonly associated with the ingestion of contaminated food products such as undercooked pork, game meat and molluscs cultivated in contaminated water, as well as occupational exposure to pigs or their effluent[2]. Patient 1 and patient 2 had genotypes with homology to genotype 3f and 3e respectively.

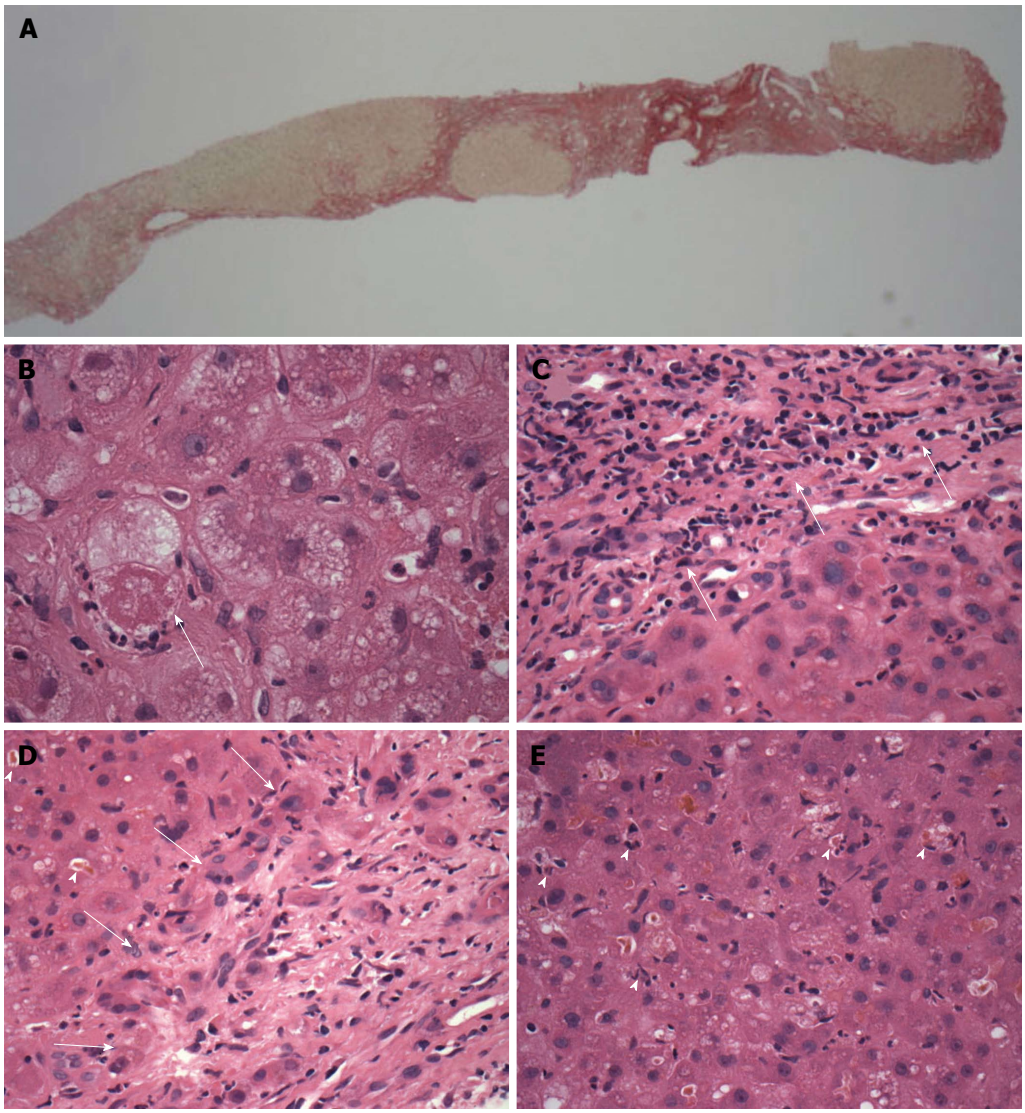

Figure 2 Patient 2, transjugular needle biopsy. A: Low magnification view of part of the biopsy, showing red-stained nodular fibrosis indicating cirrhosis (x 20 original magnification, picrosirius red stain); B: Ballooned hepatocyte (arrow) containing a Mallory-Denk body and with surrounding neutrophils (satellitosis), features of steatohepatitis. (× 600 original magnification, H and E stain). Small clusters of these cells were present in the biopsy, with sparse small droplet macro-steatosis; C: Low grade hepatitic infiltrate (arrows) of lymphocytes with occasional plasma cells in the portal area (× 400 original magnification, H and E stain); D: Prominent cholangiolitis (periportal ductules with oedema and neutrophils) (region indicated by arrows), with adjacent liver parenchyma showing canalicular cholestasis (arrowheads); E: Lobule showing mild disarray with cholestasis, increased lymphocytes and Kupffer cells within sinusoids and scattered apoptotic/necrotic cells (arrowheads).

These genotypes were isolated from European swine, however, pre-infection exposure to defined environmental and dietary risk factors is unknown and so we cannot narrow their possible source of infection further. Infection *via* blood transfusion has also been documented[2] and it was confirmed that none of the patients had recently undergone a blood transfusion.

The liver biopsy findings deserve comment given the differing genotypes. There are only limited previously published data on the liver biopsy appearances of endemic and locally acquired acute hepatitis E[23,34-36], as such cases usually have a self-limiting illness and so a liver biopsy is not commonly clinically indicated. There is a lobular hepatitis of varying severity between patients 2 and 4, from mild lobular disarray with Kupffer cell hypertrophy and scattered individually necrotic hepatocytes with adjacent neutrophils or lymphocytes, through to severe lesions with confluent necrosis and collapse. A cholestatic element is often present within lobules and portal tracts, including canalicular cholestasis, mild bile duct inflammation and typically quite prominent neutrophilic cholangiolitis around the portal tracts as seen in patient 2 (Figure 2D and E). Similar pathology has been reported by Malcolm *et al*[35] in their locally acquired cases and by others[36]. These features can easily be mis-attributed to a drug-related cholestatic hepatitis. When occurring in patients with chronic liver disease such as alcoholic cirrhosis the viral effects may be overshadowed by or mis-attributed to steatohepatitis-related changes or sepsis-related decompensation. A plasma cell-rich portal, interface and lobular hepatitis such as characterises flares of autoimmune hepatitis is not normally seen in acute hepatitis E, although loose lymphoid aggregates in portal tracts have been described in occasional patients, which might misdi-

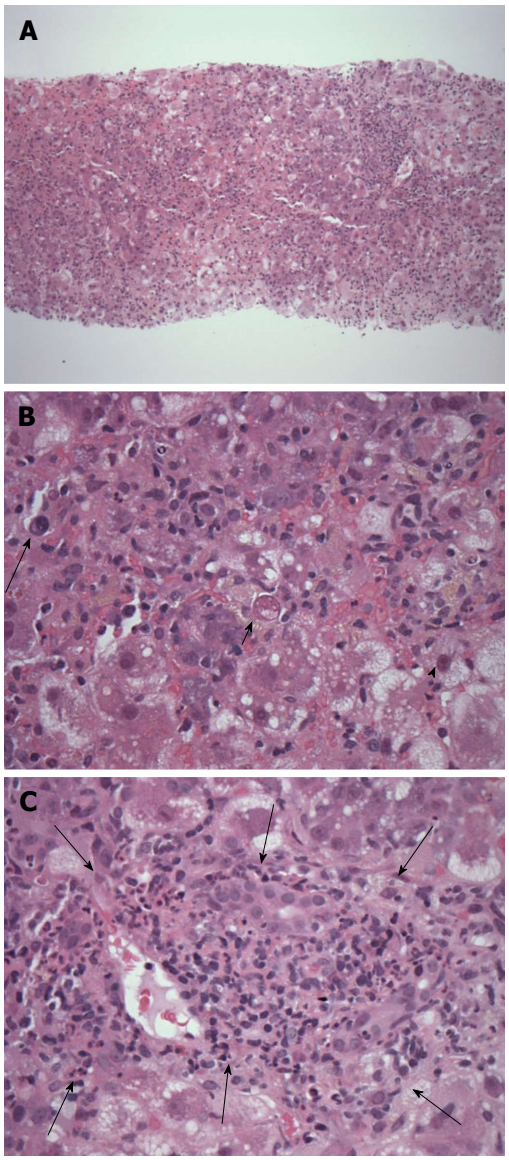

Figure 3 Patient 4, transjugular needle biopsy showing severe acute lobular hepatitis. A: Low magnification view showing the diffuse nature of the liver inflammation and injury (original magnification × 40, H and E); B: Severely inflamed lobule with numerous infiltrating inflammatory cells, including occasional plasma cells (long arrow), hepatocyte cell death (short arrow) and ballooning injury (arrowhead) (original magnification × 400, H and E); C: Shows an inflamed portal tract (delineated by arrows) expanded within by mononuclear inflammatory cells without bile duct injury and with only rare plasma cells or eosinophils. There is neutrophil cholangiolitis around the portal tract (just within the arrows) but no prominent interface hepatitis (original magnification × 200, H and E).

rect when there is pre-existing liver fibrosis. In patient 4, the absence of a prominent interface hepatitis and neutrophil cholangiolitis was comparable to a recent study on acute endemic HEV[34]. Whether autochthonous and endemic hepatitis E differ qualitatively in histological characteristics, remains uncertain given the small number of case comparisons including this study[34,35,37]. The variable underlying severity of the hepatitis and other selection factors prompting biopsy could skew the comparison and more data is required[38].

