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EDITORIAL

Multiresistant bacterial infections in liver cirrhosis: Clinical impact and new empirical antibiotic treatment policies

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Abstract

Recently, important changes have been reported regarding the epidemiology of bacterial infections in liver cirrhosis. There is an emergence of multiresistant bacteria in many European countries and also worldwide, including the United States and South Korea. The classic empirical antibiotic treatment (third-generation cephalosporins, *e.g.*, ceftriaxone, cefotaxime or amoxicillin-clavulanic acid) is still effective in infections acquired in the community, but its failure rate in hospital acquired infections and in some health-care associated infections is high enough to ban its use in these settings. The current editorial focuses on the different epidemiology of bacterial infections in cirrhosis across countries and on its therapeutic implications.

Key words: Bacterial infections; Multiresistant bacteria; Antibiotic; Liver cirrhosis

Core tip: There is a growing prevalence of multiresistant bacteria in nosocomial and in health-care associated settings worldwide. Nowadays, it is necessary that all liver units assess the presence of antibiotic resistance in their population. The classical empirical antibiotic therapy, third generation cephalosporins, can no longer be employed in areas with high prevalence of multiresistant bacterial infections. The current editorial focuses on the different patterns of resistance across countries and on its therapeutic implications.

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INTRODUCTION

Liver cirrhosis carries a big burden for health care worldwide. In Europe around 29 million people have a chronic liver disease, and mortality rate is 170000/year. Bacterial infection represents one of the main causes of decompensation. Patients with cirrhosis have alterations in the immune system and therefore they are more susceptible to develop bacterial infection, sepsis, and death[1-5]. Infection is present at admission or develops during hospitalization in around 25%-30%

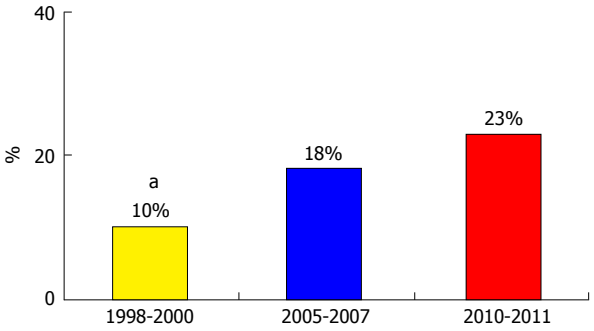

Figure 1 Increasing prevalence of multiresistant bacterial infections in a single center in Spain. Prevalence of multiresistant bacteria has doubled in the last decade (original work of the author). [a]*P* < 0.05 *vs* other periods.

of patients[5-7]. The most frequent infections in cirrhosis are spontaneous bacterial peritonitis (SBP), urinary tract infections (UTI), pneumonia, cellulitis and spontaneous bacteremia[1-3]. Bacterial infections are not only more frequent but also more severe in cirrhosis. Infection increases the probability of death 4 fold reaching 38% at 1 mo[8]. Infection can accentuate the preexisting circulatory dysfunction present in advanced cirrhosis leading to the development of hepatorenal syndrome and can also induce an excessive pro-inflammatory response that could contribute to develop multiple organ failure (acute-on-chronic liver failure) and septic shock[9]. Thus, prompt diagnosis and appropriate treatment of infection is crucial in the management of patients with cirrhosis[10,11].

GRADUAL EPIDEMIOLOGICAL CHANGES

The most common origin of infection is the community-acquired (CA) setting. The definition of healthcare associated (HCA) infection is infection which occurs previous to admission or during the first two days of hospitalization in patients in contact with the hospital setting during the last 3 mo[7].

Classically, gram-negative bacilli (GNB) accounted for the vast majority of infections (80%), but this preponderance of GNB changed at the start of the new millennium and the prevalence of gram-positive cocci (GPC) increased, accounting for almost half of the infections (47%). When infections were classified according to the origin of infection, GNB were still the most frequent bacteria causing CA infections (60%), while GPC were more prevalent in nosocomial infections (60%). This fact was explained by the increasing degree of instrumentalization of the cirrhotic patient (*i.e.*, variceal ligation, transjugular intrahepatic portosystemic shunt, and arterial chemoembolization or percutaneous ablation of hepatocellular carcinoma), and by the fact that cirrhotic patients with a critical illness are admitted into intensive care units which implies the insertion of central lines and other invasive procedures[6,7].

Furthermore, the need for long term antibiotic prophylaxis, usually with norfloxacin, also carries the risk of emerging of quinolone-resistant and cotrimoxazole-resistant bacteria. Around 50% of GNB were resistant to these antibiotics in a big study performed in 2000[7].

Multiresistant (MR) bacteria are resistant to 3 or more of the principal antibiotic families, including β-lactams[12]. The most common are extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-*E*), *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-susceptible *Enterococcus* (VSE) and vancomycin-resistant *Enterococcus* (VRE).

In 2000, only 1.2% of bacteria were resistant to third generation cephalosporins (TGC). Unfortunately, many recent studies from different countries show a growth in the prevalence of MR bacterial infections in cirrhosis. Fernández *et al*[7] reported a steady growth in the prevalence of MR bacterial infections, this prevalence rose from 10% to 23% during the period 1998-2011 in a single Spanish center[7] (Figure 1). MR bacteria were extremely frequent in the nosocomial setting, causing 35%-39% of infections, but the prevalence was very low in the CA setting, 0%-4%; and prevalence of MR bacteria in the HCA setting was intermediate, between 14% and 20%[7] (Figure 2).

DIVERSITY OF MR BACTERIA TYPES ACROSS COUNTRIES

Epidemiology of multiresistance differs among different countries and even among hospitals located in the same area. ESBL-*E* are predominant in Southern Europe and Asia[7,13-25], meanwhile MRSA and VRE are prevalent in the United States[26]. Carbapenemase-producing *K. pneumoniae* is a current problem described in some hospitals in Italy[27]. Table 1 shows the prevalence of different MR bacterial infections across countries in the cirrhotic population. The marked epidemiological differences observed among countries and centers suggest that local epidemiology should be evaluated regularly and that empiric antibiotic treatment of nosocomial infections in cirrhosis should be adjusted in accordance with the specific local pattern of multiresistance.

FAILURE OF TGC TREATMENT IN NOSOCOMIAL INFECTIONS AND CLINICAL OUTCOME

Early and appropriate antibiotic therapy is fundamental in the management of infections in patients with cirrhosis. Since the late 1980's, TGC have been recommended as empirical antibiotic therapy of the main infections in cirrhotic population because they cover a wide variety of bacteria and they are safe[28-31].

Nevertheless, recent studies demonstrate that β-lactams are not effective in an important part of infections in cirrhosis, especially in nosocomial infections[7]. TGC have scarce efficacy in nosocomial infections (40%). This

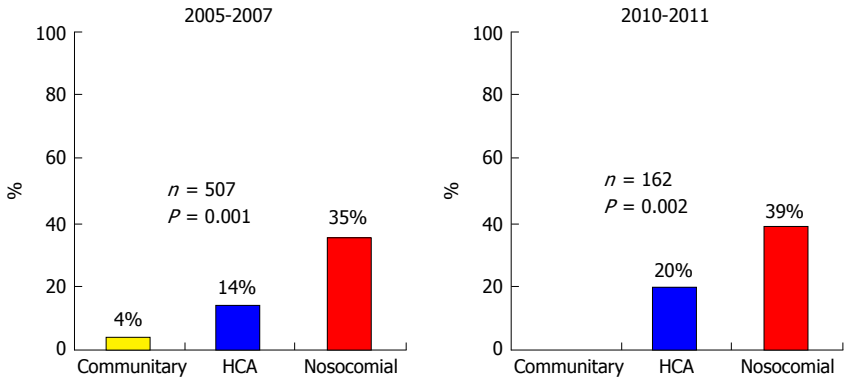

Figure 2 Prevalence of multiresistant bacterial infections according to the site of acquisition. Multiresistant bacteria are present predominantly in the nosocomial and healthcare associated (HCA) infections. *P* value in both graphics refers to χ^2 linear-by-linear association statistic (original work of the author).

Table 1 Prevalence of different multiresistant bacteria across countries

Type of multiresistant bacteria	Prevalence rate
ESBL	South Korea, 4%-29%[13,14,19,20,24]
	Italy, 8%-20%[17,21]
	Spain and United States, 6%-9%[7,15,26]
	France, Denmark and Germany, < 5%[22,23,25]
MRSA	Italy, 7%[17,21]
	United States, 5%[26]
	Spain, 3%-4%[7,15]
	France, 2%[23]
	Denmark, Germany, 0%[22,25]
Pseudomonas aeruginosa	Spain, South Korea, 2%-3%[7,13-15,19,20,24]
	Germany, 1%[22]
	Denmark, France, 0%[23,25]
VSE	Denmark, 12%[25]
	Germany, 10%[22]
	France, 5%[23]
	Spain, 1%-7%[7,15]
VRE	United States, 9%[26]
	Spain, France, Denmark, Germany, 0%[7,15,22,23,25]

ESBL: Extended-spectrum β-lactamase-producing *Enterobacteriaceae* (bacteria with chromosomal β-lactamases are also included); MRSA: Methicillin-resistant *Staphylococcus aureus*; VSE: Vancomycin-susceptible *Enterococcus*; VRE: Vancomycin-resistant *Enterococcus*.

fact is reproduced in the main types of infections as SBP, UTI and spontaneous bacteremia with efficacies of 26%, 29% and 18%, respectively. Efficacy of empirical antibiotic therapy is also lower in HCA infections compared to community-acquired infections (73% *vs* 83%), especially in pneumonia and UTI. Other groups from Italy, Germany and Turkey have also reported a reduced efficacy of TGC in SBP, with rates of failure from 18% to 41%[21,22,32].

MR bacterial infections have a poor outcome because they entail a higher incidence of treatment failure (70% *vs* 92%, $P < 0.0001$), they lead more frequently to septic shock (26% *vs* 10%; $P < 0.0001$), and cause a much higher hospital mortality rate (25% *vs* 12%; $P = 0.001$) than those infections produced by susceptible bacteria. The delay in the initiation of an appropriate antibiotic treatment is one of the main explanations for this fact[7].

RISK FACTORS FOR MR BACTERIAL INFECTIONS

Risk factors for MR bacteria constitute an important tool to identify the group of infected patients who would benefit from changing the empirical antibiotic therapy in order to cover MR bacteria. The main risk factors for the development of MR bacterial infections in the general population are current or recent hospitalization, healthcare contact (*i.e.*, hemodyalisis) and prior exposure to β-lactams or quinolones[33-37]. The same risk factors have been reported in cirrhosis. Hospital acquired and healthcare associated infections, long-term norfloxacin prophylaxis, recent infection by MR bacteria, recent use of β-lactams within the last 3 mo or systemic antibiotics in the past 30 d, upper gastrointestinal bleeding and diabetes mellitus[7,15,26].

CHANGING THE EMPIRICAL ANTIBIOTIC TREATMENT OF BACTERIAL INFECTIONS IN CIRRHOSIS

Taking into account the high failure rate of TGC in a large percentage of nosocomial infections and in a subgroup of healthcare associated infections due to the emergence of MR bacteria in these settings, it is clear that empirical antibiotic treatment in cirrhosis should be chosen in accordance with severity and type of infection, and also according to the origin of infection and to the existence of risk factors for MR bacterial infection[38-40]. The AASLD guideline was updated in 2012[41] and an EASL position statement came into light in 2013[42], both recommending substantial changes to empirical antibiotic treatment in the nosocomial setting.

Regular epidemiology assessment should be carried out in all centers and policies regarding empiric antibiotic treatment in cirrhosis should be updated and tailored according to the specific epidemiological pattern of

Table 2 Suggested empirical antibiotic therapy of bacterial infections in cirrhosis

Type of infection	Community-acquired infections	Nosocomial and HCA[1] infections	Local epidemiological pattern
SBP, SBE and SB	Third generation cephalosporin Or amoxicillin/clavulanic acid	Piperacilin/tazobactam Or carbapenem Plus glycopeptides (or linezolid)	Low prevalence of MR bacteria ESBL-*E* MRSA and VSE (when VRE)
Urinary tract infections	Third generation cephalosporin Or amoxicillin/clavulanic acid	Without sepsis: Nitrofurantoin or fosfomycin With sepsis: Piperacilin/tazobactam Or carbapenem Or plus glycopeptides (or linezolid)	VSE Low prevalence of MR bacteria ESBL-*E* MRSA and VSE (when VRE)
Pneumonia	Amoxicillin/clavulanic acid Or ceftriaxone + macrolide Or levofloxacin Or moxifloxacin	Piperacilin/tazobactam Meropenem or ceftazidime + ciprofloxacin Plus vancomycin (or linezolid)	Low prevalence of MR bacteria ESBL-E and *P. aeruginosa* MRSA and VSE (when VRE)
Skin and soft tissue infections	Amoxicillin/clavulanic acid Or third generation cephalosporin plus oxacillin	Meropenem or ceftazidime + oxacillin Plus glycopeptides (or linezolid or daptomycin)	ESBL-E and *P. aeruginosa* MRSA and VSE (when VRE)

[1]HCA infections with at least two of the following risk factors for MR bacteria: long-term norfloxacin prophylaxis, recent infection by MR bacteria in the last 6 mo, recent use of β-lactams in the last 3 mo. SBP: Spontaneous bacterial peritonitis; SBE: Spontaneous bacterial empyema; SB: Spontaneous bacteremia; HCA: Health-care associated; ESBL-*E*: Extended-spectrum β-lactamase producing *Enterobacteriaceae*; MRSA: Methicillin-resistant *Staphylococcus aureus*; VSE: Vancomycin-susceptible *Enterococcus*; VRE: Vancomycin resistant *Enterococcus*.

multiresistance in the area.

CURRENT EMPIRICAL ANTIBIOTIC TREATMENT OF COMMUNITY-ACQUIRED INFECTIONS

TGC are still effective in infections acquired in the community in cirrhosis with resolution rates of around 80%[7]. Quinolones should not be used in patients on norfloxacin prophylaxis or in zones with a high prevalence of quinolone-resistant bacteria[1,6]. For the empirical therapy of UTI we can employ trimethoprim-sulfamethoxazole, quinolones or β-lactams. β-lactams are the baseline treatment of pneumonia (in combination with levofloxacin, moxifloxacin or a macrolide) and cellulitis as well[38-42] (Table 2).

CURRENT EMPIRICAL TREATMENT OF NOSOCOMIAL INFECTIONS

Empirical antibiotic therapy should be selected in accordance with the local epidemiological pattern of multiresistance[38-42]. In zones with a high prevalence of ESBL-*E*, carbapenems should be employed as empirical treatment of spontaneous infections such as SBP, spontaneous bacteremia and spontaneous empyema. A glycopeptide (vancomycin or teicoplanin) should also be added to this empirical treatment in zones with a high prevalence of MRSA or VSE. In the United States and other countries with a high prevalence of VRE, glycopeptides should be substituted for linezolid or daptomycin[38-42]. However, in zones with a low rate of MR bacteria, piperacillin-tazobactam can be employed to treat spontaneous infections[38-42] (Table 2).

Nosocomial UTI without sepsis should be treated with oral nitrofurantoin or fosfomycin. UTI with sepsis should be treated with carbapenems plus glycopeptides to cover ESBL-*E* and VSE (Table 2)[38-42]. Again, in zones with a scarce prevalence of MR bacteria, piperacillin-tazobactam can be employed in UTI with sepsis (Table 2).

In zones with a high prevalence of MR bacteria, nosocomial cellulitis should be covered against MRSA and *Pseudomonas aeruginosa.* Thus, ceftazidime or carbapenem plus a glycopeptide can be employed. In areas with low rate of MR bacteria, cloxacillin or amoxicillin-clavulanic acid can be employed[38-42] (Table 2).

Empirical treatment of hospital acquired pneumonia should follow the local guidelines recommended for the general population.

CURRENT EMPIRICAL TREATMENT OF HEALTHCARE-ASSOCIATED INFECTIONS

TGC and amoxicillin-clavulanic acid are effective when employed in SBP (resolution rate: 71% compared to 78% in CA infections) or cellulitis (resolution rate: 81% *vs* 82% in CA infections). However, their efficacy is low in HCA pneumonia (33%) and UTI (59%)[7,38]. Therefore, empirical antibiotic therapy for HCA UTI and pneumonia and for patients with HCA spontaneous infections with two or more risk factors of infection caused by MR bacteria or those patients with severe sepsis or septic shock should follow the same scheme recommended in nosocomial infections (Table 2)[38,40].

In general, de-escalation to the most suitable antibiotic should be done soon after the report from microbiological tests (about 50% of cases) in order to

reduce the emergence of antibiotic resistance[38,40].

CONCLUSION

In conclusion, recent data demonstrate that TGC are not appropriate for the treatment of nosocomial infections in cirrhosis because of the high prevalence of MR bacteria in this setting. New antibiotic strategies for these infections should be adjusted in accordance with the local epidemiological pattern of multiresistance. In areas with high prevalence of ESBL-*E*, guidelines should include the use of carbapenems. In zones with high prevalence of MRSA and VSE glycopeptides are needed and in zones with high prevalence of VRE linezolid or daptomycin are needed as initial treatment of nosocomial infections in cirrhosis. Early de-escalation of antibiotics according to the microbiological results is also mandatory.

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EDITORIAL

Cognitive dysfunction and hepatitis C virus infection

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Abstract

Cognitive dysfunction in patients with chronic hepatitis C virus (HCV) infection is a distinct form of minimal hepatic encephalopathy (MHE). In fact, the majority of HCV-positive patients, irrespective of the grading of liver fibrosis, display alterations of verbal learning, attention, executive function, and memory when they are evaluated by suitable neuropsychological tests. Similarities between the cognitive dysfunction of HCV patients and MHE of patients with different etiologies are unclear. It is also unknown how the metabolic alterations of advanced liver diseases interact with the HCV-induced cognitive dysfunction, and whether these alterations are reversed by antiviral therapies. HCV replication in the brain may play a role in the pathogenesis of neuroinflammation. HCV-related brain dysfunction may be associated with white matter neuronal loss, alterations of association tracts and perfusion. It is unclear to what extent, in patients with cirrhosis, HCV triggers an irreversible neurodegenerative brain damage. New insights on this issue will be provided by longitudinal studies using the protocols established by the diagnostic and statistical manual of mental disorders fifth edition for cognitive disorders. The domains to be evaluated are complex attention; executive functions; learning and memory; perceptual motor functions; social cognition. These evaluations should be associated with fluorodeoxyglucose positron emission tomography and magnetic resonance imaging (MRI) protocols for major cognitive disorders including magnetic resonance spectroscopy, diffusion tensor imaging, magnetic resonance perfusion, and functional MRI. Also, the characteristics of portal hypertension, including the extent of liver blood flow and the type of portal shunts, should be evaluated.