In summary, in 4 of 80 (5%) of patients with acute liver injury severe enough to warrant assessment and treatment at the Scottish Liver Transplant Unit, the cause was hepatitis E. Only one of these patients had a history of travel to an area traditionally considered hyperendemic for HEV. This is in line with the sero-prevalence in the Scottish population and higher than the rate of viraemia[31]. This study shows that clinicians should have a low threshold for considering hepatitis E as a possible diagnosis in any patient with severe acute liver injury. This should include those with possible paracetamol hepatotoxicity, irrespective of their age or travel history.

COMMENTS

Background

Hepatitis E virus (HEV), the etiological agent responsible for hepatitis E infection, is now recognised as an emerging zoonotic disease in industrialized countries. HEV genotypes 3 and 4 are responsible for sporadic cases of autochthonous hepatitis E infection in countries such as the United Kingdom, United States, France, Italy and Japan. HEV can cause a mild, self-limiting infection but it can also cause more serious health problems such as cirrhosis of the liver and fulminant hepatitis. The ability of HEV genotype 3 and 4 strains to cross the species barrier has been documented and there is a growing body of evidence that HEV can be transmitted to humans *via* the consumption of infected or contaminated food products.

Research frontiers

In developed countries, there have been very few previous studies of HEV genotype 3 in patients with acute liver injury severe enough to warrant assessment and treatment at a liver transplant unit.

Innovations and breakthroughs

Recent reports have highlighted the importance of HEV diagnosis in a number of clinical situations. In this study, we confirm the need for increased diagnostic testing in patients presenting with drug induced liver injury. In addition, liver pathogenesis clearly differs with the genotype causing the infection. Finally, routes of infection need further clarification.

Applications

Misdiagnosis of hepatitis E infection in drug induced liver injury has been noted in patients previously in South East England (13%) and the United States (3%). However, hepatitis E virus is still not given precedence when diagnosing these individuals. In the authors' study, 5% of individuals tested were misdiagnosed and viraemic. It is an important clinical point that the diagnosis of drug induced liver injury is not secure without first excluding hepatitis E, irrespective of travel history, particularly in patients with elevated transaminases. This data contributes to the increasing need to screen patients for the presence of the HEV.

Terminology

HEV is a member of the Hepeviridae and Genotype 3 recognised as a zoonotic infection. The role of HEV in severe acute liver disease has yet to be defined.

Peer review

This is a well written manuscript, dealing with the prevalence of hepatitis E among a series of patients subjected to liver transplantation, in whom the etiology of liver failure was masked by coexisting cirrhosis and/or drug overdose. Authors correctly stress the importance of excluding HEV infection in Western countries, irrespective of the travel story.

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CASE CONTROL STUDY

Pooled genetic analysis in ultrasound measured non-alcoholic fatty liver disease in Indian subjects: A pilot study

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Abstract

AIM: To investigate genetic susceptibility in Indian subjects with non-alcoholic fatty liver disease (NAFLD) by performing a pooled genetic study.

METHODS: Study subjects (n = 306) were recruited and categorized into NAFLD and control groups based on ultrasound findings of fatty infiltration. Of the 306 individuals, 156 individuals had fatty infiltration and thus comprised the NAFLD group. One hundred and fifty (n = 150) individuals were normal, without fatty infiltration of the liver, comprising the control group. Blood samples, demographic and anthropometric data from the individuals were collected after obtaining informed consent. Anthropometric data, blood glucose, lipids and liver function tests were estimated using standard methods. Genome wide association studies done to date on NAFLD were identified, 19 single nucleotide polymorphisms (SNPs) were selected from these studies that were reported to be significantly associated with NAFLD and genotyping was performed on the Sequenom platform. Student's t test for continuous variables and χ^2 test was applied to variant carriers from both groups. Required corrections were applied as multiple testing was done.

RESULTS The mean age of the control group was 39.78 ± 10.83 and the NAFLD group was 36.63 ± 8.20 years. The waist circumference of males and females in the control and NAFLD groups were 80.13 ± 10.35; 81.77 ± 13.65 and 94.09 ± 10.53; 92.53 ± 8.27 cms respectively. The mean triglyceride and alanine transaminase (ALT) levels in the control and NAFLD groups were 135.18 ± 7.77 mg/dL; 25.39 ± 14.73 IU/L and 184.40 ± 84.31 mg/dL; 110.20 ± 67.05 IU/L respectively. When χ^2 test was applied to the number of individuals carrying the variant risk alleles between the control and NAFLD group, a significant association was seen between rs738409 of the patatin-like phospholipase domain containing 3 (*PNPLA3*) gene (P = 0.001), rs2073080 of the *PARVB* gene (P = 0.02), rs2143571 of *SAMM50* gene (P = 0.05) and rs6487679 of the pregnancy zone protein (*PZP*) gene (P = 0.01) with the disease. Variant single nucleotide polymorphisms (SNPs) in *NCAN* and *PNPLA3* gene were associated with higher levels of ALT, whereas variant SNPs in APOC3, PNPLA3, EFCAB4B and COL13A1 were associated with high triglyceride levels. Apart from the above associations, rs2073080, rs343062 and rs6591182 were significantly associated with high BMI; rs2854117 and rs738409 with high triglyceride levels; and rs2073080, rs2143571, rs2228603, rs6487679 and rs738409 with high ALT levels.

CONCLUSION: Pooled genetic analysis revealed an association of SNPs in PNPLA3, PARVB, SAMM50 and *PZP* genes with NAFLD. SNPs in NCAN and *PNPLA3*

gene were associated with higher levels of ALT, whereas variant SNPs in APOC3, PNPLA3, EFCAB4B and COL13A1 were associated with high triglyceride levels.

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Key words: Non-alcoholic fatty liver disease; Genome wide association studies; Genetic association; Hepatic steatosis; Genotyping; Single nucleotide polymorphisms; Susceptibility

Core tip: Non-alcoholic fatty liver disease (NAFLD) describes a range of conditions caused by build-up of fat within liver cells in the absence of alcohol consumption. Although obesity, diabetes, age, hypertension and hypertriglyceridemia contribute to the disease, genetics also has an important role to play. Furthermore, in 26%-35% of patients, genetic component is believed to contribute to NAFLD. By identifying significant single nucleotide polymorphisms from genome wide association studies reported from different ethnic populations for NAFLD and performing a pooled genetic association study, this study has identified important genetic risks that could help in identifying individuals with susceptibility at an early stage, thus aiding in better management of the disease.

Ravi Kanth VV, Sasikala M, Rao PN, Steffie Avanthi U, Rajender Rao KR, Nageshwar Reddy D. Pooled genetic analysis in ultrasound measured non-alcoholic fatty liver disease in Indian subjects: A pilot study. *World J Hepatol* 2014; 6(6): 435-442 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i6/435.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i6.435

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a global epidemic, the incidence of which is reported to be as high as 25%-30% in different populations[1]. Differences in prevalence, clinical profile, histological severity and outcome of NAFLD in different ethnic groups suggest a genetic contribution; and NAFLD in 26%-35% of patients is believed to be contributed by genetic component[2,3]. In recent years, genetic heritability has been a major focus of research, although changing dietary habits and modifying life style have been demonstrated to benefit patients with hepatic steatosis[4]. Genome wide association studies (GWAS) from different ethnic populations revealed a strong association of PNPLA3 variant[3,5], apart from few other variants[6-8], and an independent study identified APOC3 variants associated with higher triglyceride levels and risk of NAFLD in migrant Indians[9].

A recent GWAS[6] of hepatic steatosis revealed loci in or near the neurocan (*NCAN*), glucokinase regulatory protein, lysophospholipase-like protein 1 and protein phosphatase 1, regulatory subunit 3B (*PPP1R3B*) genes that have associations with glycemic traits, serum lipid levels, hepatic steatosis, hepatic inflammation/fibrosis, or a combination of these. Specific genotypic information in the form of single nucleotide polymorphisms (SNPs) which confer susceptibility for an individual have to be identified so that early preventive measures can be initiated, especially in children and adolescents. Patatin-like phospholipase domain containing 3 (PNPLA3) missense variant was studied and compared with MR spectroscopy for predicting NAFLD[4]. However, since it is now known that multiple SNPs are associated with the disease, identifying other susceptibility SNPs apart from PNPLA3 would enhance the predictive capability.

The prevalence of NAFLD in the Indian population is estimated to be around 25%-30%[10-16]. In addition, the prevalence of hepatic steatosis in non-obese (lean NAFLD) was shown to range between 11%-31.7% according to a recent study[17]. Increase in the incidence of obesity, metabolic syndrome and the presence of lean non-alcoholic steatohepatitis (NASH) in the Indian population warrants genetic susceptibility studies in Indian NAFLD subjects. In this preliminary pilot study, we selected SNPs (Table 1) from already reported GWAS across different populations and genotyped the same in Indian subjects.

MATERIALS AND METHODS

A total of 450 individuals with fatty infiltration were recruited for the study during 2011-2012 (1 year) from hepatology clinics of the hospital. As shown (Figure 1), 156 individuals were found to be eligible for the pooled genetic analysis. Statistical power analysis was not used to compute the sample size as this is a pilot study. Although liver biopsy is considered to be the gold standard for identifying NAFLD and NASH, lack of indication for asymptomatic individuals, the costs involved, risk of complications and ethical concerns limit its use in these types of studies. Therefore, subjects were recruited based on ultrasound findings of hepatic steatosis as per earlier reports[18,19]. Healthy subjects (n = 150) from the institute who volunteered to be part of the study were recruited as controls based on the sole criteria of the absence of fatty liver on ultrasonography and normal alanine transaminase (ALT) levels. Written informed consent was obtained from each individual. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Committee. Demographic and anthropometric details [height, weight, magnetic resonance imaging (BMI) and waist circumference] were collected in a structured pro forma. Whole blood (5 mL) was collected in pre coated EDTA containers from the study group and stored at -20 ℃ until further analysis. Biochemical investigations like ALT, viral markers and lipid profiles were estimated as per standard methods.

Definitions

Individuals with BMI less than 18.5 kg/m^2 were defined

Table 1 List of single nucleotide polymorphisms included in the study

SNP No	rsID	Risk allele	Associated gene	Associated with	Ref.
1	rs738409	G	*PNPLA3*	Hepatic steatosis	[6]
2	rs4240624	A	*PPP1R3B*	Hepatic steatosis	[6]
3	rs2228603	T	*NCAN*	Hepatic steatosis	[6]
4	rs780094	A	*GCKR*	Hepatic steatosis	[6]
5	rs12137855	C	*LYPLAL1*	Hepatic steatosis	[6]
6	rs2645424	C	*FDFT1*	NAFLD activity score	[8]
7	rs343062	T	-	Degree of fibrosis	[8]
8	rs1227756	G	*COL13A1*	Lobular inflammation	[8]
9	rs6591182	G	-	Lobular inflammation	[8]
10	rs887304	A	*EFCAB4B*	Lobular inflammation	[8]
11	rs2499604	A	Intronic *ZP4-TRNAP23P*	Serum levels of alanine aminotransferase	[8]
12	rs6487679	C	*PZP*	Serum levels of alanine aminotransferase	[8]
13	rs1421201	C	-	Serum levels of alanine aminotransferase	[8]
14	rs2710833	T	-	Serum levels of alanine aminotransferase	[8]
15	rs2854116	A	*APOC3*	Hypertriglyceridemia in Asians	[9]
16	rs2854117	G	*APOC3*	Hypertriglyceridemia in Asians	[9]
17	rs2143571	A	*SAMM50*	NAFLD	[7]
18	rs2073080	T	*PARVB*	NAFLD	[7]
19	rs1390096	A	*HS3ST1-HSP90AB2P*	NAFLD	[7]
20	rs11206226	A	*YIPF1*	NAFLD	[7]

PNPLA3: Patatin-like phospholipase domain containing 3; NCAN: Neurocan; GCKR: Glucokinase regulatory protein; LYPLAL1: Lysophospholipase-like protein 1; PPP1R3B: Protein phosphatase 1, regulatory subunit 3B; PZP: Pregnancy zone protein.

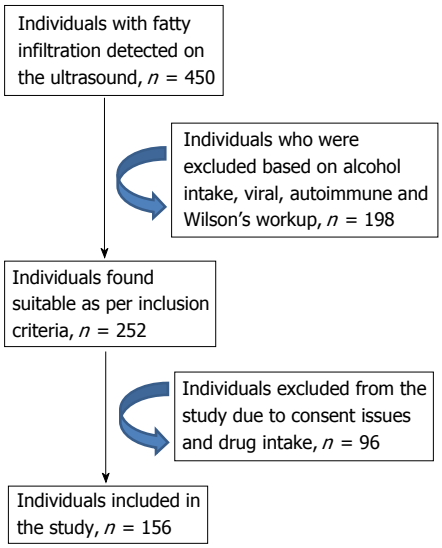

Figure 1 Flowchart showing sample recruitment.

as underweight; 18.5-22.9 kg/m^2 were defined as normal and BMI more than 23 kg/m^2 were defined as obese. Lean NAFLD was defined as hepatic steatosis in individuals with normal BMI (< 22.9 kg/m^2) according to Asian standards[20]; likewise hypertriglyceridemia (greater than 150 mg/dL), low levels of high density lipoprotein (HDL less than 40 mg/dL in males and 50 mg/dL in females), hypertension (greater than 130/85 systolic and diastolic blood pressure level in mmHg or on anti hypertensive drugs) and high fasting glucose levels (greater than 100 mg/dL of fasting blood sugar levels) were considered as cut offs. The cut off for waist circumference was > 90 cm and > 80 cm in males and females respectively, as per Asian standards[21]. A cut off of 30 IU/L was considered for ALT[22].

Genotyping

DNA was isolated from blood using standard protocols. The concentration and integrity of DNA was measured with NanoDrop 1000 spectrophotometer (Thermo Scientific, USA) and agarose gel electrophoresis respectively. The DNA with 260/280 ratios between 1.8-2.0 and agarose gel image showing a high molecular weight intact DNA band were included for further genotyping analysis. The samples were genotyped for the 19 SNPs on the Sequenom platform (Sequenom®, San Diego, CA, United States) using the manufacturer's protocol. Primers for one SNP (rs2854116) could not be designed because of proximal SNPs present very near to the target SNP and so was not included in the study. The raw data files generated by Sequenom MassARRAY were analyzed for the intensity peaks of calibrant to ascertain the quality of the data. An overall call rate of > 95% was maintained. Five percent of the samples were duplicated across the plate, their genotypes compared and they had 100% concordance. Negative controls (master mix without DNA) were also included.

Correlation of demographic and anthropometric phenotypes, like BMI, waist circumference, liver enzymes (ALT) and triglyceride levels, to the genotype was done to identify significant risk factors.

Statistical analysis

The data collected was edited for consistency and completeness and entered into MS-Excel for further analysis. Patient characteristics were compared using Student's *t* test for continuous variables and proportion test for categorical variables. χ^2 test was used on the number of variant

Table 2 Demographic and clinical characteristics of the study group

Parameter	Controls (n = 150)	Patients (n = 156)	P value
Mean ± SD	39.78 ± 10.83	36.63 ± 8.20	0.004
Age, yr	18-63	19-62	
Range			
Males	110 (73.33%)	138 (88.46%)	-
Females	40 (26.66%)	18 (11.53%)	-
Waist circumference			
Males	80.13 ± 10.35	94.09 ± 10.53	0.0001
Females	81.77 ± 13.65	92.53 ± 8.27	0.01
BMI (kg/m^2)	24.04 ± 7.77	27 ± 5.86	0.001
Triglycerides (mg/dL)	135.18 ± 7.77	184.40 ± 84.31	0.0001
HDL (mg/dL)	41.86 ± 9.70	39.56 ± 13.02	0.2923
ALT (IU/L)	25.39 ± 14.73	110.20 ± 67.05	0.0001
AST (IU/L)	25.99 ± 8.46	69.14 ± 37.77	0.0001
Hypertensives	4 (2.6%)	18 (11.53%)	-
Diabetics	19 (12.66%)	27 (17.3%)	-

BMI: Body mass index; HDL: High density lipoprotein; ALT: Alanine transaminase; AST: Aspartate amino transferase.

carriers in the control and NAFLD groups for identifying SNPs associated with NAFLD. To correct for multiple comparison testing, the Benjamini and Hochberg false discovery rate correction[23] was applied to "P values". All SNPs were divided into risk and non-risk groups and 2X2 contingency tables were prepared to estimate odds ratio for all variables like age, gender, BMI, ALT levels *etc*. Multiple logistic regression was used to identify independent predictor variables for NAFLD. The data was analyzed using Statistical Package for Social Sciences (SPSS Version 17). In this study, a P value $\leqslant$ 0.05 was considered statistically significant. Haplotype analysis was carried out using software[24]. An excel sheet was prepared as per instructions with "0" representing wild type allele and "1" representing heterozygous or mutant variants.

RESULTS

The clinical characteristics, such as age, waist circumference, BMI, triglyceride and ALT levels, of the groups are presented in Table 2. Categorization of the study population yielded two groups based on ultrasonographic detection of hepatic steatosis in the liver, namely the NAFLD (n = 156) and control group (n = 150). The inclusion of individuals in the control group was based on the absence of hepatic steatosis and retrospectively it was seen that few of the individuals in the control group were obese (BMI > 23 kg/m^2). So the group was divided based on BMI and a comparison of both clinical characteristics and the genotype was made (data not shown) between the normal and obese controls. Such an analysis did not show any significant differences between the normal and obese control group with respect to the genotype. However, the waist circumference in males (P = 0.0001) and females (P = 0.02) and the triglyceride levels (P = 0.0057) were high in the obese controls, apart from BMI (P = 0.0001), and the difference in all the other characteristics studied was statistically not significant.

Association between clinical characteristics, SNPs and risk of NAFLD

When an analysis was done between the control and NAFLD group, a significant difference in clinical characteristics was noted in BMI (P = 0.001), waist circumference of males (P = 0.0001) and females (P = 0.01), high triglyceride levels (P = 0.0001) and ALT (P = 0.0001) levels in the NAFLD group.

In the single allelic analysis, tests for associations between NAFLD and the SNPs revealed that variants in PARVB, SAMM50, NCAN, intronic SNP (rs2499604), *APOC3*, pregnancy zone protein (*PZP*) and *PNPLA3* genes were associated with NAFLD; however, after correction for multiple testing was applied, only variants in PARVB, SAMM50, PZP and PNPLA3 were significant (Table 3).