Key words: Cognitive impairment; Neuropsychological tests; Magnetic resonance imaging spectroscopy; Magnetic resonance imaging spectroscopy; Hepatitis C virus infection

Core tip: Cognitive dysfunction in patients with chronic hepatitis C virus (HCV) infection is a distinct form of minimal hepatic encephalopathy. It is unclear to what extent HCV triggers an irreversible neurodegenerative brain damage. New insights on this issue will be provided by longitudinal studies using the protocols established by the DSM-5 for cognitive disorders associated with FDG-PET and magnetic resonance imaging protocols for major cognitive disorders. Also, the characteristics

of portal hypertension, including the extent of the liver blood flow and the type of portal shunts, should be evaluated.

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INTRODUCTION

Minimal hepatic encephalopathy (MHE) is defined as the presence of test-dependent brain dysfunction in patients with chronic liver disease who are not disoriented and do not display asterixis[1,2]. MHE is clinically relevant, as it affects the quality of life and the job performance of patients with chronic liver disease, and because it is a recognized risk factor of overt hepatic encephalopathy[3-5]. Cognitive dysfunction in patients with chronic hepatitis C virus (HCV) infection is a distinct form of MHE. In fact, the majority of HCV-positive patients, irrespective of the grading of liver fibrosis, display alterations of verbal learning, attention, executive function, and memory when they are evaluated by suitable neuropsychological tests[6-8].

WHAT WE KNOW

Recent years have witnessed significant insights into the pathogenesis and management of this disorder. However, several questions remain unanswered. In particular, commonalities between the cognitive dysfunction of HCV patients and MHE of patients with different etiologies are unclear. It is also unknown how the metabolic alterations of advanced liver diseases interact with the HCV-induced cognitive dysfunction, and whether these alterations are reversed by antiviral therapies.

HCV sequences were detected in the cerebrospinal fluid of 8 of 13 patients in whom a lumbar puncture had been performed for diagnostic purposes[9]. Ten of these patients were co-infected with human immunodeficiency virus, and were affected by aseptic meningitis (5 cases), reactive meningitis (1 case), neurotoxoplasmosis (3 cases), tuberculosis (2 cases), neurosyphilis (1 case) and multiphocal leukoencephalopathy (1 case). In 4 patients different virus strains were found in serum and in peripheral mononuclear blood cells (PBMC). Of note, virus strains detected in cerebrospinal fluid were similar to those detected in PBMC. This finding led to the hypothesis that infected PBMC cross the blood brain barrier and, by this mechanism, HCV infects the brain. Subsequent studies[10,11] confirmed the tissue compartmentalization of HCV quasispecies, and showed that microglial cells and, to a lesser extent, astrocytes harbored HCV-RNA sequences and HCV specific proteins. Recently, it has been shown that brain microvascular endothelium expresses HCV receptors, and HCV replicates within endothelial cell lines[12]. These findings support the hypothesis that HCV distinct viral strains replicate in the brain. Although the level of viral replication is generally low (apart from the cases with alterations of the blood-brain barrier), it may play a significant role in the pathogenesis of the neuroinflammation. Compared with HCV-negative controls, HCV-positive patients demonstrated significantly higher levels of proinflammatory cytokines within the brain[13,14]. Additional evidence of neuroinflammation in HCV positive patients is suggested by magnetic resonance spectroscopy studies[15,16]. In fact, choline/creatine ratios (a putative indicator of inflammation) were significantly higher in the basal ganglia and white matter of HCV positive patients compared to HBV positive patients and normal controls[17]. This finding was associated with elevated myo-inositol/creatine ratios (a putative marker of glial density)[18]. Subsequent quantitative analysis of brain metabolites[19] showed that the spectra related to choline and myo-inositol were significantly higher in the basal ganglia of patients with HCV chronic hepatitis. Compared to controls, the spectrum of N-acetyl aspartate (NNA) and NNA-glutamate, which is related to the neuronal density and nitrogen removal, was also significantly higher in basal ganglia of HCV patients. When the same metabolites were evaluated according to the Fatigue Impact Scale Score, it was found that both markers of inflammation and neuronal density were inversely related to the grade of fatigue. Similar to the elevated NNA/creatine ratio observed in the contra-lesional pre-frontal regions of stroke patients[20], a possible explanation of this finding is a compensatory mechanism of HCV-related brain inflammation. When compensatory mechanisms fail, fatigue, and possibly other signs of neurocognitive impairment, take place. HCV-related brain dysfunction does not seem limited to functional alterations. Evidence of white matter neuronal loss, alterations of several commissural and association tracts, cortical hypoperfusion, and basal ganglia hyperperfusion was recently provided by magnetic resonance (MR) spectroscopy associated with perfusion weighted imaging and diffusion tensor imaging[21]. Of note, this study was conducted in patients without cirrhosis. Scanty data are available on the natural history of neurocognitive dysfunction when chronic hepatitis progresses to cirrhosis. In patients with cirrhosis unrelated to HCV, MHE was associated with widespread microstructural disintegration of the white matter, and with focal cortical damage. These findings have been related to hyperammonaemia-induced neuroinflammation[22-24]. It is unclear to what extent HCV infection exacerbates this condition, and whether it triggers an irreversible neurodegenerative brain damage.

FUTURE DIRECTIONS

In order to get insights on these issues, longitudinal studies of well characterized cohorts of patients are

needed. Such studies should be based on extended neuropsychological evaluation, standard and advanced MR imaging (MRI) protocols, and accurate characterization of portal hypertension. In the current clinical practice the diagnosis of MHE is not uniform. Current guidelines[1,2] recommend that this diagnosis should be based on at least two of the following tests: the portosystemic encephalopathy syndrome test[25], the critical flicker test[26], the continous reaction time test[27], the inhibitory control test[28], the the Stroop test[29], the SCAN test[30]. These tests are mostly aimed at evaluating attention, working memory, and visuospatial ability. However, if we assume that HCV-related brain impairment could also represent the harbinger of a slowly progressing neurodegenerative alteration[31], when evaluating these patients we should apply the protocols established by the DSM-5 for cognitive disorders[32]. Accordingly, cognitive domains to be evaluated are the following: complex attention (including sustained attention, divided attention, selective attention and processing speed); executive functions (including planning, decision making, working memory, mental flexibility, abstract reasoning skills, and judgment); learning and memory; perceptual motor functions; social cognition. In addition, further study should be performed, using FDG-PET and MRI protocols for major cognitive disorders including magnetic resonance spectroscopy, diffusion tensor imaging, magnetic resonance perfusion, and functional MRI[33]. Finally, the characteristics of portal hypertension should also be evaluated. In this regard, it is worth to mention that not only the amount of blood diverted from the liver, but also the type of shunting is of relevance in the pathogenesis of hepatic encephalopathy[34,35]. In conclusion, the mechanisms of neurocognitive disorders in patients with chronic HCV infection have been partially elucidated. The impact of this condition on the long-term outcome of these patients should be further clarified.

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REVIEW

Primary biliary cirrhosis: Pathophysiology, clinical presentation and therapy

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Abstract

Primary biliary cirrhosis (PBC) is an autoimmune, slowly progressive, cholestatic, liver disease characterized by a triad of chronic cholestasis, circulating anti-mitochondrial antibodies (AMA), and characteristic liver biopsy findings of nonsuppurative destructive cholangitis and interlobular bile duct destruction. About 10% of PBC patients, however, lack AMA. A variant, called PBC-autoimmune hepatitis (AIH) overlap, is characterized by the above findings of PBC together with findings of elevated serum alanine aminotransferase, elevated serum immunoglobulin G, and circulating anti-smooth muscle antibodies, with liver biopsy demonstrating periportal or periseptal, lymphocytic, piecemeal necrosis. PBC is hypothesized to be related to environmental exposure in genetically vulnerable individuals. It typically occurs in middle-aged females. Prominent clinical features include fatigue, pruritis, jaundice, xanthomas, osteoporosis, and dyslipidemia. The Mayo Risk score is the most widely used and best prognostic system. Ursodeoxycholic acid is the primary therapy. It works partly by reducing the concentration and injury from relatively toxic bile acids. PBC-AIH overlap syndrome is treated with ursodeoxycholic acid and corticosteroids, especially budesonide. Obeticholic acid and fibrate are promising new, but incompletely tested, therapies. Liver transplantation is the definitive therapy for advanced disease, with about 70% 10-year survival after transplantation. Management of pruritis includes local skin care, dermatologist referral, avoiding potential pruritogens, cholestyramine, and possibly opioid antagonists, sertraline, or rifaximin. Management of osteoporosis includes life-style modifications, administration of calcium and vitamin D, and alendronate. Statins are relatively safe to treat the osteopenia associated with PBC. Associated Sjogren's syndrome is treated by artificial tears, cyclosporine ophthalmic emulsion to stimulate tear production; and saliva substitutes, cholinergic agents, and scrupulous oral and dental care. Complications of cirrhosis from advanced PBC include esophageal varices, ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, and hepatoma formation.

Key words: Primary biliary cirrhosis; Ursodeoxycholic acid; Cirrhosis; Liver transplantation; Cholestatic liver disease

Core tip: Primary biliary cirrhosis (PBC) is an autoimmune, slowly progressive, cholestatic, liver disease characterized by a triad of chronic cholestasis, circulating anti-mitochondrial antibodies, and characteristic liver biopsy findings of nonsuppurative destructive cholangitis and interlobular bile duct destruction. Prominent clinical features include fatigue, pruritis, jaundice, xanthomas, osteoporosis, and dyslipidemia. Ursodeoxycholic acid is the primary therapy. Obtecholic acid and fibrate are promising new, but incompletely tested, therapies. Liver transplantation is the definitive therapy for advanced disease, with about 70% 10-year survival after transplantation. Complications of cirrhosis from advanced PBC include esophageal varices, ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, and hepatoma formation.

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INTRODUCTION

Primary biliary cirrhosis (PBC) is an autoimmune, slowly progressive, cholestatic, liver disease[1]. A triad of chronic biochemical cholestasis, circulating anti-mitochondrial antibodies (AMA), and characteristic liver biopsy findings, as described below, is diagnostic of PBC[2]. Development of PBC is hypothesized to be related to environmental exposure in genetically vulnerable individuals, but further studies are needed to understand its complex etiology[3]. This work reviews the epidemiology, pathophysiology, clinical presentation, treatment, and prognosis of PBC, with a focus on recent advances.

HISTORY

PBC, as it is now known, was first reported by Addison *et al*[4] in 1851. The term PBC was, however, coined by Ahrens *et al*[5] in 1950. Walker *et al*[6] first described the association between AMA seropositivity and PBC.

EPIDEMIOLOGY

PBC is ten-fold more common in women than men[1]. The reason for this difference is unknown, but a relatively recent study revealed that X chromosome monosomy was more common in women with PBC. This finding suggests that genes related to X-linked immunodeficiencies can lead to granuloma formation and elevated IgM levels, both of which occur in PBC[7].

The median age of diagnosis is about 50 years[8]. There is significant geographic variation, with a much higher prevalence in the United States, at 400 per million[8], or northern Europe, at 200-250 per million, than in Africa, Asia, or Australia, at 20 per million[9]. This difference, however, could largely arise from detection bias; far more epidemiologic studies have been conducted in North America and Europe than in Africa and Asia[10]. Though differences in case-finding methodology, disease awareness, and access to health care may have confounded these studies, the immense geographic disparities suggest that environmental and/or genetic factors affect PBC. Environmental agents, including exposure to sunlight, chemicals, toxins, bacteria and viruses, may differ across geographic regions, and such differences may play a role in pathogenesis[11]. The educational level of individuals with PBC is similar to that of controls, but PBC is apparently more prevalent in patients who belong to higher socioeconomic strata for unclear reasons[12].

PBC is strongly associated with recurrent urinary tract infections[10,12-14]. In an epidemiologic study, 48% of PBC patients had prior recurrent urinary tract infections *vs* 31% of controls; this difference was statistically significant[12]. *Escherichia coli* (*E. coli*), the most frequent cause of recurrent urinary tract infections in women, has been studied in relation with PBC. *E. coli* infections trigger autoimmune responses, perhaps by molecular mimicry of proteins in *E. coli* with the human pyruvate dehydrogenase complex (PDC-E2), which induces B- and T-cell cross-reactive responses that characteristically occur in patients with PBC[10,12,14,15]. Other microorganisms implicated in PBC include *Novosphingovium aromaticivorans, Lactobacillus delbrueckii, Toxoplasma gondii, Mycobacteria* and retroviruses, though these associations are weaker than that for *E. coli*[14]. Numerous studies in the United States and United Kingdom suggest a significant relationship between smoking tobacco and PBC[10,12,15-17]. Chemicals inhaled in tobacco smoke are postulated to decrease immunologic tolerance[11]. Some data suggest patients who use hair dyes are at increased risk of PBC[15], but the evidence is conflicting[10]. PBC is apparently negatively correlated with alcohol consumption[15].

Genetic and familial factors play a major role in PBC[18]. The concordance rate is 63% in monozygotic twins[19], and the concordance rate is 4% in first degree relatives of patients with PBC. Moreover, sisters of a woman with PBC have a 14-fold higher risk of PBC compared to the general population[12].

Autoimmune disorders occur more frequently in patients with PBC, including autoimmune thyroid disorders, Raynaud syndrome, and Sjogren syndrome[12]. These disorders generally precede the onset of PBC by about 4 years. Patients with thyroid disorders should be tested for the presence of AMA. A recent meta-analysis suggested that human leukocyte antigen (HLA)-DR 7 and -DR 8 are risk factors for PBC, whereas HLA-DR 11 and -DR 13 are protective factors[20]. Other genetic

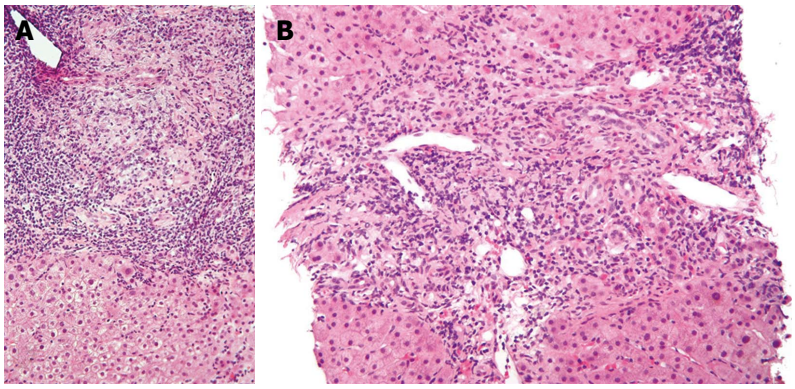

Figure 1 Lower (A) and higher (B) magnification photomicrographs of a liver biopsy sample in a patient with early primary biliary cirrhosis shows a moderately severe, mixed inflammatory infiltrate consisting mostly of lymphocytes and plasma cells concentrated around small bile ducts in the portal area. Note the absence of inflammation around hepatocytes (H and E stains). Both figures obtained with permission (granted under the GNU Free Documentation License, Version 1.2) from: http://commons.wikimedia.org/wiki/File:Primary_biliary_cirrhosis_intermed_mag_2.jpg. Accessed March 10, 2015.

variants associated with PBC include polymorphisms of cytotoxic T-lymphocyte antigen[21,22], tumor necrosis factor (TNF)[23], vitamin D receptor[24], and HLA loci 11 and 12.

DIAGNOSIS

PBC is diagnosed provided two of the following three criteria are satisfied: (1) AMA titer > 1:40; (2) alkaline phosphatase (AP) > 1.5 times the upper limit of normal for > 24 wk; and (3) compatible liver histology, demonstrating nonsuppurative destructive cholangitis and interlobular bile duct destruction[25-27].

AMA

AMA is the most specific serological marker among > 60 autoantibodies analyzed in PBC patients[28,29]. It is detected in > 90% of patients with PBC, whereas it is detected in < 1% of the general population[1,29]. Patients with hepatitis C virus infection, however, have an 8% prevalence of AMA positivity. The antibody targets are members of a family of enzymes, the 2-oxo-acid dehydrogenase complexes, which include PDC-E2, branched chain 2-oxo-acid dehydrogenase complex, and 2-oxo-glutaric acid dehydrogenase complex. These enzymes catalyze oxidative decarboxylation of ketoacid substrates and are located on the inner mitochondrial membrane[27,30,31].

AMA is routinely detected in clinical laboratories by enzyme-linked immunoassay. Its titer does not correlate with PBC severity or activity. It is reasonable to determine serum liver function tests annually in seropositive individuals who initially have normal serum liver function tests[25]. When PBC is strongly suspected in AMA-negative patients, PBC-specific antinuclear antibodies (ANA), should be determined, including sp100 and gp210[7,32]. Elevated serum AP levels and characteristic liver histology, as aforementioned, also help in diagnosing PBC[25]. Occasionally, magnetic resonance cholangiopancreatography or endoscopic retrograde cholangiopancreatography may be necessary to exclude primary sclerosing cholangitis (PSC) or other alternative etiologies for cholestatic liver disease[33]. PSC is strongly suspected in a patient with elevated serum AP who has inflammatory bowel disease[34].

The time from detection of AMA to development of PBC is about 6 years (range 1-19 years)[35]. Only about 10% of patients who are AMA seropositive, but lack clinical features of PBC, subsequently develop PBC. A recent Greek study examined the significance of AMA, ANA-specific antibodies (anti-gp210 and anti-sp100), and antichromatin antibodies[36]. Autoantibody positivity, with increased autoantibody titers of anti-gp210 and anti-sp100 during follow-up, were related to advanced PBC. Mildly elevated anti-sp100 titers were associated with improved long-term prognosis (P = 0.025), and better response to ursodeoxycholic acid (UDCA) (P = 0.016)[35].