Significant SNPs associated with clinical traits

To identify SNPs which may be associated with clinical traits like triglyceride and ALT levels but not necessarily to the disease, the individuals in the study group were divided into two groups, namely individuals with normal and those with high levels of the mentioned clinical traits irrespective of the disease status. Such an effort identified significant SNPs which are likely to be associated with clinical traits. SNPs in NCAN (P = 0.04) and PNPLA3 (P = 0.001) were significantly associated with high ALT levels and SNPs in *APOC3* (P = 0.01), *PNPLA3* (P = 0.05), *EFCAB4B* (P = 0.04) and *COL13A1* (P = 0.02) genes were significantly associated with high triglyceride levels.

Odds of developing NAFLD

Among the various characteristics like age, BMI and the SNPs that were studied, rs2073080 in PARVB, rs343062 (intronic) and rs6591182 (intronic) were significantly associated with higher odds of obese individuals with NAFLD. Likewise, SNPs in various genes studied were associated with clinical parameters like ALT and triglyceride levels (Table 4).

Haplotype analysis

Since PNPLA3, SAMM50 and PARVB are found on the same locus on chromosome 22, haplotype analysis was done for the 3 SNPs and it was noted that heterozygous or homozygous variants in these genes were overrepresented in the NAFLD group compared to the control group (8 in controls against 63 in the NAFLD group) (Table 5).

Multivariate logistic regression analysis

Multiple logistic regression analysis was applied to the data to estimate the risk of an individual for NAFLD. The dependent variables were the NAFLD group and controls. The variables that were significant in the univariate analysis, namely age (less than 40 years), BMI, waist circumference, triglyceride levels, HDL, hypertension,

Table 3 Comparison of variant carriers between patients and controls

SNP-gene name	Allele frequency controls (n = 150)		Allele frequency patients (n = 156)		χ^2	*P* value	Corrected *P* value[1]	OR	95%CI lower-upper
	Major	Minor	Major	Minor					
rs1227756 COL13A1	0.49	0.51	0.44	0.56	0.02	0.88	0.93	1.04	0.54-2.03
rs12137855 LYPLAL1	0.77	0.23	0.73	0.27	0.41	0.51	0.62	1.20	0.68-2.12
rs1390096 HS3ST1-HSP	0.68	0.32	0.66	0.34	0.007	0.93	0.93	0.97	0.55-1.70
rs1421201 intronic	0.88	0.12	0.85	0.15	1.60	0.20	0.36	1.55	0.78-3.10
rs2073080 PARVB	0.81	0.19	0.69	0.31	8.42	0.003	0.02	2.36	1.31-4.22
rs2143571 SAMM50	0.80	0.20	0.69	0.31	6.25	0.01	0.05	2.07	1.16-3.69
rs2228603 NCAN	0.97	0.03	0.92	0.08	4.09	0.04	0.12	3.29	1.10-9.84
rs2499604 intronic	0.53	0.47	0.60	0.40	3.76	0.05	0.13	1.92	0.98-3.75
rs2645424 FDFT1	0.5	0.50	0.56	0.44	1.21	0.27	0.40	0.69	0.36-1.32
rs2710833 intronic	0.60	0.40	0.66	0.34	3.09	0.07	0.17	0.56	0.30-1.07
rs2854117 APOC3	0.58	0.42	0.55	0.45	4.24	0.03	0.12	1.83	1.02-3.28
rs343062 intronic	0.55	0.45	0.52	0.48	1.96	0.16	0.32	1.53	0.84-2.80
rs4240624 PPP1R3B	0.94	0.06	0.91	0.09	1.21	0.27	0.40	1.56	0.69-3.52
rs6487679 PZP	0.89	0.11	0.75	0.25	9.65	0.001	0.01	2.81	1.44-5.48
rs6591182 intronic	0.63	0.37	0.59	0.41	0.99	0.31	0.44	1.39	0.72-2.67
rs738409 PNPLA3	0.92	0.08	0.60	0.40	46.37	0.0001	0.001	12.66	5.45-29.38
rs780094 GCKR	0.76	0.24	0.74	0.26	0.48	0.48	0.62	1.22	0.69-2.15
rs887304 EFCAB4B	0.82	0.18	0.83	0.17	0.19	0.65	0.73	0.87	0.47-1.59
rs11206226 YIPF1	1.00	0.00	1.00	0.00	-	-		-	-

[1]Benjamini and Hochberg false discovery rate correction was applied to the "*P* value". PNPLA3: Patatin-like phospholipase domain containing 3; NCAN: Neurocan; GCKR: Glucokinase regulatory protein; LYPLAL1: Lysophospholipase-like protein 1; PPP1R3B: Protein phosphatase 1, regulatory subunit 3B; PZP: Pregnancy zone protein.

diabetes and SNPs, were included for the multivariate analysis (Table 6).

DISCUSSION

The main objective of this study was to identify susceptibility SNPs for NAFLD in Indian subjects utilizing pooled genetic SNP data from various GWAS performed in different populations to date. Variants in *SAMM50*, *PARVB*, *PZP* and *PNPLA3* genes were significantly associated with NAFLD, thus suggesting involvement of multiple loci in Indian NAFLD.

A significant association of PNPLA3 (rs738409) (P = 0.001) with NAFLD was observed in Indian subjects and is consistent with the genetic association of PNPLA3 in other populations, like Caucasians, European descent, Hispanics and Japanese[6-8]. Furthermore, this SNP was also significantly associated with higher ALT (P = 0.001) and triglyceride levels (P = 0.05), suggesting that individuals with the variant may be at higher risk for NAFLD. In addition to this SNP, PZP rs6487679 located on the 12th chromosome, demonstrated to have a role in clearance of transforming growth factor-beta from human plasma and hepatic fibrogenesis[25], was also significantly associated with NAFLD in Indian subjects. This finding corroborates with similar earlier findings in non-Hispanic Caucasians[8].

rs2073080 of the beta-parvin (PARVB) located on chromosome 22 that codes for a protein beta-parvin in humans[26] was significantly associated with the disease (P = 0.018) in the present study. Not much is known about the polymorphism, but in general the protein is believed to play a role in cytoskeleton organization and cell adhesion apart from having a role in tumor suppression (Entrez Gene: *PARVB*). The association of rs2143571 of the SAMM50 sharing the same locus on chromosome 22

Table 4 Significant single nucleotide polymorphisms associated with higher odds of body mass index, triglycerides and alanine transaminase based on clinical characteristics

Variable	SNP-gene	OR	*P* value	95%CI lower-upper
BMI (obese and non-obese)	*rs2073080-PARVB*	1.81	0.0470	1.00-3.25
BMI (obese and non-obese)	*rs343062-intronic*	2.25	0.0100	1.21-4.21
BMI (obese and non-obese)	*rs6591182-intronic*	2.05	0.0340	1.05-4.00
TG (abnormal and normal)	*rs2854117-APOC3*	2.31	0.0040	1.29-4.13
TG (abnormal and normal)	*rs738409-PNPLA3*	1.94	0.0170	1.12-3.37
ALT (abnormal and normal)	*rs2073080-PARVB*	1.92	0.0200	1.10-3.36
ALT (abnormal and normal)	*rs2143571-SAMM50*	1.77	0.0200	1.10-3.36
ALT(abnormal and normal)	*rs2228603-NCAN*	3.23	0.0180	1.22-1.37
ALT (abnormal and normal)	*rs6487679-PZP*	1.92	0.0300	0.05-3.51
ALT (abnormal and normal)	*rs738409-PNPLA3*	5.07	0.0001	0.03-10.92

BMI: Body mass index; TG: Triglycerides; ALT: Alanine transaminase; SNP: Single nucleotide polymorphisms.

Table 5 PARVB-SAMM50-PNPLA3 haplotype data

Total counts	Haplotype	Number in controls	Number in patients
138	000	90	48
31	001	6	25
10	010	10	0
4	011	2	2
4	100	2	2
48	110	32	16
71	111	8	63

"Total Counts" column consists of the number of haplotypes generated in both controls and patient group combined. In the haplotype column, 0 stands for wild type allele and 1 for either heterozygous or homozygous variant carrier. The sequence of the genes is *PARVB*, *SAMM50* and *PNPLA3*. The individuals count for the haplotypes for controls and patient group is given in the Number in controls and Number in patients column.

Table 6 Results of multiple logistic regression analysis

Variable	Regression coefficient	Standard error	*P* value	OR	95%CI Lower	95%CI Upper
PZP	0.880	0.415	0.034	2.41	1.05	5.44
PNPLA3	2.289	0.480	< 0.0001	9.86	3.85	25.29
Triglyceride levels	1.502	0.391	0.000	4.48	2.08	9.67
Constant	-0.619					

PNPLA3: Patatin-like phospholipase domain containing 3; PZP: Pregnancy zone protein.

as PNPLA3 and PARVB encoding sorting and assembly machinery component 50 homolog was also significantly associated with NAFLD in the Indian subjects[27]. This protein has a function in the assembly of beta-barrel proteins into the outer mitochondrial membrane. A recent genome wide scan[7,28] also identified similar SNPs in PNPLA3, SAMM50 and PARVB in the Japanese population which was significantly associated with NAFLD. Our results corroborate with this study, indicating that these 3 variants are commonly seen in an Asian population.

Apart from the promoter polymorphism of the *APOC3* gene and variant in *PNPLA3* gene, EFCAB4B and COL13A1 polymorphisms were identified as significantly associated with higher triglyceride levels. Polymorphisms in NCAN and PNPLA3 were associated with higher ALT levels. Although previous studies[6,7] reported an association of the above mentioned SNPs with NAFLD, their associations with triglycerides and ALT levels have been identified for the first time in Indian subjects.