LIVER HISTOLOGY

Though liver biopsy is not mandatory for diagnosis, it helps stage the disease and differentiate PBC from other cholestatic liver disorders[27,37,38]. PBC has 4 histologic stages: (1) portal inflammation with or without florid bile duct lesions; (2) increase in size of periportal lesions with interface hepatitis; (3) distortion of hepatic architecture with numerous fibrous septa; and (4) cirrhosis. These stages occur sequentially with disease progression. The term "florid bile duct lesion" describes focal lesions that exhibit intense inflammatory infiltration and necrosis around bile ducts (Figure 1). The inflammatory infiltrate consists primarily of lymphocytes and mononuclear cells closely apposed with the basal membrane of cholangiocytes undergoing necrosis. The infiltrate may also contain macrophages and polymorphonuclear cells, and occasionally epithelioid granulomas, especially in early PBC[39]. The inflammatory infiltrates often compress and occlude portal venules. Generally, terminal hepatic venules are retained in their central location with progression to fibrosis and sometimes even to cirrhosis.

Table 1 Primary biliary cirrhosis - autoimmune hepatitis overlap can present in the following ways

Immunoserological overlap: *e.g.*, positive ANA/anti-smooth muscle antibody titers and elevated IgG in conjunction with AMA-positive PBC; or AMA positivity in AIH
Biochemical overlap: AST/ALT > 5 times upper limit of normal in patients with PBC or PSC; or AP > 3 times upper limit of normal in patients with AIH (or GGT > 5 times upper limit of normal in children)
Radiological overlap: clinical features of AIH with cholangiographic abnormalities indicative of inflammatory cholangiopathy; cholangiographic features of primary sclerosing cholangitis are randomly distributed annular strictures out of proportion to upstream dilatation[33]
Histological overlap: lymphoplasmacytic infiltrate and interface hepatitis on liver biopsy with bile duct lesions indicative of either PBC or PSC
Varying combinations of the above, including sequential presentations

ANA: Antinuclear antibodies; PBC: Primary biliary cirrhosis; AMA: Anti-mitochondrial antibodies; AIH: Autoimmune hepatitis; PSC: Primary sclerosing cholangitis; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transpeptidase.

Table 2 Clinical features of primary biliary cirrhosis

Clinical features	Prevalence	Mechanism
Fatigue	20%-85%[55,56,58]	Excessive manganese deposits in globus pallidum, elevated inflammatory cytokines
Pruritus	20%-75%[35,58]	Cholestasis, increased opiodergic tone
Jaundice	10%-60%[58]	Cholestasis
Xanthomas	15%-50%[58]	Hypercholesterolemia and hyperlipidemia[56]
Osteoporosis	35%[59]	Disturbances in bone remodeling due to metabolic changes in PBC
Dyslipidemia	> 75%[60]	Reduction in biliary secretion of cholesterol. Toxic effects of unconjugated bilirubin

PBC: Primary biliary cirrhosis.

Ductopenia, or bile duct paucity, is defined as presence of bile ducts in < 50% of portal tracts[27]. Interface hepatitis consists of lymphocytic piecemeal necrosis and biliary piecemeal necrosis which is associated with cholestasis[27]. Lymphocytic piecemeal necrosis consists of hepatocellular necrosis or apoptosis associated with lymphohistiocytic cells. This lesion is similar to that found in autoimmune hepatitis (AIH). Biliary piecemeal necrosis exhibits a striking reaction of ductular proliferation, accompanied by edema, neutrophil infiltration, periductular fibrosis, and necrotic hepatocytes.

PBC VARIANTS

PBC variants constitute about 5% of cases[40]. The five types of variants are listed in Table 1[33]. The two major variants are PBC-AIH overlap and AMA-negative PBC.

PBC-AIH overlap

The PBC-AIH overlap syndrome consists of a range of clinical entities of AIH together with clinical, laboratory, or histological characteristics of PBC or PSC[41-43]. These two overlapping conditions rarely present simultaneously; PBC generally precedes AIH by 6 mo to several years[43]. No stringent criteria exist for this overlap syndrome. In the most widely accepted criteria, overlap syndrome is diagnosed when PBC is diagnosed by the aforementioned criteria and AIH by the presence of the following criteria: (1) serum alanine aminotransferase $\geqslant$ 5 × upper limit of normal, serum immunoglobulin G (IgG) levels $\geqslant$ 2 × upper limit of normal, or positive test for anti-smooth muscle anti-bodies; and (2) liver biopsy demonstrating moderate or severe periportal or periseptal, lymphocytic, piecemeal necrosis[44,45]. The simplified International Autoimmune Hepatitis Group criteria for diagnosis of overlap syndrome are not currently recommended[43]. The Paris criteria for diagnosis of PBC-AIH overlap include an AP-to-aminotransferase ratio < 1.5, elevated serum IgG level, and anti-smooth muscle antibody (anti-SMA) titer > 1:80[25,26,46].

AMA negative PBC

AMA-negative PBC and classic PBC have similar clinical findings and laboratory characteristics[40,47-49]. They have similar age and sex distributions, similar incidence of complications, and similar time-to-liver-transplantation and survival. The relationship between AMA-negative PBC and autoimmune cholangiopathy is controversial. Some authorities use these terms interchangeably[49,50], whereas other authorities view them as disparate entities, with autoimmune cholangiopathy representing PBC with negative AMA titers, but positive ANA or anti-SMA titers[40]. Before labeling individuals as AMA-negative PBC, methods to detect circulating AMA should be assiduously employed, including sensitive immunochemical testing with bead microassays, use of three mitochondrial autoantigens[51], and serial testing[52]. Liver biopsy is mandatory to diagnose PBC in the setting of AMA seronegativity[50]. Other etiologies of liver disease in the differential diagnosis should be excluded, including sclerosing cholangitis, IgG4 cholangitis, drug induced liver injury, *etc.*[50]. Additionally, other causes of elevated serum AP should be investigated, including hepatic causes such as infectious hepatitis, granulomatous liver disease, infiltrative liver disease, and idiopathic adult ductopenia; and non-hepatic causes such as pregnancy, bone disease, heart failure, renal failure, leukemia, lymphoma, *etc.*[53].

CLINICAL FEATURES

The clinical features and epidemiology of PBC are described in Table 2[54-60]. The most common clinical symptoms are fatigue and pruritus. Patients with fatigue and pruritus at onset are more likely to progress to cirrhosis and are less likely to respond to UDCA[61]. Pruritus is often severe and disabling, and associated with a poor quality of life. Both symptoms are described in detail below.

Most patients with PBC have mildly elevated serum

Table 3 Drug therapy for primary biliary cirrhosis

Drug	Mechanism(s) of action	Adverse effects
Ursodeoxycholic acid	Protection of cholangiocytes, stimulation of biliary secretions of bile acids	Diarrhea, hepatic decompensation (rare)
Corticosteroids	Anti-inflammatory, especially useful for interface hepatitis	Cataracts, hyperglycemia, osteoporosis, immunosuppression, poor wound healing, weight gain
Budesonide	Anti-inflammatory, especially useful for interface hepatitis	Nausea, dyspepsia; systemic toxicity is much less than for other corticosteroids[73]
Obeticholic acid	Reduces bile acid synthesis, downregulates bile acid uptake proteins	Pruritus
Fibrates	Activates peroxisome proliferator-activated receptors	Myalgias, rhabdomyalysis, elevated liver enzymes[72]

aminotransferase levels[62,63], and increased level of immunoglobulins, especially IgM. A study of 25 patients with PBC *vs* age- and sex-matched controls revealed that IgM was uniformly elevated in patients with PBC[64]. The increase in aminotransferase levels primarily reflects the degree of periportal and lobular necrosis and inflammation, whereas hyperbilirubinemia reflects the degree of ductopenia and biliary piecemeal necrosis[27].

NATURAL HISTORY

The natural history of PBC, including the percentage of patients requiring liver transplantation or dying, has recently become less severe due to earlier diagnosis[25], and introduction of UDCA therapy[65,66].

PROGNOSTIC MODELS

The Mayo Risk score is the most widely used and best prognostic system[67]. It is superior to the Child-Pugh score in predicting prognosis[67]. It incorporates patient age, serum bilirubin concentration, albumin concentration, prothrombin time, and degree of edema. The Mayo Risk score = 0.04 (Age) + 10.87 Log_e (Bilirubin) - 22.53 Log_e (Albumin) + 12.38 Log_e (Prothrombin time) + 10.86 (Edema score). An edema score of 0=no edema without diuretics, 1 = edema with diuretics, and 0.5 = otherwise. This risk score is used to calculate expected patient survival for up to 7 years of follow-up[68]. A Mayo Risk score of 7.8 is considered the optimal time to evaluate a patient for liver transplantation[69,70].

TREATMENT

The aim of PBC therapy is to reverse injury from bile duct inflammation to relieve symptoms, prevent disease progression, relieve laboratory abnormalities, and prevent the consequences of chronic cholestasis, including pruritus, fatigue, osteoporosis, and fat-soluble vitamin deficiencies[71]. The mechanism of action and therapeutic effects of drugs for PBC are summarized in Table 3[72,73].

Ursodeoxycholic acid

Chronic cholestasis results in intrahepatic and systemic accumulation of potentially cytotoxic bile acids that initially promote hepatocyte proliferation, but subsequently cause liver damage, and ultimately cause hepatocyte apoptosis, biliary fibrosis, and cirrhosis[74]. UDCA has three mechanisms of action: (1) protection of cholangiocytes from cytotoxicity of hydrophobic bile acids by modulating the composition of mixed phospholipid-rich micelles, reduced bile acid cytotoxicity, and, possibly, reduced concentration of hydrophobic bile acids in cholangiocytes; (2) stimulation of biliary secretion of bile acids; and (3) protection of hepatocytes against bile acid-induced apoptosis, by inhibiting mitochondrial membrane permeability transition (MMPT)[75,76]. UDCA consists of water insoluble crystals that are absorbed by passive nonionic diffusion mostly in the small intestine with a small amount absorbed in the colon, reabsorbed from portal blood *via* first-pass metabolism, conjugated mainly with glycine and taurine, and actively secreted into bile. Administration of UDCA increases bile acid saturation in bile in a dose-dependent manner, resulting in increased clearance of bile acids from blood, and reduced symptoms from cholestasis, particularly pruritus. These beneficial effects occur with the recommended daily-administered dose of 13-15 mg/kg per day which increases the solubility of bile acids in bile by 40%-50%. However, efficacy is not established beyond this recommended dose[74].

UDCA is the only therapy approved by the United States Food and Drug Administration (FDA)[77]. It is well tolerated at the recommended dose. Diarrhea occurs in 2%-9% of cases[78]. Hispanics reportedly respond less well to UDCA than non-Hispanics[79]. UDCA can occasionally cause right upper quadrant pain, and rarely hepatic decompensation, when administered to patients with end-stage PBC[78]. A prospective study of 297 Dutch patients with PBC showed that UDCA, when administered to patients with early histologic disease, significantly improved transplant-free survival (1 year = 99.7%, 5 year = 87%, and 10 year = 71%) than that predicted by the Mayo model[80]. The AASLD recommends administration of UDCA in patients with PBC with abnormal liver function tests, regardless of histological stage[36]. A meta-analysis of 1038 patients in seven randomized clinical trials with long-term follow-up demonstrated UDCA decreased the incidence of liver transplantation [odds ratio (OR) = 0.65, P = 0.01], and decreased the combined rates

of mortality or liver transplantation (OR = 0.76, P = 0.05). A meta-analysis of French, Canadian, and Mayo Clinic trials[81] demonstrated beneficial effects of UDCA on patient survival and time-to-liver transplantation. This benefit was observed in patients with moderate-to-severe disease, but not in patients with mild disease (serum bilirubin concentration < 1.4 mg/dL, stage Ⅰ or Ⅱ histological abnormalities)[81]. After liver transplantation, UDCA, compared to placebo, does not affect retransplantation rates, acute cellular rejection, or mortality related to allograft rejection[77].

UDCA protects against hepatoma development in patients with PBC, as demonstrated in a study of 930 patients[82,83]. Patients with PBC treated with UDCA experience an approximately three-fold decline in risk of hepatoma as compared to untreated patients. However, failure to improve liver function test abnormalities after one year of UDCA therapy represents a risk factor for hepatoma development[84].

Biochemical response to UDCA

A biochemical response to UDCA has been variably described as: (1) a Mayo risk score < 4.5, or serum AP level < 2 × upper limit of normal after 6 mo of treatment[85]; (2) a reduction in serum AP level to < 40% of baseline or to within normal limits at one year[86]; or (3) serum AP < 3 × upper limit of normal, AST < 2 × upper limit of normal, and bilirubin < 1 mg/dL after 1 year of UDCA therapy. Patients satisfying criterion 3 had a 90% rate of transplant-free survival at 10 years[87].

Corticosteroids

UDCA alone may not produce a biochemical response in patients with features of AIH, and such patients often require concomitant immunosuppressive therapy. Meta-analysis of seven randomized, controlled trials of patients with PBC with features of AIH showed that UDCA combined with corticosteroids significantly improved serum parameters of liver function and histologic grades, but did not significantly improve mortality or liver transplantation rate[88]. Long term use of corticosteroids can cause adverse effects, including hyperglycemia, osteoporosis, cataracts, weight gain, increased risk of opportunistic infections, *etc*.[89].

Budesonide

Budesonide is a corticosteroid that demonstrates first-pass metabolism in the liver, decreases systemic exposure to corticosteroids by 90% and reduces systemic toxicity as compared to other corticosteroids[90]. Randomized clinical trials have shown that budesonide at 6-9 mg/kg per day in combination with UDCA can improve serum biochemical parameters of liver function and liver histology, especially in patients with grade Ⅰ-Ⅲ fibrosis[91,92]. One study, however, showed only marginally significant improvement in serum AP levels with budesonide at the cost of increased systemic toxicity. This study did not, however, assess the grade of fibrosis in study patients and the finding of marginal improvement might reflect poor prognosis of PBC patients with cirrhosis[93]. Patients with cirrhosis administered budesonide have worse side effects than those without cirrhosis, and have an increased risk of portal venous thrombosis. Hence, patients with grade Ⅳ fibrosis or cirrhosis are generally not administered budesonide[94].

Obetocholic acid

Obetocholic acid, a farnesoid-X-receptor (FXR) agonist, is present in liver, intestine, kidneys, and adrenals. It plays an important role in the enterohepatic circulation of bile acids. It reduces bile acid synthesis by its action on 7 alpha hydroxylase, down-regulates bile acid uptake proteins, and increases expression of bilirubin exporter pumps[95,96]. An international, double-blind, placebo-controlled clinical trial in patients with PBC showed substantial improvement in various liver enzyme levels including AP, gamma glutamyl transpeptidase, and alanine aminotransferase with various drug doses[97-99]. A long-term, phase Ⅲ trial of obetocholic acid in UDCA-treated patients is currently in progress (EudraCT Number: 2011-004728-36)[71].

Fibrates

Fibrates can benefit patients who respond suboptimally to UDCA, as reflected by significant improvement in cholestasis, cytolysis, and pruritus after adding fibrates[71,100-105]. Its mechanisms of action are incompletely understood. Fibrates activate the peroxisome proliferator-activated receptors, and apparently stimulate multidrug resistance protein 3 located primarily in liver, which in turn protects the hepatobiliary system by inducing phosphatidylcholine transport across the bile canalicular membrane to render bile less toxic[100]. Fibrates substantially improve serum biochemical tests of liver function, especially serum AP, though improvement in survival is yet to be demonstrated.

Other therapies

As PBC presumably has an immunologic component, numerous immunosuppressants and immunomodulators other than corticosteroids have been tested for treating PBC. No evidence supports their efficacy (Table 4[106-120]). Similarly, numerous other drugs have failed to demonstrate efficacy in clinical trials and are not currently recommended[26,27].

Rituximab, an anti-CD20 monoclonal antibody, produces selective B-cell depletion that could potentially ameliorate autoimmune disease by decreasing autoantibody production and antigen presentation by B cells[121]. However, this biologic therapy has little efficacy[121,122]. A human monoclonal antibody directed against interleukin 12 (IL-12)/IL-23 (ustekinumab) is currently being investigated in PBC in a phase Ⅱ trial (EudraCT Number: 2011-000554-31)[71]. Definitive data about safety and efficacy of ustekinumab in PBC are currently lacking.

Table 4 Drugs without efficacy in primary biliary cirrhosis as demonstrated in clinical trials

Drug	Ref.
Azathioprine	[106]
Chlorambucil	[107]
Methotrexate	[108-110]
Mycophenolate mofetil	[111]
Cyclosporine	[112]
Penicillamine	[113,114]
Colchicine	[115,116]
Malotilate	[117]
Thalidomide	[118]
Silymarin	[119]
Statins	[120]

PBC-AIH overlap is treated with UDCA and immunosuppression using corticosteroids or azathioprine. These agents produce a favorable serum biochemical response[43,44,123-125]. In such patients, UDCA alone increases the rate of fibrosis[124].

Liver transplantation

Liver transplantation is the only definitive treatment for PBC. It improves survival[126]. As per the United Network for Organ Sharing (UNOS) database, between 1995 and 2006, the number of liver transplants increased by a mean of 249 per annum in the United States, but the number of liver transplants performed for PBC decreased steadily by a mean of 5.4 cases per annum[127]. In Europe, PBC is still the third most common reason for liver transplantation, with a relative rate of 9%[128]. One-, 5- and 10-year survival for PBC in Europe are 86%, 80% and 72%, respectively[128]. These rates are higher than those for patients transplanted for hepatitis B, hepatitis C, alcoholic cirrhosis, or other autoimmune liver diseases.

As per European Association for the Study of the Liver (EASL) guidelines, any PBC patient with a serum bilirubin > 5.9 mg/dL, a Mayo Risk score > 7.8, and/or a Model for End-Stage Liver Disease (MELD) score of > 12 should be evaluated for potential liver transplantation[129]. Potential liver transplant candidates should be referred early for evaluation at a liver transplantation center to determine eligibility and assure timely listing as a liver transplant candidate.

As for all patients with end stage liver disease, clinical indications for liver transplantation in PBC include refractory ascites, recurrent spontaneous bacterial peritonitis, recurrent variceal hemorrhage, hepatic encephalopathy, hepatorenal syndrome type Ⅰ, and hepatocellular carcinoma, subject to the Milan criteria[130]. Indications specific to PBC include refractory pruritus and chronic fatigue[128]. After determining that a patient is a liver transplant candidate, patients are assigned to receive donor livers from UNOS in America according to the MELD score.

The incidence of recurrent PBC after liver transplantation is about 30% at 10 years and about 40% at 15 years[127,131-133]. Median time to recurrence is 3-5.5 years. Diagnosis of recurrent PBC after liver transplantation is often challenging because AMA is persistently positive in most patient after transplantation[134], and elevated serum AP can be due to various etiologies after transplantation including acute rejection, chronic rejection, viral infection, drug toxicity, graft *vs* host disease, bile duct pathology, or hepatic vein/artery pathology, in addition to recurrent PBC. Liver biopsy is essential for diagnosing recurrent PBC[128]. Diagnostic criteria for recurrent PBC include: (1) PBC was an indication for liver transplant (obligatory); (2) graft histopathology suggests recurrent PBC, including: epithelioid granulomas, mononuclear inflammatory infiltrate, formation of lymphoid aggregates, and bile duct damage; and (3) other causes of graft failure have been excluded. Recurrent PBC is definitively diagnosed when all 3 criteria are met, including the presence of at least 3 of the 4 histologic features in criterion 2. Recurrent PBC is likely diagnosed when only 2 histologic features in criterion 2 are present[128,132]. Anti-parietal cell antibodies may be a marker for recurrent PBC[135]. There are conflicting data about whether donor's age, recipient's age, cold ischemic time, warm ischemia time, number of HLA mismatches, and specific immunosuppressive regimens are risk factors for recurrence[136-140].

The appropriate therapy for recurrent PBC is unclear. UDCA has been advocated as a therapy[141,142]. Anecdotal evidence suggests improvement in serum biochemical parameters of liver function following UDCA administration. UDCA most likely acts in recurrent PBC just like it acts on the native liver before transplantation.

SYMPTOMATIC THERAPY

Fatigue

Fatigue occurs in up to 70% of patients[55]. It is associated with excessive daytime sleepiness and autonomic dysfunction. Cerebral structural abnormalities, related to excessive manganese deposits in the globus pallidum, may be a contributing factor[143-146]. Patients with fatigue had significantly higher serum levels of manganese[143], and increased manganese deposits in the brain, likely secondary to impaired biliary excretion. Other implicated factors include elevated inflammatory cytokines (IL-1, IL-6, TNF)[147], elevated progesterone levels[148,149], and impaired peripheral muscle function[150,151]. Fatigue at clinical presentation may be associated with an aggressive clinical course, rapid progression to cirrhosis, and poor response to UDCA[61].

Fatigue severity is measured according to the fatigue impact scale (FIS)[152] or PBC-40 (PBC-40 question profile)[153]. The FIS is a detailed and cumbersome tool, which takes approximately 3 min to complete in a non-fatigued person, but may take considerably more time in severely fatigued patients. There are 40 items on the scale, each of which is scored from 0 (no problem) to 4 (extreme problem), providing a continuous scale score of 0-160[154].

There is no specific treatment for the fatigue. Some

data suggest that modafinil improves fatigue symptoms in PBC patients, without major side effects[155], but it is currently not approved by the FDA for this indication. Randomized, prospective studies are needed to establish efficacy. Some PBC patients experience a symptom complex of fatigue, symptoms of cognitive dysfunction, and social and emotional dysfunction[156]. Various agents such as ondansteron, fluoxetine, and antioxidants have been studied to ameliorate this fatigue, but none have demonstrated efficacy[147].

Pruritus

Pruritus from cholestasis is mostly generalized, associated with scratching, sometimes violent, and sleep deprivation. It may even lead to suicidal ideation in extreme cases[157]. Intensity of pruritus is not correlated with PBC severity[157]. Treatment of this pruritus involves a multifaceted, individualized approach. Proper skin care is essential. Patients with pruritus from liver disease do not have primary pruritic skin lesions. However, lesions secondary to scratching, including excoriations, and sometimes prurigo nodularis (formation of localized or generalized, eroded, excoriated, and intensely pruritic cutaneous nodules from the scratching) can occur[158]. Patients have difficulty avoiding scratching because the pain induced by the scratching often relieves the pruritus. Patients should be referred to a dermatologist to exclude primary skin diseases that can contribute to the pruritus. All potential pruritogens should be removed from the body. Cholestyramine is commonly used to treat this type of pruritis[159]. The mechanism of action of cholestyramine to relieve pruritus is incompletely understood. It is a non-absorbable resin that binds anions, including bile acids and cholesterol, in the small intestine thereby promoting their fecal excretion. Its side effects, most commonly bloating, are generally minor. Cholestyramine should be administered immediately before and after breakfast to bind pruritogens that accumulate in the gallbladder during an overnight fast and that are otherwise poured into the small bowel after breaking the overnight fast. Physical removal or plasma separation of pruritogens have been attempted to treat extreme, refractory pruritus. A transient relief from pruritus has been reported with anion adsorption and plasma separation[160], using extracorporeal liver support systems such as Prometheus or MARS (molecular adsorbent recirculating system[160-165]). Analysis of plasma removed *via* MARS from patients suffering from refractory pruritus revealed 60 proteins, one of which, SLURP1, was three times higher in samples extracted from patients with cholestasis than in those extracted from controls. Nasobiliary drainage can help relieve the pruritis by removing bile salts[166].

Pruritus may have a central component, and is associated with increased opioidergic tone[167]. Opioid antagonists, including naloxone and naltrexone, have been used to relieve the pruritus[157]. Treatment with opiate antagonists is initiated by progressively increasing the dose of intravenous infusion for several hours before introducing it orally to reduce the risk of opiate withdrawal[168-170]. If a patient exhibits signs of withdrawal during dosage increases, the dose can be maintained at the prior dose for a day or two with subsequent increases, until the pruritus is relieved.

Review of patient diaries in a clinical trial of PBC therapy revealed that sertraline was associated with relief of pruritus[171]. In a subsequent randomized, placebo-controlled study, sertraline was again associated with relief of pruritus, as determined by a visual analogue scale, and associated with improved skin appearance, as determined by physical examination[172].

Antibiotics have been used to treat the pruritus. In clinical trials, rifampicin has relieved pruritus in some patients with liver disease[173-175]. Rifampicin stimulates PXR, which induces drug-metabolizing enzymes and transporters[176,177]. A meta-analysis of controlled, randomized clinical trials demonstrated that rifampicin was generally safe, but is occasionally hepatotoxic[178,179]. Thus, follow-up of serum tests of liver function is necessary when patients are initiated on this drug, and this drug should be stopped if drug hepatotoxicity is suspected. Metronidazole may ameliorate refractory pruritus in patients with PBC[180]. It is best used as short-term therapy because long-term administration can cause peripheral neuropathy[181].

Osteoporosis

Hepatic osteodystrophy refers to metabolic bone disease in patients with chronic liver disease[182]. PBC patients have a 20%-44% prevalence of osteoporosis. The prevalence increases with disease progression, and up to 80% of patients with cirrhosis have osteoporosis. As in the general population, risk factors for osteoporosis in PBC include old age, female gender, smoking, excessive alcohol consumption, underweight physique (body mass index < 19.0 kg/m^2 in adults), early menopause (< 45 years of age), positive family history of osteoporosis, and corticosteroid therapy[182].

The mechanism of osteoporosis is unclear. PBC apparently produces metabolic changes that affect osteoprotegerin (OPG)-receptor activator of nuclear factor-κB - ligand (RANKL), the major mechanism of bone remodeling. Cirrhosis of any etiology, including PBC, impairs the function of osteoblasts, reduces the production of growth factors (especially insulin-like growth factor-1), and increases the synthesis of oncofoetal fibronectin. Unconjugated bilirubin and lithocholic acid, which accumulate in cirrhosis, may be directly toxic to bone precursors and osteoblasts. This subject has been extensively reviewed by Raszeja-Wyszomirska *et al*[182]. Malnutrition, vitamin deficiencies, especially of vitamins D and K, could also increase the risk of osteoporosis. Osteoporosis is diagnosed by dual energy X ray absorptiometry (DEXA) scans of lumbar spine and femur. These scans should be performed at disease diagnosis with surveillance annually thereafter

according to EASL guidelines or surveillance every 2-3 years as per AASLD guidelines[27].

Treatment guidelines for the osteoporosis is not established. Treatment is begun at an early phase (DEXA score: -1 SD to -2.5 SD). Patients should be advised about lifestyle modifications[183], including regular weight-bearing exercises, avoidance of smoking, alcohol, *etc*. Patients should be prescribed calcium at 1000-1500 mg/d and vitamin D at 400-800 IU/d. Bisphosphonates, particularly alendronate, are commonly administered. Alendronate at 70 mg/wk significantly improves bone mineral density after 1 year of therapy[184]. Patients should not lie down for at least 30 min after ingesting alendronate to avoid esophageal reflux or ulcers induced by this medication. In a pilot study, 9 patients administered raloxifene exhibited a slight increase in bone mineral density in the lumbar spine[185]. Therapies including zoledronic, ibandronic acid, anabolic therapy with strontium ranelate or recombinant human parathormone 1-34, and denosumab-IgG2 monoclonal antibody against RANKL have not been studied for osteoporosis in PBC.

SPECIAL SITUATIONS

Associated autoimmune disorders

Prevalence of autoimmune disorders associated with PBC ranges from 2% to 20%[44,186-190]. Sjogren's syndrome and Raynaud's syndrome are strongly associated with PBC[27]. Clinical symptoms of Sjogren's syndrome include ocular and oral dryness for > 3 mo, use of artificial tears > 3 times per day, and the need to drink liquids to swallow solid food. All PBC patients with suspicious symptoms should undergo Schirmer's test, a highly specific test for Sjogren's syndrome[191]. Patients with PBC should also undergo tests to exclude celiac disease, rheumatological disorders, and thyroid disease[192].

Management of Sjogren's syndrome includes general measures to improve eye care, such as household humidification, use of artificial tears, including hydroxypropyl methylcellulose or carboxymethylcellulose, or cyclosporine ophthalmic emulsion to increase tear production[193]. General measures to improve oral health include regular visits to the dentist, mouth rinsing with water, use of fluoride-containing toothpaste, daily dental flossing, and avoidance of eating sugars between meals[27]. Patients with xerostomia are prescribed saliva substitutes. Cholinergic agents, such as pilocarpine and cevimeline, are empirically used in Sjogren's syndrome[194]. Cevimeline, a cholinergic agent with a high affinity for M3 muscarinic receptors, relieves the perception of dry mouth and decreases the need for artificial saliva[194]. Oral candidiasis, a complication of dry mouth, requires specific antifungal therapy. Care must be exercised when swallowing esophagotoxic pills, such as potassium supplements, tetracycline, or alendronate, because of the sicca syndrome and occasional esophageal dysmotility. Drinking plenty of water while in the upright position is helpful[27]. Patients with Sjogren's syndrome may experience vaginal dryness. Vaginal lubricants, such as K-Y jelly or vaginal inserts, are helpful. Cortisone creams should be avoided. Estrogen preparations are recommended in postmenopausal women.

Pregnancy

A case control study of 267 pregnant patients with PBC and 367 healthy pregnant controls revealed that most PBC patients have uneventful pregnancies[195]. Up to 60% of patients develop post-partum flares[196]. UDCA is safe to administer during pregnancy in patients with pruritus (FDA pregnancy category B).

Hyperlipidemia

Complications of chronic cholestasis include osteoporosis (described above) and hyperlipidemia. The hyperlipidemia in PBC is, however, apparently not associated with adverse cardiovascular effects[60,197-199]. It is unusual for cholesterol-lowering agents to be needed, but statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) are a safe therapy, even when serum liver function tests are abnormal[200]. Fibrates have been used safely in some patients[201], but occasionally cause paradoxical elevations of serum cholesterol[202].

COMPLICATIONS FROM CIRRHOSIS FROM ADVANCED PBC

Patients with cirrhosis from advanced PBC are subject to all the usual complications of cirrhosis, including hepatoma development, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatorenal syndrome, and esophageal variceal bleeding. The diagnosis and treatment of these complications are briefly listed in Table 5[126,189,203-222], which includes references for further reading on these complications.

FUTURE TRENDS

The future of PBC promises to be exciting. Genetic, immunologic, and epidemiologic data should further elucidate the pathogenesis of PBC, especially in the era of genome-wide association studies and epigenetics. Such advances may help hepatologists screen and diagnose PBC early, to improve survival, and institute preventive measures to reduce exposure to environmental factors that accelerate the disease. New drugs with molecular targets, such as obeticholic acid and ustekinimab, show considerable promise. Great advances have recently been made in improving transplant-free survival and this trend should continue for the next several years. Also, the prognosis after transplantation should continue to improve with improved immunosuppression and surgical techniques. Future agents might reverse advanced

Table 5 Complications of cirrhosis or portal hypertension in patients with primary biliary cirrhosis

Complication	Special considerations in PBC	Ref.
Hepatoma	Like other cirrhotics, patients with PBC have increased risk of developing hepatomas	[189,203-205]
	In patients with PBC who have not undergone a liver biopsy to document the diagnosis of cirrhosis, hepatoma screening should be initiated when the Mayo score > 4.1	[126]
	Surveillance for hepatoma in patients with cirrhosis from PBC should be performed every six months by abdominal ultrasound or an alternative modality of abdominal imaging	[206]
Spontaneous bacterial peritonitis	Diagnosed by abdominal paracentesis revealing > 250 polymorphonuclear leukocytes/mm^3 in ascitic fluid	[207]
	Treated with a short course of multiple antibiotics, generally including either a third-generation cephalosporin or flouroquinolones	[208]
Hepatic encephalopathy	Diagnosed clinically by confusion, delirium, or stupor on physical examination, depending on degree of hepatic encephalopathy; possible presence of asterixis on physical examination; and elevated serum ammonia level in a cirrhotic patient	[209]
	Treatment options include rifaximin, lactulose, supportive care, and reversal of underlying precipitating causes, such as dehydration, infection, or gastrointestinal bleeding	[209-211]
HRS	Type 1 HRS defined as doubling of serum creatinine level, reaching a level > 2.5 mg/dL in < 2 wk. Type 2 HRS defined as a less severely elevated serum creatinine level. Must exclude other causes of renal failure, especially hypovolemia in both types of HRS	[212,213]
	Treatment includes avoidance of nephrotoxic medications; short-term trial of volume expansion; and administration of vasopressin analogues, such as terlipressin, and α-adrenergic agonists, such as norepinephrine or midodrine. Ultimate treatment for type 1 HRS refractory to therapy is liver transplantation	[213-215]
Esophageal varices	Usually occur only after Mayo score becomes > 4.1. Patients with advanced PBC can develop portal hypertension before developing established cirrhosis from nodular regenerative hyperplasia	[216-220]
	Esophageal varices usually diagnosed and graded by esophagogastroduodenoscopy	
	Specific therapies for esophageal varices include: endoscopic banding, endoscopic injection therapy, and non-selective beta-blockers. Transjugular intrahepatic shunt is recommended for refractory variceal bleeding, especially when the MELD score < 18	[221,222]

PBC: Primary biliary cirrhosis; MELD: Model for end-stage liver disease; HRS: Hepatorenal syndrome.

fibrosis in PBC, thereby reducing complications from portal hypertension and cirrhosis.

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REVIEW

Incidence, risk factors and outcome of *de novo* tumors in liver transplant recipients focusing on alcoholic cirrhosis

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Abstract

Orthotopic liver transplantation (OLT) is an established life-saving procedure for alcoholic cirrhotic (AC) patients, but the incidence of *de novo* tumors ranges between 2.6% and 15.7% and is significantly increased in comparison with patients who undergo OLT for other etiologies. Tobacco, a known carcinogen, has been reported to be between 52% and 83.3% in AC patients before OLT. Other risk factors that contribute to the development of malignancies are dose-dependent immunosuppression, advanced age, viral infections, sun exposure, and premalignant lesions (inflammatory bowel disease, Barrett's esophagus). A significantly more frequent incidence of upper aerodigestive (UAD) tract, lung, skin, and kidney-bladder tumors has been found in OLT recipients for AC in comparison with other etiologies. Liver transplant recipients who develop *de novo* non-skin tumors have a decreased long-term survival rate compared with controls. This significantly lower survival rate is more evident in AC recipients who develop UAD tract or lung tumors after OLT mainly because the diagnosis is usually performed at an advanced stage. All transplant candidates, especially AC patients, should be encouraged to cease smoking and alcohol consumption in the pre- and post-OLT periods, use skin protection, avoid sun exposure and over-immunosuppression, and have a yearly otopharyngolaryngeal exploration and chest computed tomography scan in order to prevent or reduce the incidence of *de novo* malignancies. Although still under investigation, substitution of calcineurin inhibitors for sirolimus or everolimus may reduce the incidence of *de novo* tumors after OLT.

Key words: *De novo* malignancies; *De novo* tumors tobacco consumption; Alcoholic cirrhosis; *De novo* cancer; Liver transplant

Core tip: Incidence of *de novo* tumors is significantly increased in patients who undergo liver transplantation for alcoholic cirrhosis. The association of alcohol and tobacco consumption and immunosuppression contribute to the development of *de novo* malignancies, mainly located in upper aerodigestive tract, lung and skin.

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INTRODUCTION

The occurrence of *de novo* tumors is considered the second cause of late mortality after orthotopic liver transplantation (OLT)[1-3]. Initially, similar frequency of *de novo* tumors (carcinomas of the lung, prostate, breast, colon, and uterine cervix) was published among transplant recipients in comparison with the non-transplant population[4]. Subsequently, a higher incidence of posttransplant lymphoproliferative disease (PTLD) and skin cancer was established in OLT patients *vs* non-immunosuppressed population[2,5-7]. Moreover, the incidence of other tumors is controversial so that, depending on the series of OLT, an increased incidence of upper aerodigestive (UAD) tract[1,5,6,8-16], colon[5,6,17], and kidney tumors[5] can be found. It has been reiterated that the most important contributing factors for increased incidence of *de novo* tumors are the long period of follow-up of the recipients and the presence of risk factors, such as abuse of alcohol and tobacco, sun exposure, overimmunosuppression, advanced age, inflammatory bowel disease, hepatitis B virus (HBV) and hepatitis C virus infections, Epstein-Barr virus, herpes virus 8, and human papilloma virus[5,8,11,14,18-21]. Alcoholic cirrhosis (AC) constitutes the leading cause of end-stage liver disease in Western countries, and many of these patients may potentially benefit from OLT if they fulfill the usual criteria for this technique[22]. However, the OLT patients for AC show an increased incidence of *de novo* malignancies after transplant[9,11-16,23-32].