When lean and obese controls were compared for significant differences in clinical characteristics and genotype, BMI between the groups was significantly different, with a higher BMI in the obese group as expected. The triglyceride levels were also significantly higher in the obese control group without hepatic steatosis compared to the lean controls; however, there were no significant differences in the genotype with respect to the 19 SNPs studied. Based on this, these individuals were found to be suitable to be included in the control group for further analyses.

The incidence of lean NAFLD in the present study (19.87%) is in agreement with an earlier study from North India[17] which had more or less a similar incidence (13.2%) and there was no significant difference in the incidence between the two groups ($P = 0.08$).

When odds were computed based on obese and non-obese status in the study group, PARVB and two intronic SNPs (rs343062 and rs6591182) were significantly associated with higher odds of NAFLD in the obese, suggesting that an obese individual with these variants is at a higher risk of hepatic steatosis compared to a non-obese individual. This important finding has a clinical implication in that, if an individual with the above mentioned variants can be identified at an early age, the significant modifying risk factors like higher waist circumference and triglyceride levels can be managed, thus reducing the predisposing risk component because of the variants in SNPs and thereby delaying the onset of hepatic steatosis.

Haplotype data for the three SNPs on PNPLA3, SAMM50 and PARVB suggests that three SNPs might be linked as heterozygous or mutant variant carriers were overrepresented in the NAFLD group (63 counts) compared to the control group (8 counts) (Table 5).

A recent study[29] from North India reported a higher frequency of CG and GG genotypes of rs738409 polymorphism in the *PNPLA3* gene in North Indians and a significant association of the genotype to ALT ($P = 0.003$) and AST levels ($P = 0.04$). The values of triglycerides were slightly higher in the cases but were not significantly different in comparison to controls. This study is in agreement with the above study from the North Indian center with respect to the PNPLA3 polymorphism and

its association with NAFLD and ALT levels. Our study also found a significant association of higher triglyceride levels with rs738409 polymorphism. However, the present study has looked at an additional 18 polymorphisms, which is by far the most comprehensive pooled genetic analysis taken up in Indian subjects with NAFLD.

To estimate the strength of the relationship between several independent variables and a continuous dependent variable, multiple logistic regression analysis was done with significant SNPs and patient characteristics like triglyceride levels, BMI from the univariate analysis as the independent variables and NAFLD as the dependent variable. While high levels of BMI, triglyceride levels, waist circumference both in males and females, ALT levels and variant SNPs in PARVB, SAMM50, NCAN, intronic SNP rs2499604, PZP and PNPLA3 were significantly associated with NAFLD when univariate analysis was done, only variants in *PZP* and *PNPLA3* genes and high triglyceride levels were significantly associated with NAFLD when multivariate analysis was done, suggesting that these three are independent risk factors to predict hepatic steatosis and that the others probably interact with the modifying risk factors like BMI and waist circumference in the causation of NAFLD.

In conclusion, an analysis between the control and NAFLD groups revealed significant differences in BMI, triglyceride levels, waist circumference in both males and females and ALT levels with higher levels associated with the NAFLD group. Variant SNPs in *NCAN* and *PNPLA3* genes were significantly associated with high ALT levels, which are the clinical phenotype of hepatic necroinflammation state, and SNPs in APOC3, PNPLA3, EFCAB4B and COL13A1 were associated with higher triglyceride levels.

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COMMENTS

Background

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of conditions associated with lipid deposition in the hepatocytes, ranging from simple steatosis (fatty liver) to non-alcoholic steatohepatitis (fatty changes with inflammation and hepatocellular injury or fibrosis), to advanced fibrosis and cirrhosis. It is the most common cause of liver disease, with a prevalence of 25%-30% in the general population. The presence of metabolic syndrome is the most common risk factor for NAFLD and it is now believed that NAFLD is the hepatic manifestation of metabolic syndrome. The other important risk factors are obesity, type-2 diabetes, total parenteral nutrition, jejunoileal bypass operation and use of certain medications. However, genetics play an important role in NAFLD and it is believed that 26%-35% of the patients who develop NAFLD have an underlying genetic component. So, it is important to identify the genetic aspects of the disease and their environmental interactions for better management of the disease.

Innovations and breakthroughs

Studies have identified that variant single nucleotide polymorphisms (*SNPs*) in genes, namely PNPLA3, NCAN, glucokinase regulatory protein, lysophospholipase-like protein 1, FDFT1, COL13A1, SAMM50, PARVB and pregnancy zone protein (PZP), were associated with NAFLD. A pooled genetic study was carried out by identifying significant SNPs from genome wide association studies and this study identified SNPs which are associated with Indian NAFLD. Apart from these associations, variant SNPs which contribute to hypertriglyceridemia, and alanine transaminase levels were also identified. By genotyping for these SNPs, an individual's predisposing risk can be identified at an early age and lifestyle-based modifications would ensure delayed onset of fatty infiltration.

Applications

Susceptibility loci for Indian NAFLD have been identified for the first time. The genotype data can be used in early identification and better management of the disease.

Terminology

Genome wide association study is the examination of many common genetic variants known as SNPs (single nucleotide polymorphisms) in two sets of individuals. One set of individuals with disease and the other set without the disease are compared for a large number of SNPs (approximately 9 lakhs) and analysis is done to identify those SNPs with a higher frequency in the disease group and these SNPs are said to be associated with the disease.

Peer review

This well written and interesting pilot study of genetic susceptibility of NAFLD in an Indian population has shown that multiple SNPs and loci are involved in the development of NAFLD. Variant SNPs in *PZP* and *PNPLA3* genes were found to be independent risk factors for the development of NAFLD. PARVB, SAMM50, neurocan and intronic SNP rs2499604 were significant risk factors along with other associations. So, genetics play an important role along with metabolic factors in the development of NAFLD. These findings may add a new level to the existing knowledge about the genetic basis of NAFLD, especially in the Indian population, and be valuable for clinical interference.

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Reuse of liver grafts following the brain death of the initial recipient

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Abstract

AIM: To determine if there is a reasonable prospect of success of a re-use liver transplantation.

METHODS: We systematically searched for reports of liver graft re-use using electronic searches of PubMed and Web of Knowledge. We performed hand searches of references lists of articles reporting re-use of grafts.

RESULTS: A systematic review of the literature reveals 28 liver transplantations using previously transplanted grafts. First and second recipients ranged in age from 4 to 72 years and 29 to 62 years respectively. Liver disease in the first recipient was varied including 5 (18%) patients with fulminant liver failure who died subsequently of cerebral edema. The second transplantation was performed after a median interval of 5 d (one day-13 years). Viral hepatitis was present in 3 (11%) of the initial recipients and in 8 (29%) of final recipients. Hepatocellular carcinoma was present in 6 (21%) of the final recipients. Early survival after the final transplantation was 93%, whereas long-term survival was 78% with a mean follow-up of 23.3 (3-120) mo.

CONCLUSION: Outcomes of transplantation using previously transplanted grafts in this select population are similar to those seen with conventional grafts.

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Key words: Reuse; Liver graft; Brain death; Liver transplantation

Core tip: Reuse of a previously transplanted liver graft may be considered if the first recipient suffers neurological death at some time after liver transplantation.

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INTRODUCTION

The growing disparity between the demand for and supply of organs for transplantation has restricted the availability of grafts for patients whose indications for transplantation fall outside of conventional guidelines and it has led to new strategies to increase donor utility. On rare occasions, a donor situation is such that it is not acceptable for routine transplantation but a novel rationale is present for an expectation of success so that the graft

may be offered to candidates who would otherwise be excluded from transplantation. We encountered this situation for a patient with hepatitis C virus (HCV) and hepatocellular carcinoma (HCC) that had advanced beyond criteria for transplantation when another liver recipient unexpectedly suffered neurological death from intracerebellar bleeding, 13 d after transplantation. Organ donation for allocation to patients on the conventional liver transplantation waiting list had been declined by the organ procurement organization. To determine if there was a reasonable prospect of success of a re-use transplantation, we undertook a systematic survey of the literature.

MATERIALS AND METHODS

We systematically searched for reports of liver graft re-use using electronic searches of PubMed (1966 to January 2013) and Web of Knowledge (1981 to January 2013). The following key words were used: "liver transplantation"; "reuse"; "graft" or "liver graft". The search was limited to the English literatures and humans. We performed hand searches of references lists of articles reporting re-use of grafts. We collected the data to determine the age range of each donor and recipient, their liver disease and cause of death (if applicable), the interval between the initial and final liver transplantation and the outcome of the final transplantation.

RESULTS

Systematic review of the literature revealed 14 papers describing 27 liver recipients of previously transplanted grafts with an early survival rate of over 90% for both the patients and the re-used grafts[1-14]. No review of this aspect of liver transplantation was located. We proceeded with the re-use liver transplantation in London, ON. The initial recipient was a 55-year-old man with end-stage liver disease secondary to hepatitis C. His blood type was O and hepatitis B core antibody was positive. The initial donor for this recipient was a 69-year-old man with blood type O, who developed brain death from intracranial hemorrhage. Unfortunately, he suffered a huge intracerebellar bleed on the 4th day after transplantation and was declared brain dead on day 13. The second recipient was 54-year-old man with hepatitis C cirrhosis and a history of ruptured HCC two years earlier. He had been put on capecitabine 1000 mg/m^2 and multiple liver lesions were embolized by angiogram 7 mo earlier. Even though his HCC appeared to be stable and there was no evidence of extrahepatic disease, long-term survival without liver replacement was considered unlikely. The opportunity was discussed with the patient and his family including its known risks and uncertainties. The blood type of the second recipient was B and hepatitis B serology was negative. At retrieval surgery 14 d after the initial transplant, the liver graft was found to be larger than before with a stiff texture (Figure 1). The liver graft was perfused with Histidine Tryptophan Ketogluterate (HTK) solution *via* the portal vein. Arterial perfusion was done on the back-table confirming good flow of the perfusate. In the final recipient wide resection of tissue surrounding the liver was performed including areas of diaphragm, peritoneum, omentum, extrahepatic nodes and lymphatic tissue. Occlusive thrombus was removed from the native portal vein. Cold ischemic and warm ischemic times were 9 h and 1.5 h, respectively. His postoperative course was straightforward except for temporary renal impairment. His transaminases went up more than 4000 IU/L, but graft function improved significantly thereafter. His induction immunosuppressive therapy was basiliximab and steroid, and he was maintained on sirolimus and steroid thereafter. Prophylaxis for hepatitis B started according to our protocol. He was put on capecitabine again on day 4. He was discharged 15 d after transplantation. Evidence of recurrent hepatitis C virus was diagnosed 8 mo later. Although the graft continued to function well, he expired 16 mo after transplantation due to recurrence of HCC.

Figure 1 Reuse graft after the retrieval. It was slightly enlarged with a stiff texture.

Data from the 28 reuse transplantations are given in Table 1. Initial donors and recipients ranged in age from 4 to 72 years and 29 to 62 years respectively. Liver disease in the first recipient was varied with the notable exception of higher than expected incidence of fulminant liver failure in 5 (18%) patients. These patients became donors when brain death from cerebral edema was diagnosed after liver transplantation. The commonest cause of death of the initial recipient was cerebrovascular accident 4 d (median, one day-13 years) after transplantation. Brain anoxia was the cause of death in one patient but is not recorded in the remaining patients. The second transplantation was performed 5 d (median, one day-13 years) after the initial transplantation. Viral hepatitis was present in 3 (11%) of the initial recipients and in 8 (29%) of final recipients. HCC was present in 6 (21%) of the final recipients. One reused graft failed to function and a second graft failed from hepatic artery thrombosis giving an initial patient and graft survival of 93%. Long-term survival is 78% with a mean follow-up of 23.3 (3-120) mo.

DISCUSSION

The outcomes described in this report of liver transplantation using previously transplanted grafts is comparable

Table 1 Reuse of liver grafts following the brain death of the initial recipient

Location	Donor			Interval (d) initial	Recipient		Outcome	
(ref. NO.)	Age (yr)	Liver disease	Cause of death	to final transplant	Age (yr)	Liver disease	Early after second transplantation	Long-term
London, Canada (current report)	55	HCV	CVA	14	54	HCV/HCC	No complications	Died of recurrent HCC at 16 mo
Madrid, Spain[1,2]	57	PBC	CVA	1	29	CR post LTx for PSC and CCC	No complication	Died of recurrent CCC at 48 mo
Madrid, Spain[2]	54	PSC	CVA	2	32	CR post LTx for HCV	Sepsis	Died at 4 mo
Madrid, Spain[2]	51	CR post LTx (cause N/A)	CVA	2	56	HCV/HCC	AR	Alive at 25 mo
Creteil, France[3]	24	CR post LTx for cryptogenic cirrhosis	CVA	5	52	Alcoholic	AR	Alive at 6 mo
Essen, Germany[4]	N/A	Cryptogenic cirrhosis	CVA	1	46	Recurrent HBV post LTx	AR	Alive at 5 mo
Barcelona, Spain[5]	55	Alcoholic	CVA	5	58	HCV	No complication	Alive at 14 mo
Brussels, Belgium[6]	47	ALF (acetaminophen)	Cerebral edema	2	53	HCV/HCC	AR	Alive at 22 mo
Lille Cedex, France[7]	21	ALF (acetaminophen)	Cerebral edema	2	61	HCV	No complication	Alive at 11 mo
UNOS #1[8]	6	N/A	N/A	1	N/A	N/A	N/A	Alive at 111 mo
UNOS #2[8]	60	Cryptogenic cirrhosis	CVA	8	44	N/A	N/A	Alive at 62 mo
UNOS #3[8]	21	N/A	N/A	1	N/A	N/A	N/A	Alive at 3.5 mo
UNOS #4[8]	49	N/A	N/A	4.9 yr	N/A	N/A	failed at 0.1 mo (cause N/A)	-
UNOS #5[8]	48	N/A	N/A	5	N/A	N/A	N/A	Failed at 11 mo
UNOS #6[8]	56	HCV	Anoxic brain injury	2	N/A	HCV, alcoholic	No complication	Alive at 25.4 mo
UNOS #7[8]	49	N/A	N/A	6	N/A	N/A	N/A	Alive at 4.8 mo
UNOS #8[8]	35	ALF (acetaminophen)	Cerebral edema	3	56	PSC	No complication	Alive at 12 mo
UNOS #9[8]	46	N/A	N/A	2	N/A	N/A	N/A	Alive at 11 mo
UNOS #10[8]	25	N/A	N/A	2.8 yr	N/A	N/A	N/A	Alive at 5.9 mo
UNOS #11[8]	44	N/A	N/A	17	N/A	N/A	N/A	Alive at 3.0 mo
Barcelona, Spain[9]	55	Alcoholic	CVA	5	58	HCV	No complication	Alive at 120 mo
Barcelona, Spain[9]	58	Alcoholic	CVA	14	55	Budd Chiari synd	No complication	Alive at 13 mo
Barcelona, Spain[9]	58	Alcoholic	CVA	10	47	Ischemic cholangitis	AR	Alive at 7 mo
Creteil, France[10]	72	Alcoholic	CVA	13 yr	61	Cryptogenic cirrhosis,	No complication	Alive at 12 mo
Montreal, Canada[11]	26	ALF (acetaminophen overdose)	Cerebral edema	2	62	Hemochromatosis HCC	No complication	Alive at 30 mo
Berlin, Germany[12]	53	Cryptogenic cirrhosis	Cerebral edema	24	43	Alcoholic, HCC	Biliary obstruction by stones	Alive at 6 mo
Stuttgart, Germany[13]	38	Budd Chiari synd	CVA	5 yr	51	Polycystic liver disease	No complication	Alive at 18 mo
Malatya, Turkey[14]	4	ALF (hepatitis A)	Cerebral edema	5	31	Cryptogenic cirrhosis, HCC	HAT at one month, died at 1.3 mo	-

HCV: Hepatitis C virus; CVA: Cerebrovascular accident; HCC: Hepatocellular carcinoma; PBC: Primary biliary cirrhosis; CR: Chronic rejection; LTx: Liver transplant; PSC: Primary sclerosing cholangitis; CCC: Cholangiocarcinoma; N/A: Not available; AR: Acute rejection; HBV: Hepatitis B virus; UNOS: United Network for Organ Sharing; ALF: Acute liver failure; HAT: Hepatic artery thrombosis.

to transplantation from conventional donors. There may be a publication bias where poor outcomes have been excluded from reportage. The inclusion of patients from mandatory databases and the large number of centers reporting from several jurisdictions may mitigate this risk of publication bias.

There are several causes that lead to severe brain damage in liver transplant recipients[15,16], and some of those circumstances make re-use of liver grafts possible. In the series described here, cerebrovascular accident and cerebral edema are the commonest causes of death of the donor of the previously transplanted graft. Brain death from cerebral edema is a particular concern in candidates with fulminant liver failure as recovery from coma may unpredictably occur after a considerable interval from successful liver

replacement. Knowledge that these grafts may be available for re-use should a recovery not occur, may permit the teams to give candidates with fulminant failure the benefit of the doubt.

Moreno González *et al*[2] considered several factors to be important for successful reuse of liver grafts: all reused grafts should be obtained from young and stable initial donors, excellent graft function in the first recipient, early reuse (within 48 h), short preservation times, biopsy showing minimal preservation injury, negative donor-recipient crossmatch, ABO compatibility, absence of viral, bacterial, and fungal infection. While it is wise to be prudent, the current report suggests that criteria for donation after transplantation may be similar to conventional donation after neurological death. The age of donor here ranged from 4 to 72 years. The interval between transplantations was up to several years. Biopsy before re-use was not routinely reported but should be considered. All of the teams reported efforts to shorten cold and warm ischemic times. Extension of criteria to include donation after cardiac death has not been reported.

There is limited experience of re-use of HCV infected grafts with only two reports in this series. Both of the final recipients experienced recurrence of HCV. One died from recurrent HCC at 16 mo (our case) but the other is well at 25.4 mo after transplantation[8]. Biopsy of HCV infected grafts should be performed before re-use using the same protocols as for initial transplantation.

Clinical indications for the re-use of the liver grafts is varied in the current series but the incidence of HCC, chronic rejection and recurrent hepatitis suggest that candidates may have been offered this unconventional form of transplantation because access to the conventional list was limited. There has been no established guideline so far for the recipients' indication of reuse liver transplantation. A marginal recipient whose general condition is deteriorating or whose stage of malignancy is almost beyond the criteria for liver transplant and suitable donor is not available may take advantage of the reuse liver transplant. If so, the results presented here confirm that the courage shown by the patients was properly rewarded. Even though the results in this select group of transplantations are good, the world wide experience is so limited that we do not advocate for previously transplanted grafts to be included in the conventional donor pool. This report will hopefully guide medical teams faced with unusual circumstances where a liver recipient unexpectedly dies after transplantation in a manner that permits organ donation.