The objective of this review is to analyze the incidence, risk factors, location and characteristics of *de novo* tumors in AC patients who underwent OLT, and also to evaluate the prognosis and survival after diagnosis of malignancies.

RESEARCH

We performed MEDLINE search considering the most important series related with *de novo* tumors after OLT that were reported in English literature. We analysed the incidence of *de novo* malignancies in OLT recipients in comparison with the non-transplant population as control group, and also we will mainly focuss on the different series which studied the incidence, risk factors and predisposing conditions for developing *de novo* tumors, locations, survival after diagnosis, surveillance, and immunosuppression changes as prevention or therapeutics measures for control these tumors. In addition, we analysed the incidence of *de novo* tumors in comparative studies between alcoholic and non-alcoholic recipients of OLT.

INCIDENCE

Overall incidence of *de novo* tumors after OLT ranges between 2.6% and 33.6%[1,2,5-8,11,12,16,17,25,26,32-44] (Table 1). The disparity of *de novo* tumors incidence among these series is attributed to exclusion of some type of tumors, such PTLD in the study of Saigal *et al*[26] (incidence of 2.6%). The increased incidence of *de novo* tumors after OLT is mainly due to the intensive surveillance, and the life-long immunosuppressive therapy the transplant recipients receive[1,5]. In our series of 528 adult transplant recipients with a mean follow-up of 6.7 years, the cumulative risks for development of non-cutaneous malignancies at 5, 10, and 15 years post-OLT, were 9%, 18%, and 25%, respectively[45]. A recent series reports a 15-year cumulative incidence of *de novo* tumors of 34.7% as compared to 8.9% for the non-transplant population, and emphasizes the continuously increasing incidence of tumors over time following OLT[44]. The mean interval between OLT and tumor diagnosis was reported to be between 19.2 and 82.7 mo, and the mean age of recipients at the time of diagnosis was between 53 and 59.5 years[14,32,37,38,44]. A significantly higher incidence has been observed in patients who underwent OLT for AC in comparison with other non-AC diseases[9,11-13,15,23,24,26,28,29] (Table 2). In our series of 701 adult recipients of OLT, the incidence of *de novo* tumors in AC patients was significantly higher (25% in AC patients *vs* 9.4% in non-AC recipients; $P < 0.001$)[28].

RISK FACTORS AND PREDISPOSING CONDITIONS FOR *DE NOVO* TUMORS AFTER OLT FOR AC PATIENTS

Recipient age

Liver transplant series showed recipients older than 40 years[5], and older than 51 years old at the time of OLT[25] as having independent risk for *de novo* malignancies. In two recent studies it was also observed that older age and smoking were independently associated with a higher risk of malignancy[38,46], especially lung, head and neck, kidney and urinary tract[46].

Tobacco and alcohol consumption

Prevalence of tobacco use among the non-transplant population is between 20% and 30%, and as high as 40% in OLT recipients[47].

Smoking fewer cigarettes over a long period seems more damaging than smoking more cigarettes over a shorter period[47]. Tobacco discontinuation is usually required for heart and lung transplantation candidates, but in OLT candidates the requirement of smoking discontinuation is less clear and remains controversial. The association of alcohol and tobacco consumption

Table 1 Overall incidence of *de novo* tumors after liver transplantation *n* (%)

Ref.	No. OLT	Follow-up period	Time from OLT-DNT	Pts with DNT	DNT incidence	Overall SIR
Jonas *et al*[18]	458	50/22 mo	43 mo	33	34 (7.2)	-
Jain *et al*[1]	1000	78 mo	36 mo	57	58 (5.7)	7.6 (UAD)
Kelly *et al*[33]	888	29.3 ± 25.2 mo	24 ± 16.8 mo	39	43 (4.8)	-
Peyrègne *et al*[8]	251	50.5 mo	24.3 mo	11	12 (4.8)	-
Galve *et al*[34]	1827	20 ± 18 mo	30.7 ± 22 mo	70	70 (3.8)	-
Sheiner *et al*[2]	121	499 persons/yr	19.2 mo	18	19 (15.7)	3.9
Haagsma *et al*[5]	174	61 mo	-	21	23 (13.2)	-
Xiol *et al*[25]	137	69 mo	12-104 mo	22	30 (21.9)	-
Jiménez *et al*[35]	505	8-168 mo	47.8 mo	57	62 (12.2)	-
Saigal *et al*[26]	1140	69 mo	45 mo	30	30 (2.6)	-
Sanchez *et al*[6]	1421	67 mo	-	125	125 (8.8)	-
Benlloch *et al*[11]	772	51 mo	40 mo	41	41 (5.3)	-
Herrero *et al*[36]	187	65 mo	49.5 mo	49	63 (33.6)	-
Oo *et al*[12]	1778	-	57 mo	141	141 (7.9)	2.07
Aberg *et al*[7]	540	3222 persons/yr	61 mo	47	50 (9.2)	2.59
Jiang *et al*[17]	2034	-	42.2 ± 33.8 mo	113	113 (5.5)	2.5
Baccarani *et al*[37]	417	81.6 mo	51 mo	43	43 (10.3)	2.7
Chatrath *et al*[38]	534	68 ± 38.4 mo	48 ± 26.4 mo	73	80 (14.9)	3.1
Collett *et al*[39]	6846	-	-	-	-	2.2
Tjon *et al*[40]	385	> 4 mo	-	50	66 (17.1)	2.2
Engels *et al*[41]	6291	-	-	-	-	2.2
Krynitz *et al*[42]	1221	61.2 mo	-	150	150 (12.3)	3.4
Sampaio *et al*[43]	43216	-	31.2 mo	1923	1923 (4.4)	-
Ettorre *et al*[32]	1675	62.4 mo	38.4 mo	98	100 (5.9)	1.4
Schrem *et al*[16]	2000	-	82.7 mo	115	120 (6)	1.94
Wimmer *et al*[44]	609	57.3 mo	68.4 ± 44.4 mo	71	87 (14.3)	-

OLT: Orthotopic liver transplantation; DNT: *De novo* tumors; UAD: Upper aerodigestive tumor.

Table 2 Comparative studies between alcoholic cirrhotic and non-alcoholic cirrhotic patients who underwent orthotopic liver transplantation, incidence of *de novo* tumors *n* (%)

Ref.	No. OLT	Follow-up period	DNT overall incidence	DNT-AC	DNT-non-AC	*P*-value	DNT excluded
Duvoux *et al*[9]	90	45.2 ± 21.2 mo	11 (12.2)	8 (26.7)	3 (5)	0.01	-
Jain *et al*[23]	834	9 4 ± 11 mo	81 (9.7)	36 (19.4)	45 (6.9)	< 0.05 (UAD)	-
Bellamy *et al*[24]	513	81.7 mo	57 (11.3)	33 (26)	24 (6.1)	0.0001	PTLD
Saigal *et al*[26]	1140	69 mo	30 (2.6)	10 (7.5)	20 (1)	0.001	-
Benlloch *et al*[11]	772	40 mo	41 (5.3)	18 (9.4)	23 (3.9)	0.01	Skin DNT
Oo *et al*[12]	1778	-	141 (7.9)	15 (8.8)	126 (7.8)	0.001	-
Dumortier *et al*[13]	594	-	42 (7)	37 (12.1)	5 (1.7)	0.05	-
Jiménez *et al*[28]	701	9-206 mo	109 (15.5)	69 (25)	40 (9.4)	0.001	-
Biselli *et al*[29]	147	-	11 (7.5)	7 (14.3)	4 (4)	0.042	-
Zanus *et al*[15]	638	48 mo	43 (6.3)	16 (11)	27 (5)	0.02	-

AC: Alcoholic cirrhosis; OLT: Orthotopic liver transplantation; PTLD: Post-transplant lymphoproliferative disease; DNT: *De novo* tumors; UAD: Upper aerodigestive tumor.

has been published to be as high as 90% in alcoholic patients[48,49]. It has been documented that 52% of AC patients were active smokers before OLT and 44% after OLT[50]. In our series of OLT for AC, 83.3% of patients were smokers *vs* 43% of non-AC patients[28]. Smokers show an increased risk of cardiovascular disease, stroke and cancer[51]. Moreover, tobacco consumption has also been associated with squamous cell carcinoma (SCC) of the skin in the non-transplant population[52] and OLT recipients[20,27]. In addition, malignancies seem to develop much earlier after OLT in tobacco users[50]. In a comparative study among active smokers, ex-smokers, and non-smokers who underwent OLT, a significantly increased cardiovascular-specific mortality and sepsis mortality but not malignancy-related mortality was demonstrated in the active smokers group[53]. On the other hand, other authors showed a significantly higher 10-year cumulative rate of *de novo* tumors in active smokers (12.7%) *vs* non-smokers (2.1%), but without an effect of smoking on skin cancer or cardiovascular disease[50]. Liver transplant recipients who ceased smoking had a lower incidence of such tumors in comparison with patients who continued to smoke[46].

A synergistic effect has been demonstrated when patients are exposed to combined alcohol and tobacco consumption, resulting in a more than 7-fold increased risk of tumors[54,55]. In the general population, tobacco and alcohol abuse are well-known risk factors for oral,

pharyngeal, laryngeal, esophageal, upper airway, bladder and cervix tumors[54,56-60]. In a more recent review of the non-transplant population, a causal association was established between alcohol intake and cancers of the oral cavity, pharynx, larynx, esophagus, liver, colon, rectum, and in women, breast[61,62]; an association is suspected for cancers of the pancreas and lung[61]. However, the carcinogenic effects of alcohol have not been fully defined and probably differ by target organ. Alcoholic drinks might act as a solvent for carcinogens (*e.g.,* tobacco-derived), facilitating penetration through the mucosa of the upper aerodigestive organs[63]. Heavy alcohol intake seems to affect folate metabolism which changes DNA methylation and the control of expression of genes with a potential role in carcinogenesis (colon, rectum and breast)[64]. For breast cancer, alcohol carcinogenicity is thought to be due to increased estrogen concentration[65]. Production of reactive oxygen species and nitrogen species is a possible mechanism of alcohol-related liver carcinogenesis[66].

Alcoholic cirrhotic patients have a longer history of tobacco use than the general population[67] and also a higher tobacco consumption than patients undergoing OLT for other etiologies[9,35]. Multivariate analysis of a series from the Mayo Clinic showed an increased probability of developing any solid organ *de novo* malignancy with increased age, a history of smoking, and AC or primary sclerosing cholangitis as indications for OLT[14]. In our experience, there is a significantly higher incidence of *de novo* tumors (overall and partial incidence of skin, upper aerodigestive, and lung tumors) in AC patients compared with non-AC patients, a feature that is also related with a significant consumption of alcohol and tobacco in the AC group[27,28,35].

After 10 years of smoking, OLT recipients presented a significantly higher risk of non-skin tumors[51]. It has also suggested that alcohol abuse can produce genetic alterations that potentiate those induced by tobacco smoke[60], and tobacco can also alter the cellular immune system by decreasing the number of natural killer cells[68,69].

The association of immunosuppressive drugs and smoking may have adverse additive effects, mainly in liver transplant patients for AC with a long history of alcohol and tobacco abuse, where there has been a demonstrated higher incidence of UAD and lung *de novo* tumors[1,9-11,14,18,23-25,27,28,45,46,70,71] and even bladder[46] or skin tumors[23,27].

Infections

Kaposi's sarcoma (KS) is a tumor exclusively seen in the immunocompromised patients and is clearly related with type 8 human herpes virus[72]. Human papillomavirus increases the risk for anal, genitourinary, oropharyngeal, and skin tumors, and even for cervical cancer in kidney transplant recipients[73]. PTLDs are associated with Epstein-Barr virus-infected B-lymphocytes and have been reported to have an incidence between 1.7% and 4% after OLT[21,74]. The incidence of PTLD is lower in OLT recipients in comparison with other solid organ transplants[74].

Immunosuppression

The influence of immunosuppression on the development of *de novo* tumors has been directly related to the intensity as well as the cumulative dose of immunosuppressive drugs[75].

Cyclosporine (CyA) and tacrolimus promote the spread of tumors in immunodeficient mice, probably by increasing the production of growth factors that enhance angiogenesis, tumor growth and metastasis[76]. The pathogenic process triggered by immunosuppressors consists of direct damage to the host DNA and impairment of the recipient's immunosurveillance, which reduce their antitumor and antiviral immunity[77,78]. A retrospective study suggested a dose-dependent immunosuppressive drug relationship with *de novo* tumor development[11]. By contrast, other authors did not find that immunosuppression is an independent risk factor for *de novo* malignancy[26]. Several studies found a higher *de novo* tumor risk for CyA-based[33,38,45] or tacrolimus-based[36,44], whereas others did not observe significant differences between CyA- and tacrolimus-based immunosuppressive therapy[18,38,79]. It was pointed out that CyA therapy increased malignancy risk when C_2 monitoring (blood concentration at 2 h post-dose) was performed and the patient consequently received a significantly higher CyA dose[40]. Azathioprine has also been described as an independent risk factor for higher incidence of *de novo* malignancy, mainly due to inhibition of DNA repair, and its metabolite 6-thioguanine has been shown to accumulate in skin cells *in vitro*[80]. A recent report revealed that standardized incidence ratio for *de novo* tumors were similar for patients who received tacrolimus- or CyA-based immunosuppressive protocols as long-term immunosuppression (mean = 7.4 years) with or without mycophenolate mofetil, azathioprine, or prednisolone as a co-medication[16]. As summary, there are no randomized control studies designed to evaluate the influence of different immunosuppression schedules over the development of post-OLT *de novo* malignancies.

Premalignant conditions

Barrett's esophagus is a premalignant condition with increased risk for development of esophageal tumor; a rapid progression to high-grade of dysplasia has been reported after OLT[81], as well as the development of esophageal carcinoma[82].

There has been suggested an increased incidence in colorectal *de novo* tumors in patients with ulcerative colitis or sclerosing cholangitis who underwent OLT[19,83].

Screening for premalignant conditions should be performed in a pre-OLT evaluation, and patients with evidence of a premalignant state should be followed carefully after OLT for detection of malignancy[84]. Because of the increased incidence of *de novo* tumors

Table 3 Location and risk factors for the most frequent *de novo* tumors in patients who underwent orthotopic liver transplantation for alcoholic cirrhosis

Tumor location	Risk factors
UAD	Alcoholic cirrhosis[9-11,13-15,23,24,30]
	Tobacco consumption[9,14,38,46]
	Barrett's esophagus[82]
Lung	Alcoholic cirrhosis[14,28]
	Tobacco consumption[1,28,38,46]
Skin	Alcoholic cirrhosis[24,26,27]
	Tobacco consumption[20,27,51]
	Age > 40 yr[20] or age > 51 yr[25]
	Male, red hair, brown eyes[20]
	Sun exposure[20,36]
	Sclerosing cholangitis[20]
	CyA immunosuppression[20]
Kidney and genitourinary tract	Tobacco consumption[46]

CyA: Cycosporine A; UAD: Upper aerodigestive tumor.

in AC (frequently smokers), these candidates should be subjected to thorough evaluation to rule out tumor or premalignant condition before OLT[15]. Thus, these patients should be screened for oropharyngeal/laryngeal, esophageal, lung, bladder, and skin tumors as the most frequent tumors associated with alcohol and tobacco consumption.

LOCATION OF *DE NOVO* TUMORS IN OLT RECIPIENTS FOR AC

We analyzed the subset of *de novo* tumors that are usually developed in patients who undergo OLT because of AC. Thus, a significantly more frequent incidence has been published of UAD tract, lung, skin, and bladder tumors in liver transplant patients for AC. PTLD is the second most frequent *de novo* tumor after OLT, but it is not associated with AC. Other types of solid tumors, such as gastric, pancreatic, colorectal, prostate, breast, and uterine cervix tumors show a similar incidence after OLT for AC and non-AC recipients. Risk factors for most frequent *de novo* tumors after OLT for AC are shown in Table 3.

Upper aerodigestive tract tumors

In this group a subset of tumors located in the floor of the mouth, tonsil, tongue, pharynx, larynx and esophagus is included, which are significantly more frequent in male and smoker recipients who underwent OLT for AC[1,9,10,14-16,23,24,30,33,36]. In some series, the most common solid tumors were tumors of the UAD tract[32,38]. The incidence of UAD tumors in OLT recipients was published to be between 0.3% and 3.5% in several series[1,6,10,11,18,33]. The risk of the development of oropharyngeal/laryngeal malignancies was highest in AC patients, with 5- and 10-year risks of 3.2% and 4.6%, respectively, *vs* 0.16% and 0.32%, respectively, for non-AC patients[14]. In patients who underwent OLT for AC, the rate of UAD tumors was 25.5 times higher than in patients with other etiologies[23]. The mean time from OLT to diagnosis of UAD tract tumors was reported to be between 24 and 62 mo after OLT[1,6,10,11,18,33]. In one series, UAD tract malignancies occurred exclusively in patients transplanted for AC[9]. In one of our studies, the incidence was also significantly higher in the AC group (8.1%) *vs* the non-AC group (0.8%), and among the patients who suffered UAD tract tumors 70% were heavy smokers and 75% had a history of heavy drinking[10]. In patients who underwent OLT for AC, immunosuppressors may enhance the effects of alcohol and tobacco, which are well-known risk factors for the development of UAD tract tumors[85,86].