Nowadays transplant programs are increasingly accepting marginal donors such as old donors, donors with fatty liver, or other conditions such that delayed graft function or poor outcome might be anticipated after the transplant compared to the transplants from non-marginal donors. The local Ethical committee should be ideally called before accepting the reuse liver, and this paper will help the committee understand the feasibility of the rare form of transplants.

COMMENTS

Background

The growing disparity between the demand for and supply of organs for transplantation has restricted the availability of grafts for patients whose indications for transplantation fall outside of conventional guidelines and it has led to new strategies to increase donor utility.

Innovations and breakthroughs

Reuse of a previously transplanted liver graft may be considered if the first recipient suffers neurological death at some time after liver transplantation.

Applications

This report will hopefully guide medical teams faced with unusual circumstances where a liver recipient unexpectedly dies after transplantation in a manner that permits organ donation.

Peer review

This is a very novel article focused on the Reuse of liver grafts following the brain death of the initial recipient. Subject to certain restrictions, there maybe some bias. However, liver transplantation secondary use, which provide a new method to solve the liver source,and it deserves further study.

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CASE REPORT

Grade 4 febrile neutropenia and Fournier's Syndrome associated with triple therapy for hepatitis C virus: A case report

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Abstract

The use of triple therapy for hepatitis C not only increases the rate of sustained virological responses compared with the use of only interferon and ribavirin (RBV) but also leads to an increased number of side effects. The subject of this study was a 53-year-old male who was cirrhotic with hepatitis C virus genotype 1 A and was a previous null non-responder. We initially attempted retreatment with boceprevir (BOC), Peg-interferon and RBV, and a decrease in viral load was observed in the 8th week. In week 12, he presented with disorientation, flapping, fever, tachypnea, arterial hypotension and tachycardia. He also exhibited leucopenia with neutropenia. Cefepime and filgrastim were initiated, and treatment for hepatitis C was suspended. A myelogram revealed hypoplasia, cytotoxicity and maturational retardation. After 48 h, he developed bilateral inguinal erythema that evolved throughout the perineal area to the root of the thighs, with exulcerations and an outflow of seropurulent secretions. Because we hypothesized that he was suffering from Fournier's Syndrome, treatment was replaced with the antibiotics imipenem, linezolid and clindamycin. After this new treatment paradigm was initiated, his lesions regressed without requiring surgical debridement. Triple therapy requires knowledge regarding the management of adverse effects and drug interactions; it also requires an understanding of the importance of respecting the guidelines for the withdrawal of treatment. In this case report, we observed an adverse event that had not been previously reported in the literature with the use of BOC.

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Key words: Hepatitis C; Treatment; Boceprevir; Telaprevir; Adverse events

Core tip: Triple therapy is a recently developed strategy for the treatment of hepatitis C that requires extensive knowledge of adverse effects and drug interactions. It also requires an appreciation of the importance of respecting the guidelines for treatment withdrawal. The case report presented here describes a serious adverse event associated with this new therapy that has not previously been reported in the literature. This finding emphasizes the importance of adequately managing patients according to international clinical protocols, and our study allows for an exchange of experience among experts in the conduct of real-life cases.

Oliveira KCL, Cardoso EOB, de Souza SCP, Machado FS, Zangirolami CEA, Moreira A, Silva GF, de Oliveira CV. Grade 4 febrile neutropenia and Fournier's Syndrome associated with triple therapy for hepatitis C virus: A case report. *World J Hepatol* 2014; 6(6): 448-452 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i6/448.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i6.448

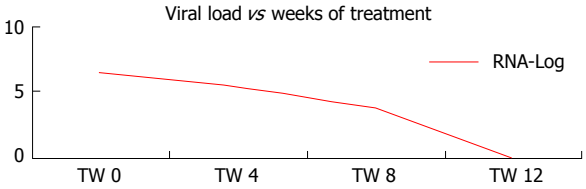

Figure 1 Viral kinetics in response to clinical treatment. TW 0: 0th week of treatment.

INTRODUCTION

The hepatitis C virus (HCV) is the principle cause of chronic liver disease, cirrhosis of the liver and hepatocarcinoma (CHC) throughout the world[1-4]. It is estimated that 120 to 200 million individuals are chronically infected with HCV worldwide, with chronic infections by the HCV genotype 1 being the most prevalent globally (40% to 80%)[1]. Although the incidence of acute hepatitis C has diminished significantly since screening for HCV in the donors of blood and its derivatives began in 1990, the number of patients who present with decompensated cirrhosis and CHC is expected to increase, reaching a peak in approximately 2020[1].

Fifty to 80% of individuals with an acute HCV infection will develop the chronic form of this infection. Of the infected individuals, 2% to 20% will develop cirrhosis within the first 20 years, and most evidence suggests that the disease progression may then increase in a nonlinear fashion. From the point at which cirrhosis is established, the rate of CHC development ranges from 1% to 6% per year. Numerous factors have been associated with rapid progression to cirrhosis, such as a greater age at the time of infection, being male, alcohol consumption, co-infections with the human immunodeficiency virus or hepatitis B virus, non-alcoholic fatty liver disease and tobacco smoking[1].

Over the past 10 years, standard therapy for chronic hepatitis C has consisted of a combination of Peg-interferon alpha and Ribavirin (RBV). This treatment results in a sustained virological response (SVR) in 40% to 50% of HCV genotype 1 patients and in approximately 80% of patients with genotypes 2 or 3[1].

Two direct-acting antiviral agents, telaprevir (TVR) and boceprevir (BOC), both of which are first generation protease inhibitors (PIs), have recently been approved for the treatment of chronic hepatitis C genotype 1[1,3,5]. In Brazil, the approval of these PIs has been granted exclusively for mono-infected HCV genotype 1 patients with advanced fibrosis or compensated cirrhosis of the liver[6].

Triple therapy that comprises a PI in combination with Peg-interferon alpha and RBV increases the SVR rate to approximately 70% and shortens the required treatment duration by approximately 50% in naïve patients (*i.e.*, individuals who had not been previously treated). The SVR rates in previously treated patients depend on the response to prior treatment and the degree of liver fibrosis. Prior work has shown that this rate may vary from > 80% in previous relapse cases to approximately 15% to 30% in null responders and individuals with an advanced degree of fibrosis[1,3,7]. However, these advances occur at the expense of an increased incidence of adverse events and higher therapy costs[1,6,7].

Triple therapy for hepatitis C has been associated with a higher incidence of adverse events, a fact that can limit its tolerability. These unwanted side effects require greater monitoring of the patient compared with treatment with only Peg-interferon alpha and RBV[6,8]. The augmentation of hematological toxicity that occurs with triple therapy can also lead to a rise in the use of growth factors, which results in increased strain on the medical resources in the health system[6,7]. Furthermore, PIs carry the risk of inducing mutations that lead to HCV resistance. Extensive monitoring of patients for their virological response, attention to criteria for treatment cessation and counseling on compliance would be necessary to minimize the development of resistant variants[1].

The higher incidence of adverse events requires PI discontinuation in 10% to 21% of patients. Adverse events that occur at a higher frequency among individuals who received triple therapy include anemia, neutropenia, dysgeusia (BOC), gastrointestinal discomfort, fatigue, cutaneous eruption (TVR) and perianal discomfort (TVR)[1,6].

The present work aimed to report a case of febrile neutropenia and the development of Fournier's Syndrome in a cirrhotic patient with HCV genotype 1 A. This patient was a null responder to two prior treatments (a change in viral load was undetectable following previous treatments, with no decrease in HCV-RNA of at least 2-log after 12 wk of treatment). The patient's retreatment for HCV included triple therapy with BOC, Peg-interferon alpha and RBV.

CASE REPORT

This study describes the case of a 53-year-old male with cirrhosis induced by HCV genotype 1 A. This patient was a null non-responder to two previous treatments (Peg-interferon alpha and RBV for 48 wk), and he denied the previous use of alcohol and other drugs. He was treated again with BOC, a double dose of Peg-interferon alpha [180 micrograms (μg)] and RBV. He obtained a sharp drop in viral load in the 8th week of treatment (TW 8), and viral negativity was observed in week 12 as illustrated in Figure 1.

In the 12th week of treatment (TW 12), he presented with a fever (40 ℃), dyspnea and diarrhea. During the initial evaluation, he was confused and disoriented, with

Figure 2 Photos documenting the involvement of the right and left inguinal regions, respectively.

flapping, fever, tachypnea, arterial hypotension and tachycardia. Laboratory analyses revealed leucopenia (300 leukocytes/mm³) with neutropenia (10 neutrophils/mm³). Cefepime and filgrastim were indicated, and treatment for hepatitis C was suspended.

A myelogram demonstrated hypoplasia with cytotoxicity and maturational retardation; the chosen reposition consisted of folic acid and vitamin B12, in addition to the continuance of filgrastim.

After 48 h of antibiotic therapy, the patient started to present with bilateral erythematous lesions in the inguinal region, and these lesions evolved within 2 d with diffuse erythema throughout the perineal area extending to the root of the lower limbs, with exulcerations and an outflow of seropurulent secretions (Figure 2).

The patient underwent computed tomography (CT) of the pelvis to evaluate the depth of the lesion, and involvement of the lesion in deep planes was not observed (Figure 3). CT revealed a thickening of the skin of the inguinal region and the root of the thighs and scrotum, which was associated with a slight blurring of the adjacent fat.