The pathogenic mechanism of esophageal carcinoma remains unclear. However, experimental studies in animals suggest that oxidative damage from smoking and alcohol intake, or gastroesophageal reflux, which produces inflammation, esophagitis, and increased cell turnover, might initiate the carcinogenic process[87]. Esophageal *de novo* tumors have been diagnosed between 8 and 96 mo after transplant in patients who smoke and who underwent OLT for AC[8,88,89]. In a German series[89] of 10 *de novo* esophageal tumors, diagnosed at a mean time of 51 mo after OLT, all patients were males, 9 underwent OLT for AC and 1 for hepatocarcinoma, 3 were smokers, 9 were immunosuppressed with CyA, whereas 7 patients revealed SCC and 3 adenocarcinoma. Five of these patients were treated with chemo-radiotherapy and the other 5, who had a better general condition, underwent Ivor Lewis esophagectomy. In our published experience of 5 patients with *de novo* esophageal SCC, all were male and smokers, and underwent OLT for AC; diagnosis of SCC was performed at a mean time of 36 mo after OLT, and four patients were treated by transhiatal esophagectomy, showing a 3-year patient survival of 40%[90]. In spite of the elevated risk of OLT patients who suffer esophageal tumors, the mortality after surgical resection was reported as zero in both series[89,90]. To date, there is no experience of neoadjuvant chemo-radiotherapy associated with esophagectomy for the treatment of esophageal tumors after OLT, but the results for surgery are comparable with non-transplant patients who present with an esophageal cancer[89,90].

Lung tumors

The incidence of *de novo* lung tumors after OLT is increased, and ranges between 0.1% and 2.4%[1,11,18,25,28,36,38,91,92]. Our incidence of these malignancies is 40-fold higher than that of the non-transplant population in Spain[28]. The main risk factors for post-OLT lung tumors are the longer time elapsed since transplant[3], AC as indication for OLT[1,11,24,28,32,46], and a long period of tobacco consumption[1,14,28,36,38,91,92]. The risk of developing a lung cancer was highest in AC patients, with 5- and 10-year risks of 2.0% and 4.8%, respectively, compared to non-AC patients with 0.15% and 1.3%, respectively[14].

In several small series[91-93] all patients with *de novo*

lung malignancies had indicated the antecedent of heavy smoking, and this addiction was also present in 62.5% of patients in Pittsburgh series[1] and in 83.3% in our series[28]. According to our experience, the continuation of smoking after OLT represents an additional risk factor for lung cancer[28]. The mean time from OLT to tumor diagnosis was reported to be between 42 and 83 mo[1,6,18,28,92]. The mean age of our patients at the time of lung tumor diagnosis was significantly lower than a Spanish non-transplant population[94].

Patients with lung tumors after OLT show similar symptoms to non-transplant patients[1,28,93]. These lung tumors are usually diagnosed at advanced stages[1,18,28,36,95] in almost two-thirds of cases[28,93]. To obtain early diagnosis of a potentially curable stage of lung cancer, the goal is to perform screening with a computed tomography (CT) scan every year of recipients at increased risk, especially in the subset of older recipients, over-immunosuppressed patients and smokers of more than 20-30 pack-year who have undergone OLT for AC[28,46,93,95-98].

According to OLT series[1,18,28,33,91], lung tumor resection can only be performed at early stages (Ⅰ or Ⅱ) and when the patients are in good general condition. In several reported series[1,28,33,36,91-93], there is little information about surgical resection of *de novo* lung tumors after OLT, but among 58 collected cases of these series there were only 13 resected cases (20.6%). In unresectable patients, palliative chemo and/or radiotherapy is an alternative option[1,8,18,28,93].

Skin tumors

Nonmelanoma skin cancer is the most common tumor in the post-OLT population, with an up to 70 times higher incidence in comparison with non-transplant patients[5,10,25,99-101]. Tobacco constitutes a risk factor for skin malignancies in non-transplant patients[53]. The overall incidence in most OLT series ranges from 1% to 6.9%[1,5,11,18,27,33]. Skin tumors represent between 16% and 55% of all tumors and can develop at any time after OLT[20,27,36,40]. The cumulative incidence of nonmelanoma skin malignancies 5, 10, and 15 years after OLT has been reported to be 5.1%, 10.2% and 19.7%, respectively[40].

A long history of sun exposure, personal or family history of actinic keratosis or skin cancer, human papillomavirus, male sex, patient age, red hair, brown eyes, primary sclerosing cholangitis, hepatocarcinoma[14,20,25,36,102], AC and smoking[1,24,27] are described as risk factors for the development of skin tumors. The most frequent sites for *de novo* skin tumors were the face, lips, head, neck, and ears[1,20,27]. As in the non-transplant population, in some series the most common histologic tumor type was basal cell carcinoma[27,103], in contrast to other experiences where SCC was the most frequent[1,20]. Immunosuppressors increase the risk of skin cancer, but whether CyA shows a higher risk in comparison with tacrolimus is unclear. Thus, in one series CyA-treated patients have been associated with a higher incidence and earlier development of skin tumors[20], but another series failed to find significant differences between CyA and tacrolimus[18]. Nonmelanoma skin cancer does not affect mortality[40].

The incidence of KS ranges from 0.14% to 2.8% after OLT[99]. Viral infections, such as HBV, cytomegalovirus, and Epstein-Barr virus infections have been reported as risk factors for KS, but the main risk factor is human herpes virus 8[104,105]. Kaposi's sarcoma has been related with the degree of immunosuppression, and the lesions disappeared after immunosuppressive drugs were discontinued[106]. Almost all reported cases of KS are located on the skin, but some visceral cases with bad prognosis have also been described[72]. Full-skin examination may detect every kind of skin malignancy, such as melanoma, nonmelanoma cancer, KS or cutaneous lymphoma[107].

Genitourinary and gynecological tumors

Regular and current cigarette smokers in the non-transplant population have a higher risk of bladder cancer than those who never smoked, and there is a statistically significant dose-response relationship in bladder cancer risk between smoking duration, intensity and pack-year consumption[48]. People who discontinued smoking for 20 years or more remain at a higher risk of bladder cancer than people who never smoked, suggesting an early-stage irreversible effect of cigarette smoke[108,109].

Considering the frequent consumption of tobacco among AC patients, the incidence of bladder tumors after OLT must be increased in this group of patients. However, there is little information about the increased incidence of bladder tumors in smokers, except for a recent report where smoking and older age were associated with a higher risk of urinary tract and kidney tumors[46]. An earlier series of OLT reported a 30-fold increase in *de novo* kidney tumors[5].

It appears that the rate of non-prostate genitourinary tumors is increased in OLT patients, but the rate of prostate cancer may be comparable to that in the non-transplant population[31]. The incidence of non-prostate genitourinary cancer in OLT patients ranges between 0% and 0.4%[11,91]. Other authors did not find an increased incidence of breast, cervix or bladder tumors in OLT compared with the non-transplant population[12,32].

SURVIVAL AFTER DIAGNOSIS OF *DE NOVO* TUMORS

The increased mortality associated with *de novo* tumors is thought to be the consequence of aggressive immunosuppression that may give rise to increased proliferation and spread of the tumor, which in turn results in more advanced stages of disease at presentation, precluding surgical or chemo-radiotherapy options[14].

Patients with *de novo* non-skin cancer after OLT have diminished long-term survival in comparison with controls[23,37,40,110]. Aerodigestive tract malignancies

after OLT are greater causes of morbidity and mortality than recurrent alcohol liver disease[10,13]. *De novo* cancer-related death accounted for 21% of all deaths in patients surviving more than six months after OLT, and post-OLT survival was significantly lower in patients who developed *de novo* malignancy in comparison with patients without cancer (70% *vs* 82% at five years)[38]. In a series of 21 UAD and lung tumors diagnosed in 20 OLT recipients, 1-, 2-, and 3-year survival rates were 47.6%, 37.0% and 19.7%, respectively[10]. Moreover, in our series of 15 *de novo* lung tumors all patients died, and mean survival after tumor diagnosis was only 5.4 mo[28]. A recent study considering smoking-related malignancies (lung, head and neck, esophageal, kidney and urinary tract tumors) reports a significantly higher mortality in OLT recipients *vs* the non-transplant patients[46].

Once the tumor was diagnosed, and according to the specific site of the *de novo* tumors, the probability of death at 1 and 5 years was 33% and 48%, respectively, for gastrointestinal tumors; 59% and 84%, respectively, for lung tumors; 22% and 44%, respectively, for oropharyngeal/laryngeal tumors; and 21% and 29%, respectively, for genitourinary tumors[14].

SURVEILLANCE OF TRANSPLANT PATIENTS FOR AC

While smoking and alcohol use, age and existence of premalignant conditions generate suspicion for *de novo* tumor development, prevention and screening after OLT is of paramount importance[14,30,31]. Thus, pre-OLT screening is advised for candidates with Barrett's esophagus[111]. Moreover, all transplant patients who undergo OLT for AC and have a long history of smoking must be carefully reviewed for malignancy in the post-OLT setting, particularly in the oropharyngeal, laryngeal, lung[14,28,30,32,46], esophageal[46], skin[27], and kidney-bladder locations[46].

Periodic patient controls in outpatient clinic and patient education on the importance of preventive screenings are of vital importance. Thus, all OLT candidates should be encouraged to cease smoking and alcohol intake (for a minimum period of 6 mo to be included on the transplant waiting list). After OLT for AC, the recipients should continue with complete alcohol abstinence, avoidance of tobacco consumption, using sun protection with sunscreen and limiting sun exposure, undergo regular skin assessments, and routinely adhere to cancer screening tests[112,113]. Annual oto-pharyngo-laryngeal evaluation is advised in order to obtain early tumor detection[113]. Smokers of more than 20 pack-years who are actively smoking or have ceased tobacco abuse less than 10 years before OLT should be subjected every year to otolaryngeal evaluation and low-radiation CT scan[114]. Other authors only recommend annual chest X-rays for lung tumor screening[113].

IMMUNOSUPPRESSION CHANGES AS PREVENTION OR TREATMENT OF *DE NOVO* TUMORS

In long-term follow-up the maintenance drugs (CyA and tacrolimus) are associated with side effects such as cardiovascular complications, nephrotoxicity, neurotoxicity, diabetes, hepatocarcinoma recurrence, and the development of *de novo* malignancies[1,115,116]. The main objective is to get effective immunosuppression, while no promoting cancer development[117].

Higher degrees of immunosuppression increase the risk of tumor after transplant in a dose-dependent manner[118]. Recently, two immunosuppressive drugs, mycophenolate mofetil[119,120], and the inhibitors of mammalian target of rapamycin (mTORi: sirolimus and everolimus)[121-123] have shown protective effects against the development of cancer. However, there are no published controlled trials evaluating the effect of mTORi in preventing *de novo* tumors or recurrence of hepatocarcinoma after OLT[117,124].

Although much additional research is needed, several studies indicate that m-TORi may be effective in the prevention of malignancies, since a significantly reduced incidence of *de novo* malignancies was demonstrated when rapamycin was used alone or in combination with a reduced dose of CyA or tacrolimus[125], or combined with steroids only[126]. In addition, there is clinical evidence of the ability of sirolimus to suppress cancer progression in humans, as has been demonstrated in several kidney transplant series, 2 cases of complete remission of cutaneous Kaposi's sarcoma[127], and 12 cases of remission of *de novo* lymphoma[128]. A multicenter prospective clinical trial assessing the effectiveness of mTORi in avoiding the development of malignancies after OLT is currently under way in patients transplanted for hepatocarcinoma[129].

Nevertheless, the remarkable reduction of all *de novo* posttransplant malignancies and the excellent regression/control of the most common tumors in the early stages with mTORi immunosuppression is a strong reason to expand the role of mTORi in maintenance immunosuppressive therapy[130]. Immunosuppression protocols using sirolimus or everolimus monotherapy to replace calcineurin inhibitors (CNI) in patients who underwent OLT for hepatocarcinoma or who developed *de novo* tumors have been recommended because of their antitumor properties, absence of nephrotoxicity, well tolerated adverse events, and potent immunosuppressive effect, which prevents rejection, especially in recipients with long-term follow-up who have developed some tolerance[123,131-134]. In our preliminary experience using sirolimus monotherapy in 16 patients who developed post-OLT malignancies we did not see any case of acute rejection during a mean follow-up of 15.7 mo. The mean period elapsed from OLT to sirolimus monotherapy was 86 mo, and the mean

trough level of sirolimus was 8.9 ng/mL[131]. Recently, we published our experience of 57 patients using everolimus monotherapy (24 patients) or everolimus combined with low doses of CNI (33 patients) mainly in patients who underwent OLT for hepatocarcinoma (monotherapy, 9 patients; combined, 21 patients), or who developed *de novo* malignancies after OLT (monotherapy, 13 patients; combined, 6 patients); we observed only one case of acute rejection, improved renal function, and good tolerance of adverse effects[123]. In summary, independently of their antineoplastic efficacy, sirolimus and everolimus, in combination with low doses of CNI or as monotherapy at least one year after OLT, are safe and effective immunosuppresive drugs, which may be especially indicated in patients who underwent OLT for hepatocarcinoma or who are at high risk for development of *de novo* tumors (premalignant lesions, smokers or patients transplanted for AC).

CONCLUSION

Liver transplant recipients for AC are at higher risk than recipients with other etiologies for the development of post-OLT tumors, mainly due to frequent association of alcohol and tobacco consumption among these patients. *De novo* tumors related to AC patients are mainly located in the UAD tract, lung, skin and bladder-kidney. With the exception of skin tumors, these malignancies have very poor prognosis. Thus, strict surveillance (otolaryngeal exploration and yearly chest CT scan), and avoidance of alcohol and tobacco consumption should be advised to AC recipients in order to prevent the development of *de novo* malignancies or to obtain an early tumor diagnosis. Although the clinical antineoplastic efficacy of mTORi is not yet unambiguously demonstrated, a decreased CNI dose or substitution with mTORi after a minimun period of one year after OLT has been proposed in order to avoid hepatocarcinoma recurrence or the development of *de novo* tumors.

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REVIEW

Hepatitis B reactivation in the setting of chemotherapy and immunosuppression - prevention is better than cure

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Abstract

Due to the inherent relationship between the immune system and the hepatitis B virus (HBV) in exposed and infected individuals, immunomodulation associated with the treatment of solid tumours, haematological malignancies and inflammatory disorders has been linked to HBV reactivation (HBVr). Reactivation of HBV infection in the setting of chemotherapy and immunosuppression may lead to fulminant liver failure and death, but there is a cumulative body of evidence that these are potentially preventable adverse outcomes. As chronic hepatitis B is largely asymptomatic but also endemic worldwide, clinicians caring for patients requiring chemotherapy or immunosuppression need to be vigilant of the potential for HBVr in susceptible individuals. Serological screening and prophylactic and pre-emptive antiviral treatment with a nucleos(t)ide analogue should be considered in appropriate settings. Hepatitis B prevalence is examined in this review article, as are the risks of HBVr in patients receiving chemo- and immunosuppressive therapy. Recommendations regarding screening, monitoring and the role of antiviral prophylaxis are outlined with reference to current international associations' guidelines and the best available evidence to date.

Key words: Immunosuppression; Hepatitis B; Hepatitis B virus reactivation; Prophylaxis; Lamivudine; Chemotherapy; Entecavir; Tenofovir; Rituximab

Core tip: Hepatitis B virus reactivation is a potentially fatal but preventable complication of chemotherapy and immunosuppression. Both chronically infected [hepatitis B surface antigen (HBsAg) positive] and previously exposed (HBsAg negative/anti-HBc positive) patients are susceptible, the risk observed to be strongly associated with the potency of the immunosuppressive drug regime and the baseline virological status. The knowledge gaps that require further investigation in the optimal management of this phenomenon are discussed in this review. Recommendations regarding screening, monitoring and the role of antiviral prophylaxis are outlined with reference to current international associations' guidelines and the best available evidence to date.

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HEPATITIS B EPIDEMIOLOGY

It is estimated that 2 billion people have been infected with hepatitis B worldwide; of these, 350 million of these are chronically infected [chronic hepatitis B (CHB)][1]. Seventy-five percent of the chronically infected reside in the Asia-Pacific region, where the disease is endemic[2,3]. Across the globe, northern, western, and central Europe, North America and Australia have the lowest prevalence of chronic hepatitis B virus (HBV) infection [hepatitis B surface antigen (HBsAg) positive 0.2%-0.5%] and HBV exposure (HBsAg negative but anti-HBc positive 4%-6%); Eastern Europe, the Mediterranean, Russia, Southwest Asia, Central and South America have higher rates (2%-7% chronically infected and 20%-55% exposed) and the highest rates are documented in China, Southeast Asia and tropical Africa (8%-20% chronically infected and 70%-95% exposed)[1]. The Centre for Disease Control (CDC) advises the high-risk groups in the general population (Table 1) who should be screened and managed for chronic HBV infection[4].

HBV INFECTION AND THE IMMUNE SYSTEM

HBsAg positive patients

Chronic HBV infection is characterised by the interaction between the virus, the immune system and the liver itself. This interaction is one that may change spontaneously over time, resulting in the 4 phases of CHB infection [the Hepatitis B e-antigen (HBeAg) positive phases of immune tolerance and immune clearance; the HBeAg negative phases of immune control and immune escape] and the corresponding hepatic consequences[5]. This interaction between virus and host may also be disrupted by any drug-induced modulation of the immune system resulting in HBV reactivation (HBVr), which has the potential to cause significant liver injury.

The liver injury that occurs as a result of a HBVr may arise from two mechanisms[6]. Loss of immune control of the virus during chemo- or immunosuppressive therapy may result in uncontrolled viral replication, with rapid rises in HBV-associated proteins causing overwhelming direct cytolytic destruction of hepatocytes. Alternatively, after cessation of chemotherapy, reconstitution of the immune system may cause severe immune-mediated injury to infected hepatocytes. The exaggerated immune response against hepatocytes expressing hepatitis B viral proteins may cause overwhelming necrosis of liver cells. The reactivation may be delayed, occuring as late as six months after the cessation of chemotherapy. In the case of certain treatment regimens (*e.g.*, rituximab due to prolonged immunosuppression and immune reconstitution phases) reactivation can occur as late as 12 mo post-treatment[7,8]. HBVr presents clinically as a spectrum of asymptomatic biochemical hepatitis through to the more concerning acute symptomatic hepatitis with the potential for liver failure and death[9].

HBsAg negative, anti-HBc positive patients

Individuals known to have CHB (HBsAg positive) may spontaneously lose HBsAg at an annual rate of 0.5%; this is defined as "spontaneous clearance"[10]. Alternatively, patients may have serological evidence of past HBV exposure, both scenarios leading to an HBsAg negative/anti-HBc positive state. These patients by far outnumber those with CHB across the globe[1]. The HBV may persist in hepatocytes and other tissues in the form of covalently closed circular DNA. Although the HBV DNA may not be detectable in serum, they remain at risk of HBVr in the setting of chemo- or immunosuppressive therapy, and the clinical adverse outcomes as described above[11,12].

The significance of anti-HBs

Anti-HBs antibodies may develop in HBsAg negative/anti-HBc positive individuals indicating the development of natural immunity or in anti-HBc negative individuals who have been immunised against HBV. There is limited evidence to date that the presence of anti-HBs protects against HBVr. In one small study of 29 lymphoma patients, no patient (0/10) with an anti-HBs titre of > 100 IU/mL experienced HBVr, and lower anti-HBs titre was independently associated with HBVr[13]. In patients receiving haematopoetic stem cell transplantation, the donor anti-HBs titre was associated with a decreased risk of HBVr[14]. These findings are yet to be validated. Until then, management decisions on the prophylaxis of HBVr cannot be made on the basis of the presence or titre of anti-HBs.

DEFINITIONS OF HBVr AND ASSOCIATED CLINICAL ENDPOINTS

HBVr has been variably defined across the existing studies examining this phenomenon. The HBV DNA assays used have varied in their lower limits of detection, potentially underestimating the prevalence of HBVr and delaying the time point at which HBVr may be first detected thereby limiting the ability to directly compare the results across studies. "Hepatitis" has been variably reported as alanine aminotransferase (ALT) elevation above upper limit of normal, or by "fold" increases from baseline; whether the hepatitis is symptomatic or asymptomatic is inconsistently documented. Suggested definitions for HBVr are listed beneath, however a consensus is yet to be reached for

Table 1 Populations at high risk for hepatitis B virus infection that should be screened[4]

Individuals born in areas of high (≥ 8%) or intermediate prevalence (2%-7%) for HBV (HBsAg positive) including immigrants and adopted children
Asia, Africa, South Pacific Islands: All countries
Middle East (except Cyprus and Israel)
Eastern Europe: All countries except Hungary
European Mediterranean: Malta and Spain
The Arctic (indigenous populations of Alaska, Canada, and Greenland)
South America: Ecuador, Guyana, Suriname, Venezuela, and Amazon regions of Bolivia, Brazil, Colombia, and Peru
Caribbean: Antigua and Barbuda, Dominica, Granada, Haiti, Jamaica, St. Kitts and Nevis, St. Lucia, and Turks and Caicos
Central America: Guatemala and Honduras
Other groups recommended for screening
United States born persons not vaccinated as infants whose parents were born in regions with high HBV endemicity (8%)
Household and sexual contacts of HBsAg-positive persons
Persons who have ever injected drugs
Persons with multiple sexual partners or history of sexually transmitted disease
Men who have sex with men
Inmates of correctional facilities
Individuals with chronically elevated ALT or AST
Individuals infected with HCV or HIV
Patients undergoing renal dialysis
All pregnant women
Persons needing immunosuppressive therapy

HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HIV: Human immunodeficiency virus.

the purposes of future studies.

In HBsAg positive patients: Detectable HBV DNA in individual who previously had undetectable HBV DNA by highly sensitive assay (lower limit of detection < 20 IU/mL); ≥ 1 log rise in HBV DNA in individual who previously had a detectable HBV DNA[15]; biochemical hepatitis (ALT flare): ≥ 3 fold rise in ALT from baseline levels exceeding the reference range or an absolute ALT ≥ 100 IU/mL[15], preceded by a rise in HBV DNA.

Consensus is needed as to a grading of the severity of biochemical hepatitis and associated clinical symptoms for the purposes of reporting in future studies. A 5-point grading system was proposed at a recent single topic conference[16]: (1) Without change in ALT level (silent); (2) Increased ALT level without jaundice (mild); (3) Increased ALT level and concomitant jaundice (moderate); (4) Jaundice and signs of liver failure (severe); and (5) Fatal.

In HBsAg negative, anti-HBc positive patients: Sero-reversion (or reverse seroconversion) is the redevelopment hepatitis B surface antigenemia, HBV DNA viremia with or without hepatitis as a result of reactivation of "occult" infection triggered by chemotherapy or immunosuppression[17].

Clinical endpoints associated with the virological and biochemical changes: Jaundice, liver failure and death.

Another important clinical outcome (and relevant endpoint for future studies) is the interruption of chemo- or immunosuppressive therapy, which may be indicated upon the occurrence of HBVr. In a study of 41 patients with breast cancer, HBVr was diagnosed in 17 (41%), and treatment interruption occurred in 71% of these cases (compared with only 33% of those that did not experience HBVr, $P = 0.019$)[18]. Treatment interruption has the potential to increase morbidity and mortality associated with the underlying malignancy or disease process. Due to a lack of reporting of the occurrence of treatment interruption and the long-term outcomes of cancer- or disease-related morbidity and mortality in the majority of studies of HBVr, the impact of treatment interruption due to HBVr across diseases is not clear and requires further evaluation.

THE MAGNITUDE OF THE RISK OF HBVr

Clinically significant reactivations of HBV have been documented in both cancer and non-cancer patients receiving chemo- or immunomodulating pharmacotherapy. The majority of the studies reporting the rates of HBVr are case reports or small case series using variable definitions of HBVr, hence leading to a broad range of prevalences cited.

Reactivation of HBV has been reported in patients treated for lymphoma, other haematological malignancies and in the setting of haematopoetic stem cell transplant[14,19-22]. The prevalence of CHB in patients with lymphoma has been reported as high as 26%[15]. HBVr can occur in 38%-73% of HBsAg positive patients being treated for lymphoma, the higher HBVr rates seen in patients being treated with chemotherapy regimes including high dose corticosteroids[9,23,24]. Patients who receive a bone marrow transplant (BMT) or haematopoetic stem cell transplant (HSCT) for haematological malignancy are a special population that experience prolonged immunosuppression related to the conditioning chemotherapy leading up to the transplant, post-transplant immunosuppressive therapy as well as a potentially protracted immunodeficient state while engraftment occurs. Fatal HBVr has been observed in HBsAg positive patients, as well as HBsAg negative/anti-HBc positive patients[25,26]. In a multicentre retrospective study of patients receiving both autologous and allogeneic stem cell transplantation, the rates of HBVr at 2 years post-transplant were 66% and 81% respectively; the majority of the reactivations occurred within the first 12 mo post-transplant[27].

Therapy for solid tumours including breast, nasopharyngeal and hepatocellular cancer (the latter in the setting of either systemic chemotherapy or trans-arterial chemoembolisation) has also been associated with HBVr[28-36]. Amongst a cohort of oncology patients with solid tumours, CHB was documented in 12% of patients[15]. These investigators observed that approximately 20% of

Table 2 Immunosuppressive drug classes and corresponding risk estimates of hepatitis B virus reactivation[63,80]

Drug class	Drug	Risk estimate of HBVr for HBsAg positive	Risk estimate of HBVr for HBsAg negative/anti-HBc positive
B-cell depleting agents	Rituximab (anti-CD20)	High (30%-60%)	High (> 10%)
	Ofatumumab (anti-CD20)		
Anthracycline derivatives	Doxorubicin	High (15%-30%)	High (> 10%)
	Epirubicin		
TNF-α inhibitors	Infliximab	Moderate (1%-10%)	Moderate (1%)
	Etanercept		
	Adalimumab		
Cytokine inhibitors and integrin inhibitors	Abatacept (anti-CD80, -86)	Moderate (1%-10%)	Moderate (1%)
	Ustekinumab (anti-IL-12, -23)		
	Natalizumab (binds α4-integrin)		
	Vedolizumab [binds integrin α4β7 (LPAM-1)]		
Tyrosine kinase inhibitors	Imantinib	Moderate (1%-10%)	Moderate (1%)
	Nilotinib		
Corticosteroids	High dose, *e.g.*, prednisone ⩾ 20 mg for ⩾ 4 wk	High (> 10%)	NA
	Moderate dose, *e.g.*, prednisone < 20 mg for ⩾ 4 wk	Moderate (1%-10%)	Moderate (1%-10%)
	Low dose, *e.g.*, prednisone for < 1 wk	Low (< 1%)	Low (<< 1%)
	Intra-articular corticosteroids	Low (< 1%)	Low (<< 1%)
Traditional immunosuppression	Azathioprine	Low (< 1%)	Low (<< 1%)
	6-mercaptopurine		
	Methotrexate		

TNF: Tumour necrosis factor; IL: Interleukin; LPAM: Lymphocyte Peyer's patch adhesion molecule; NA: Not available; HBVr: Hepatitis B reactivation; HBsAg: Hepatitis B surface antigen.

CHB patients receiving chemotherapy for their malignancy experienced HBVr[15]. Forty-one percent of breast cancer patients positive for HBsAg have been reported to experience HBVr[18].

HBVr has been reported in patients receiving immunosuppression for inflammatory bowel disease[37,38], rheumatological diseases (rheumatoid arthritis, ankylosing spondylitis)[39-44], dermatological disorders (psoriasis)[45], autoimmune disorders[46,47] and in those following solid organ transplantation (*e.g.*, renal and liver)[48-52].

FACTORS ASSOCIATED WITH HBVr

Elucidating the risk factors for HBVr amongst those receiving chemo- or immunosuppressive therapy may help to identify cases that should receive antiviral prophylaxis. Patient-specific risk factors associated with reactivation include younger age, male gender and the type of treatment regimens prescribed[9,53,54].

Virological and serological status

Detectable HBV DNA, HBsAg, HBeAg and anti-HBc are important virological and serological markers strongly associated with HBVr[10-12,17,55]. High HBV DNA is the strongest of these risk factors, and HBsAg positive patients are up to 8 times more likely to experience HBVr than HBsAg negative/anti-HBc positive patients[30,56,57]. Amongst HBsAg positive patients, HBeAg positive patients have been observed to be more likely to experience HBVr than HBeAg negative patients[56]. The HBV genotype appears to be significant in that HBV genotypes C and B (prevalent in East Asia but rare in Caucasians) correlate with HBVr[9,58,59]. The latter observations may simply be a reflection of the prevalent genotypes in these geographical regions and requires further investigation.

Mutations of the HBsAg may confer risk of HBVr[60]. In a recent study of 93 patients with CHB (29 of whom developed HBVr) the HBsAg genetic features were analysed. HBsAg-mutations localised in immune-active HBsAg regions were observed in 76% patients who experienced HBVr (*vs* 3.1% controls, $P < 0.001$). Of the 13 HBsAg-mutations found in these patients, 8 are known to block HBsAg-recognition by the humoral immune pathway and the remaining 5 mutations were identified within in Class-Ⅰ/Ⅱ-restricted T-cell epitopes (potentially influencing T-cell mediated responses to HBV-escape)[60]. These observations suggest that patients infected with HBV expressing such HBsAg-mutations may be more able to overcome the normal immune response, thereby being more at risk of HBVr with chemotherapy. The clinical application of these findings is yet to be determined.

Chemotherapy/Immunosuppression drug class

The pharmacotherapy used to manage malignant and inflammatory conditions is rapidly evolving with new and targeted agents being developed. Several of these newer agents have the potential to disrupt the control that the immune system has over any underlying HBV exposure or chronic infection. Clinical evidence of HBVr with these agents has subsequently been apparent in case reports and case series. A list of the drug classes listed from most potent to least potent of the agents appears in Table 2.

The B-cell depleting agents appear to be the most potent immunosuppressants (and thereby associated with the highest risk of HBVr). Rituximab and ofatumumab are two B-cell depleting agents predominantly

used to treat haematological malignancy, however rituximab has been used for non-malignant autoimmune and neurological diseases[61,62]. Both HBsAg positive and HBsAg negative/anti-HBc positive patients who receive these agents appear to be susceptible to HBVr. The rate of HBVr with these agents in HBsAg negative/anti-HBc positive patients has been reported at 16.9%, and seroreversion rate of 20%-40%[63-65]. HBVr has occurred up to 12 mo after cessation of B-cell depleting drugs (and in a small number of cases delayed beyond 12 mo) indicating the potency of the immunosuppressive effect of this drug class and the prolonged immune reconstitution phase. A study of 63 HBsAg negative/anti-HBc positive patients with haematological malignancy who received rituximab without antiviral prophylaxis has been reported[20]. At 2 years, 41.5% had experienced HBVr which occurred at a median of 23 (range 4-100) wk after rituximab treatment[20]. These observations would indicate that any monitoring or antiviral prophylaxis prescribed to these patients may require longer duration than other classes of immunosuppressive drugs.

Tumor necrosis factor (TNF)-alpha inhibitor agents include infliximab, etanercept and adalimumab, which have been used in the management of inflammatory bowel disease, rheumatological disease and psoriasis (amongst other disorders). All 3 drugs have been associated with HBVr[66]. The absolute risk of HBVr with these agents is not clear owing to the heterogeneity of the cases and cohorts reported. A larger study of 257 cases exposed to anti-TNF agents for a variety of indications reported a HBVr rate of 39% in HBsAg positive patients and 7 fold lower rate of HBVr in anti-HBc positive patients[67].

Cytokine and integrin inhibitors, by virtue of their interaction with the immune system, have also been associated with HBVr. Drugs of this class and their target molecules are listed in Table 2. Evidence of role of these drugs in HBVr exists largely as case reports and small case series [54,55,68].

Tyrosine kinase inhibitors including imantinib and nilotinib are used to treat chronic myeloid leukaemia and gastrointestinal stromal tumours. Evidence of HBVr is limited, again, to case reports and small case series[69-74].

Corticosteroids are the most longstanding and hence most commonly used of the immunosuppressants across all the aforementioned disease processes. In addition to their effect on T-cell function, corticosteroids directly enhance HBV replication through their interaction with the HBV glucocorticoid responsive element (a transcriptional regulatory element)[75]. Although steroids are administered at a range of dosages and durations for a variety of indications, it has been observed that a 4-wk course of prednisone has been associated with HBVr in the post-withdrawal (immune reconstitution) phase and worsened liver histology[76]. Chronic steroid use in the setting of chronic airways disease is associated with HBVr in 11.1% of those treated with oral steroids and 3.2% of those treated with inhaled steroids[77]. In the aforementioned study (including 198 patients with asthma or chronic obstructive pulmonary disease) continuous oral corticosteroid therapy (> 3 mo) and high-dose (defined as > 20 mg prednisone/day) were associated with HBVr with OR of 5.7 and 4.9 respectively, when compared with HBVr in those receiving inhaled corticosteroids[77]. Low dose, short term (< 2 wk) administration of oral (systemic) corticosteroids, intraarticular injection and topical therapies have not been associated with HBVr. These data taken together indicate that corticosteroids have the potential to induce HBVr, but that the risk varies according to the dose, duration and route of administration of the drug.

"Traditional" immunomodulating drugs such as azathioprine, 6-mercaptopurine and methotrexate appear to have the lowest potential for HBVr. There are no documented cases of HBVr with the use of azathioprine or 6-mercaptopurine monotherapy. Cases of HBVr have been reported with methotrexate, however corticosteroids or other immunomodulators were co-administered in most instances, compounding the risk of HBVr[78,79].

The risk of HBVr associated with each of the drug classes administered to HBsAg positive or HBsAg negative/anti-HBc positive patients has been estimated by the American Gastroenterological Association (AGA) based on a thorough systematic review of the existing literature[63,80]. This risk stratification is summarised in Table 2. The risk of HBVr may be stratified to high (> 10% risk of HBVr), moderate (1%-10%) and low (< 1%). The current AGA recommendations are based on the risk of HBVr according to the combination of serological markers of HBV and the chemotherapy/immunosuppression regimen prescribed and, to date, are the most detailed and specific recommendations with regard to the patient risk groups in whom antiviral prophylaxis should be considered[63,80].

Hepatitis B and delta co-infection

To date, only a single case report of hepatitis delta (HDV) reactivation in association with HBVr exists[81]. This patient was co-infected with hepatitis C (HCV RNA positive), HBV (HBsAg positive, HBV DNA undetectable at baseline) and had evidence of cleared HDV infection (anti-HDV positive). A rituximab-CHEOP regime was prescribed to treat lymphoma; HBV DNA became detectable during chemotherapy. Subsequently, 15 mo after chemotherapy, HDV RNA was detected at a level of 77.6 million copies/mL. The patient was managed successfully with lamivudine, which was in turn switched to emtricitabine/tenofovir. Given the singularity of this report, there is no real evidence base to guide the management of HBV-HDV co-infection in this setting and patients should be managed according to their HBV status.

MANAGEMENT OF HBVr

HBVr occurring during chemo- or immunosuppressive therapy, if detected, may be an indication to delay

or cease therapy. Withholding chemotherapy may halt or reduce the rate of HBV replication potentially abrogating the HBVr. As discussed, in the absence of antiviral prophylaxis, HBVr may also occur in the post-chemotherapy immune reconstitution phase.

The role of antiviral therapy once HBVr is already established has been investigated by several groups. In a prospective study of patients treated for non-Hodgkin's lymphoma, lamivudine therapy started when ALT elevation was detected did not change the natural course of HBVr; 2 patients in this cohort died despite lamivudine use at the onset of HBVr[8]. Numerous case reports and series describe death due to liver failure despite the introduction of lamivudine at the onset of HBVr[82-86]. Only a few cases of successful treatment of HBVr with entecavir have been published[87-89]. Despite the paucity of data regarding the efficacy of entecavir to treat established HBVr (and no data to date regarding tenofovir), the ability of these drugs to rapidly reduce HBV DNA make them attractive alternatives to lamivudine in patients who experience HBVr to potentially abrogate the risk of liver failure and mortality. Data on the efficacy and cost-effectiveness of these approaches to management are needed.

PREVENTION OF HBVr

Given the poor outcomes associated with reactionary treatment of HBVr (*i.e.,* antiviral treatment once HBVr is already established), strong consideration must be given to the role of antiviral prophylaxis in at-risk patients who will receive chemo- or immunosuppressive therapy.

A systematic review of studies examining the role of antiviral prophylaxis in chemotherapy patients concluded that lamivudine prophylaxis (*vs* no prophylaxis) is associated with a relative risk of 0.0-0.21 for HBVr and 0.0-0.2 for death attributable to HBV[90]. Liver failure was not observed in any patient who received lamivudine prophylaxis[90]. In line with these observations, a subsequent systematic review reported that patients given lamivudine prophylaxis during chemotherapy showed an 87% decrease in HBVr compared to patients not given prophylaxis[91]. It is noteworthy to mention that the number needed to treat to prevent one reactivation was just 3 patients[91]. Treatment delay and early cessation of chemotherapy due to HBVr were also reduced by 92% in those who received lamivudine[91].