Based on these symptoms, a hypothesis of Fournier's Syndrome was postulated, and the therapy was replaced with the antibiotics imipenem, linezolid and clindamycin. The blood cultures were positive for multi-sensitive *Pseudomonas,* and the urine cultures were positive for *Staphylococcus aureus* that was sensitive to oxacillin.

The patient's lesions regressed without requiring surgical debridement, and his neutrophil count normalized with the use of filgrastim. The patient was discharged from the hospital after 14 d of antibiotic therapy.

DISCUSSION

The advent of triple therapy for chronic hepatitis C with PIs, Peg-interferon alpha and RBV in HCV genotype 1 carriers has increased the rates of SVRs in naïve patients, previous relapsers and null responders to rates of 70%, > 80% and 30%, respectively. Nevertheless, the observed parallel increase in the incidence of adverse events limits the tolerability of this therapy and raises its associated costs[1,3,6].

The hemolytic anemia that has been associated with RBV use and the suppression of hematopoiesis observed with the use of Peg-interferon alpha require extensive monitoring of hemoglobin levels and absolute neutrophil numbers to achieve adequate management of anemia and neutropenia, two frequent adverse effects that have been related to double therapy. The frequency of these adverse events is increased in patients treated with BOC or TVR, and it results in greater reductions in the doses of RBV and/or Peg-interferon alpha, the use of growth factors or even the discontinuation of PI therapy[3,4,7].

The various dermatological manifestations associated with HCV are classified into three types according to their etiology. The first type involves the direct action of the virus on the skin, and it includes the involvement of lymphocytes, dendritic cells and blood vessels. The second type occurs secondary to the interruption of the immune response, and the third type involves a non-specific cutaneous response secondary to HCV involvement in other organs[9-11].

Interferon has the clinical potential to cause adverse effects on the skin, and these effects are secondary to interferon's immunomodulatory activity[9-11].

The well-described association of adverse dermatological events with the use of TVR is less evident when BOC is chosen. Light-to-moderate cutaneous eruptions can be treated with oral antihistamines and/or topical corticosteroids, but TVR therapy must be terminated immediately for severe cutaneous eruptions (> 50% of the body surface area) or any eruption associated with significant systemic symptoms, including evidence of the involvement of internal organs, facial edema, mucosal erosions or ulcers, target lesions, epidermal dislocation, vesicles or blisters. For these types of serious adverse effects, the patient must be immediately referred for dermatological medical assistance. Drug Rash with Eosinophilia and Systemic Symptoms Syndrome and Stevens Johnson Syndrome occur in < 1% of patients treated with TVR[1,3,11].

The increase in the neutropenia incidence [absolute neutrophil count (ANC) < 750 per cubic millimeter] is similar in patients treated with TVR, Peg-interferon and RBV compared with patients treated only with Peg-interferon and RBV. However, higher costs are incurred among the more severe cases when the proposed scheme includes BOC, Peg-interferon and RBV. Approximately 23% of patients treated with BOC had grade 3 neutropenia (ANC between 500 and 750 per cubic millimeter), and approximately 7% of these individuals experienced grade 4 neutropenia (ANC < 500 per cubic millimeter) in comparison with 13% and 4%, respectively, in patients who received Peg-interferon and RBV. It may be necessary to reduce the Peg-interferon doses and to use granulocyte colony-stimulating factor in patients treated with BOC[3].

There are no reports in the literature that have reported the appearance of Fournier's Syndrome with triple therapy for HCV. However, it should be taken into consideration that this rare condition is most common in patients with diabetes, alcohol abusers and in immu-

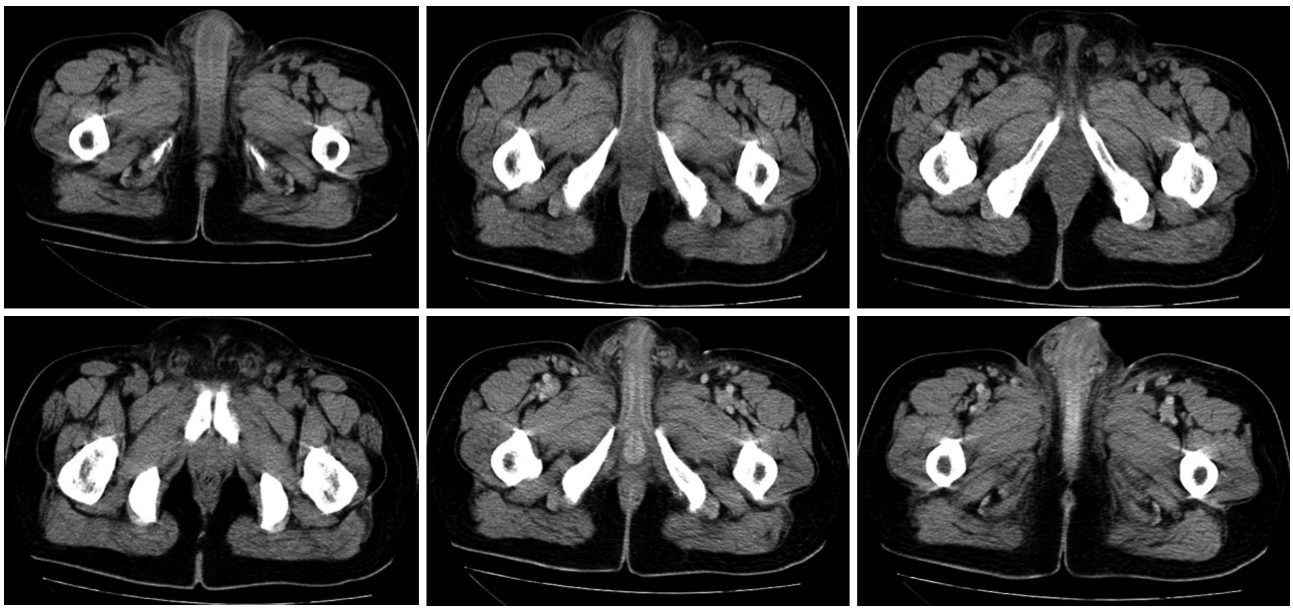

Figure 3 These images reveal a thickening of the skin in the inguinal region, as well as in the thigh roots and scrotum. These features are associated with slight blemishing of the adjacent adipose tissue.

nosuppressed individuals. Although this diagnosis would be made primarily using clinical data, imaging exams may be useful in cases of atypical presentation or when there is concern regarding the true extent of the disease. The most common sites of involvement are the genitourinary tract, the lower gastrointestinal tract and the skin. Fournier's Syndrome is a mixed infection caused by both aerobic and anaerobic bacteria; thus, the management of Fournier's Syndrome requires immediate debridement and wide-spectrum antibiotic therapy[11-15].

The present case report documents severe neutropenia associated with a serious infection, Fournier's syndrome, during triple therapy for HCV. Given the seriousness of the adverse events and the good patient outcomes observed with this treatment paradigm, this case report may have extremely important implications for patient care.

COMMENTS

Case characteristics

A 53-year-old cirrhotic patient with hepatitis C virus (HCV) genotype 1 A who had exhibited no response to prior treatment was initiated on retreatment with boceprevir (BOC), Peg-interferon (at a double dose of 180 μg) and ribavirin (RBV). However, he exhibited fever and signs of hepatic encephalopathy during the 12th week of treatment.

Clinical diagnosis

The patient exhibited fever (40 ℃), dyspnea, diarrhea, confusion and disorientation, flapping, tachypnea, hypotension, tachycardia and evolving diffuse erythema throughout the perineal area to the root of the lower limbs, with exulcerations and seropurulent secretions.

Differential diagnosis

Septic shock and an infection of the gastrointestinal tract were also considered.

Laboratory diagnosis

Leucopenia (300 leukocytes/mm³) with neutropenia (10 neutrophils/mm³) was diagnosed based on laboratory results.

Imaging diagnosis

A computed tomography scan of the abdomen showed a thickening of the skin in the inguinal region, the roots of the thighs and the scrotum, and these features were associated with a slight blurring of the adjacent fat.

Pathological diagnosis

A myelogram demonstrated hypoplasia with cytotoxicity and maturational retardation.

Treatment

The patient was initially treated with cefepime and filgrastim after the cessation of treatment for hepatitis C. This treatment was subsequently exchanged for treatment with the antibiotics imipenem, linezolid and clindamycin.

Related reports

Triple therapy for hepatitis C has been previously shown to increase the rate of sustained virological responses (SVRs), and we observed an increase in the frequency and severity of adverse events related to this treatment.

Term explanation

Triple therapy for hepatitis C involves the use of PIs (BOC-boceprevir/telaprevir-telaprevir) in combination with Peg-interferon and RBV, and it should be considered for early treatment viral kinetics to treatment previously performed. Naïve: a patient who has not received prior treatment; Relapser: a patient characterized by undetectable levels of HCV-RNA after an initial treatment, without a SVR because of a positive viral load after the discontinuation of treatment; Partial responder: a patient whose HCV-RNA levels fell by more than 2-log after 12 wk of treatment, but HCV-RNA levels were detectable at the end of the treatment period; null responder: a patient whose HCV-RNA levels fell at least 2-log after 12 wk of treatment.

Experiences and lessons

The case report presented here describes a serious adverse event associated with this new triple therapy that has not yet reported in the literature. The authors' data emphasize the importance of adequate patient management plans that are in accordance with international clinical protocols, and these findings allow experts to gain access to and experience with the conduct of this real-life case.

Peer review

Dr. Oliveira *et al* presented an interesting case report. The design of the study is adequate. The finding of the presented case report is of interest for the general reader.

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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