Most recently, a systematic review and metanalysis of 5 randomised controlled trials comparing antiviral prophylaxis to treatment at the onset of HBVr has been published[63]. Lamivudine was used in 4 studies and entecavir was used in 1 study[8,92-95]. The overall risk ratio (RR) favoured the prophylactic use of antivirals over no antivirals [RR = 0.13 (0.06-0.30)][63]. Antiviral prophylaxis was also associated with a significant risk reduction of hepatitis flare [RR = 0.16 (0.06-0.42)][63].

Owing to the fewer occurrences and lower severity of HBVrs, the use of lamivudine prophylaxis has been deemed to be a cost-effective intervention. The cancer death rate in patients who receive prophylaxis is also reduced, presumably due to the reduced rate of chemotherapy interruption or curtailment[96]. The cost-effectiveness of entecavir and tenofovir have not, as yet, been evaluated.

The duration of antiviral prophylaxis remains under debate. As discussed, delayed HBVr has been observed in patients 6-12 mo after completion of chemotherapy (in the absence of antiviral prophylaxis) in both HBsAg positive and HBsAg negative/anti-HBc positive patients, and also when the antiviral prophylaxis has been curtailed to 2 mo post-completion of antiviral therapy[8]. The duration of risk of HBVr appears to be strongly related to the potency of treatment regime, again mentioning that patients who have received B-cell depleting agents appear to be susceptible to delayed HBVr (up to 12 mo post-treatment and beyond)[20]. Hence, antiviral prophylaxis may be required for at least 6 mo after cessation of chemo- or immunosuppressive therapy and for at least 12 mo for those receiving B-cell depleting agents; subsequent monitoring for delayed HBVr after cessation of antiviral prophylaxis is essential.

Mention must be made of the role of antiviral prophylaxis in recipients of bone marrow or haematopoetic stem cell transplants. Both lamivudine and entecavir have been used with the aim of preventing HBVr in these cases[97-101]. The optimal timing of withdrawal of antiviral prophylaxis, however, remains unclear. HBVr has been observed as early as 12 wk post-discontinuation of lamivudine in the bone marrow transplant setting[98]. In a study of 16 patients who received lamivudine for a median of 73 wk (range 19-153) after stem cell transplantation, the cumulative rate of HBVr at 30 mo follow-up was 20%; 63% of the patients developed documented lamivudine resistance and one patient had virological breakthrough during the study period[99]. HBVr has been diagnosed as late as 4 years after transplantation in a patient who was anti-HBs positive at baseline[102]. It would appear that BMT/HSCT recipients are potentially at risk of HBVr for years after the transplant and consideration must be given to whether these patients require antiviral therapy long term. If therapy were to continue long term, then consideration must be given to the risk of lamivudine resistance, and hence entecavir and tenofovir may be more suitable choices for antiviral prophylaxis due to their high barrier for drug resistance. The current evidence base to address these issues is weak, and further study is required. Of the major international associations' guidelines, only the European Association for the Study of Liver Disease (EASL) guidelines (2009) provide a recommendation for this patient population: that nucleos(t)ide analogue prophylaxis is recommended for anti-HBc positive patients receiving bone marrow or stem cell transplantation (grade of recommendation C2); a duration of therapy is not specified[103].

Based on the available data, prophylactic antiviral

therapy in the appropriate candidates appears to reduce the risk of HBVr and morbidity. Further studies are required to determine the impact on overall and cancer-related survival and the cost effectiveness of the strategies employed (drug choice, duration of therapy).

CHOICE OF ANTIVIRAL AGENT

The drugs currently available for the management of chronic hepatitis B include lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir disoproxil fumarate. By far, the largest body of literature on the prevention of HBVr examines the role of lamivudine, the first of these drugs to be available. The downside of lamivudine use is the high rate of drug resistance (and potential for virological breakthrough or relapse) reported to be 20% within the first 12 mo of use. Fatal HBVr despite lamivudine prophylaxis owing to the development of the M204 drug resistance mutation has been reported in a patient who received R-CHOP for lymphoma[104]. Lamivudine may have a role where total chemotherapy and post-chemotherapy follow-up duration spans less than 12 mo (thereby reducing risk of drug resistance and virological breakthrough), the HBV DNA is undetectable at baseline and the patient is not receiving any of the "high risk" treatment regimes. The latter approach requires further evaluation, but may be an attractive strategy, *e.g.*, in countries with high prevalences of HBV where the cost of the more potent antivirals may be prohibitive.

With substantially lower antiviral resistance rates than lamivudine, entecavir and tenofovir may be a more suitable first line for HBV DNA suppression in those with high pre-chemotherapy HBV DNA levels in order to mitigate HBVr. There are five studies to date comparing entecavir to lamivudine or no prophylaxis in patients with haematological malignancy or lymphoma alone[92,105-108]. Lower rates of HBVr are generally observed with the use of entecavir in these studies, however these studies vary in their design (ranging from retrospective audit to randomised-controlled study) and hence the strength of their findings. In the single randomised controlled study (published in abstract form), 61 patients who received entecavir prophylaxis were compared to 60 patients who received lamivudine[92]. Entecavir was associated with a relative risk reduction of 0.22 (0.08-0.61) for HBVr and had significantly fewer chemotherapy interruptions (1.6% *vs* 18.3%)[92]. Further data is awaited, as are studies on the role of tenofovir in this setting, but it is expected that given the limitations of lamivudine, entecavir and tenofovir will have a greater role in the prevention of HBVr in the future.

The data regarding adefovir and telbivudine in the prevention and management of HBVr is limited to the liver transplantation setting and is outside of the scope of discussion of this review. These drugs are not recommended as first line drugs for prophylaxis of HBVr in the context of chemotherapy or immunosuppression.

SCREENING FOR HBV PRIOR TO CHEMOTHERAPY OR IMMUNOSUPPRESSION

Given the risk of HBVr in patients previously exposed or chronically infected with HBV and receiving chemotherapy or immunosuppression, it is essential for clinicians caring for such patients to be aware of this risk and screen for HBV in order to institute appropriate prophylactic therapy or monitoring. Additionally, screening may uncover previously unrecognized chronic hepatitis B infection and subsequently the complications of cirrhosis and hepatocellular cancer. These liver-related complications require long-term and directed management and may influence the management underlying malignancy/disease.

There are several approaches to screening for HBV in this patient population: (1) Screen all patients prior to chemotherapy/immunosuppression[103,109]. This strategy would identify patients who would potentially benefit from: Antiviral prophylaxis; HBV serology and HBV DNA monitoring (without antiviral prophylaxis); Immunisation against HBV; Evaluation for complications of CHB; Contact tracing of family members for CHB and their subsequent management; (2) Screen only patients at risk of HBV according to CDC "high risk" groups (Table 1)[4,110]; and (3) Screen only patients who, if serological testing was positive, would be prescribed antiviral prophylaxis[63,80,111].

Consideration must also be given to the serological test(s) to be used for screening. The approaches to serological screening for HBV include: (1) Test HBsAg, anti-HBc and anti-HBs. Test HBV DNA if HBsAg or anti-HBc are positive (the latter in case of occult HBV infection); (2) Test HBsAg, anti-HBc only. The role of anti-HBs in HBVr is unclear. Furthermore, immunisation against HBV may not be efficacious during immunosuppression. Therefore, one may argue that anti-HBs status may not be relevant prior to chemotherapy; and (3) Test anti-HBc only. If positive, proceed to test for HBsAg and HBV DNA.

There is a paucity of data on the best and most cost-effective approach to screening for HBV in patients at risk of HBVr. Each of the major international associations has made screening recommendations (summarised in Table 3), which vary across the associations. The most recent and complete of the systematic reviews performed (as at 2014) has estimated risk of HBVr according to specific chemotherapy/immunosuppressive regime, and hence recommends HBV serological screening in patients with moderate to high risk[63,80]. The clinical decision on who and how to screen will likely be influenced by the characteristics of the population being managed and the resources available to the individual, the institution

Table 3 Comparison of International Associations' guidelines on the management of hepatitis B virus in the setting of chemotherapy and immunosuppression

Association guidelines	HBV screening population	Screening test	Antiviral prophylaxis		Antiviral drug recommended for prophylaxis	Monitoring in untreated anti-HBc + ve patients
			HBsAg + ve, anti-HBc + ve	HBsAg - ve, Anti-HBc + ve		
American Gastroenterological Association 2014[63,80]	High risk of HBVr (> 10%)	HBsAg and anti-HBc; HBV DNA if serology + ve	Yes (B1)	Yes (B1) if taking:	Drug with high barrier to resistance is favoured over lamivudine (B2)	No recommendation (knowledge gap)
	B-cell depleting agents		Continue until at least 6 mo after completion of chemotherapy	B-cell depleting agents		
	Anthracycline derivatives High dose corticosteroids (≥ 20 mg prednisone for ≥ 4 wk)			Anthracycline derivatives Continue until at least 12 mo after completion of chemotherapy for B-cell depleting agents		
	Moderate risk of HBVr (1%-10%)	HBsAg and anti-HBc; HBV DNA if serology + ve	Yes (B2)	Yes (B2) if taking	Drug with high barrier to resistance is favoured over lamivudine (B2)	No recommendation (knowledge gap)
	TNF-α inhibitors		Continue until at least 6 mo after completion of chemotherapy	TNF-α inhibitors		
	Cytokine or integrin inhibitors			Cytokine or Integrin inhibitors		
	Tyrosine kinase inhibitors High dose corticosteroids (≥ 20 mg prednisone for ≥ 4 wk)			Tyrosine kinase inhibitors Continue until at least 6 mo after completion of chemotherapy		
	Low risk of HBVr (< 1%)	Routine screening not recommended	Not recommended (B2)	Not recommended (B2)	Not applicable	No recommendation (knowledge gap)
	Traditional immunosuppression Intra-articular corticosteroids Systemic corticosteroids for < 1 wk	Screen for HBV as per CDC guidelines[4]; manage accordingly				
American Association for the Study of Liver Disease 2009[110]	Anyone at high risk of HBV infection; Table 1 (Ⅱ-3)	HBsAg and anti-HBc	Yes (regardless of HBV DNA level)	No recommendation (knowledge gap)	Lamivudine (Ⅰ) or telbivudine (Ⅲ) if the anticipated treatment duration is short (< 12 mo) and baseline HBV DNA is not detectable	Monitoring recommended; no specific test/frequency provided
			Maintain for 6 mo completion of chemotherapy (Ⅲ)		Tenofovir or entecavir if anticipated treatment duration > 12 mo (Ⅲ)	
			If baseline HBV DNA > 2000 IU/mL, continue antiviral until endpoints as per immunocompetent patients (Ⅲ)			

European Association for the Study of Liver Disease 2012[103]	All candidates for chemo- and immunosuppressive therapy (A1)	HBsAg and anti-HBc; HBV DNA if serology + ve	Yes (A1) Regardless of HBV DNA level Continue until 12 mo after cessation of chemotherapy	Yes if: HBV DNA detectable Taking rituximab (C2) Bone marrow or stem cell transplantation (C2) Treat as per HBsAg + ve No if: HBV DNA undetectable; monitor	Lamivudine if HBV DNA < 2000 IU/mL and the treatment duration is short/finite (B1) Entecavir or tenofovir if HBV DNA is high, lengthy or repeated cycles of immunosuppression (C1)	ALT and HBV DNA every 1-3 mo Treat upon evidence of HBVr (C1)
Asian-Pacific Association for the Study of Liver Disease 2012[109]	All patients prior to receiving immunosuppression or chemotherapy	HBsAg (IVA) Test anti-HBc if patient due to receive biological agent	Yes HBVr prophylaxis with lamivudine; Continue until 6 mo after end of chemotherapy (ⅠA) Alternatively use entecavir or tenofovir (ⅢA) Continue HBV treatment if clinically indicated (ⅠA)	No; monitor		HBV DNA should be closely monitored and treated with nucleos(t)ide analogue when needed (IVA)
American Society of Clinical Oncology Provisional Clinical Opinion 2010[111]	Advise against routine serological screening. Screen those with High risk of HBV exposure; evidence of liver disease Therapeutic regimen with high risk of HBVr including all patients undergoing rituximab therapy Haematopoetic stem cell transplant	HBsAg; anti-HBc if receiving rituximab	Consider role of antiviral therapy Do not delay chemotherapy	No specific recommendation provided	No specific recommendation provided	No specific recommendation provided

The grade of recommendation and/or level of evidence have been noted where available. AGA and EASL guidelines: evidence grade A: High quality; B: Moderate quality; C: Low quality. Recommendation grade 1: Strong; 2: Weak. Ⅰ: Randomised controlled trials; AASLD guidelines: Ⅱ-1: Non-randomised controlled trials; Ⅱ-2: Cohort or case-control studies; Ⅱ-3: Case series; Ⅲ: Expert opinion. APASL guidelines: Quality of evidence ranked from Ⅰ (highest) to Ⅴ (lowest); strength of recommendations ranked A (strongest) to D (weakest). HBV: Hepatitis B virus; HBVr: Hepatitis B virus reactivation; CDC: The Centre for Disease Control; + ve: Positive; - ve: Negative; ALT: Alanine aminotransferase.

and nation to fund the serological testing and manage positive results. Studies evaluating the efficacy and cost-effectiveness of the various screening strategies as relevant to the main global regions are needed.

MONITORING IN ANTI-HBC POSITIVE PATIENTS WHO DO NOT RECEIVE ANTIVIRAL PROPHYLAXIS

The data presented in this review thus far indicate that not all HBsAg negative/anti-HBc positive patients will benefit from antiviral prophylaxis, *e.g.*, patients with undetectable HBV DNA who are prescribed lower potency or limited duration immunosuppressive drug regimes. In those who do not receive antiviral prophylaxis, monitoring for features of HBVr is intuitive, however there is a paucity of data as to how this monitoring should be carried out. There is a general consensus from the international associations that some form of monitoring is required. The EASL recommends ALT and HBV DNA testing every 1-3 mo and treatment upon any evidence of HBVr, but this is based on relatively weak level of evidence (C1, Table 3). An alternative approach may be to test for HBsAg in HBsAg negative/anti-HBc positive patients to monitor for seroreversion, which may occur prior to detection of HBV DNA or ALT rise. The remainder of the major societies do not make specific recommendations owing to the knowledge gap in this area. Further data is required to determine the method, frequency and duration of monitoring. Similar to issues arising regarding screening for HBV, the monitoring for HBVr in patients who do not receive prophylaxis will be guided by the prevalence of HBV and HBVr, cost-effectiveness as well as the access to testing and follow-up that varies across the globe.

MONITORING AFTER THE CESSATION OF ANTIVIRAL PROPHYLAXIS

Some patients who receive antiviral therapy at the initiation of chemotherapy or immunosuppression may need to remain on antivirals long term if there is underlying chronic liver disease and ongoing treatment criteria are met[103,109,110]. In those who receive antiviral prophylaxis without otherwise meeting ongoing treatment criteria for chronic HBV, once the decision has been made to cease antiviral prophylaxis there is no evidence base to guide how monitoring is best performed. The major associations do not make specific recommendations as to how to perform post-prophylaxis monitoring. Measurement of HBV DNA and ALT every 1-2 mo for 3-6 mo after cessation of lamivudine prophylaxis have been proposed[90], but based the observations of many of the aforementioned studies, these patients should be monitored for at least 12 mo, if not, long-term. Furthermore, relapse of the underlying malignancy requiring resumption of chemotherapy would warrant reinstitution of antiviral prophylaxis and should not be overlooked.

A COMPARISON OF THE INTERNATIONAL GUIDELINES ON THE PREVENTION AND MANAGEMENT OF HBVr

The guidelines of the major international associations for the HBV screening, antiviral prophylaxis and monitoring have been referenced, where relevant, throughout this review and are summarised in Table 3, the most recent being the technical review and guidelines of the American Gastroenterological Association[63,80]. It must be noted that some of these recommendations span back to 2009, and as such more recent data would not have been included when older recommendations were made. The application of these guidelines by the clinician warrants consideration of clinical circumstances, resources available and cost-effectiveness, which are patient and region/nation specific.

CONCLUSION

Clinicians managing patients with malignancy need to be vigilant of the potential for HBVr as a complication of chemotherapy in susceptible cases. Those at risk for HBVr must be screened serologically for the virus according to international guidelines, which are based on the best available evidence. Prophylactic antiviral therapy with lamivudine or other nucleos(t)ide analogue should be instituted prior to the start of chemotherapy. Prevention is better than cure.

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REVIEW

Control of oxidative stress in hepatocellular carcinoma: Helpful or harmful?

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Abstract

Oxidative stress is becoming recognized as a key factor in the progression of chronic liver disease (CLD) and hepatocarcinogenesis. The metabolically important liver is a major reservoir of mitochondria that serve as sources of reactive oxygen species, which are apparently responsible for the initiation of necroinflammation. As a result, CLD could be a major inducer of oxidative stress. Chronic hepatitis C is a powerful generator of oxidative stress, causing a high rate of hepatocarcinogenesis among patients with cirrhosis. Non-alcoholic steatohepatitis is also associated with oxidative stress although its hepatocarcinogenic potential is lower than that of chronic hepatitis C. Analyses of serum markers and histological findings have shown that hepatocellular carcinoma correlates with oxidative stress and experimental data indicate that oxidative stress increases the likelihood of developing hepatocarcinogenesis. However, the results of antioxidant therapy have not been favorable. Physiological oxidative stress is a necessary biological response, and thus adequate control of oxidative stress and a balance between oxidative and anti-oxidative responses is important. Several agents including metformin and L-carnitine can reportedly control mechanistic oxidative stress. This study reviews the importance of oxidative stress in hepatocarcinogenesis and of control strategies for the optimal survival of patients with CLD and hepatocellular carcinoma.

Key words: Liver cancer; Liver cirrhosis; Hepatitis B; Hepatitis C; Non-alcoholic steatohepatitis; Reactive oxygen species

Core tip: Oxidative stress is a key biological response that correlates with the progression of chronic liver disease. However, oxidative stress is an essential survival mechanism and thus to erase it is an unsuitable approach to disease control. As hepatocarcinogenesis is closely associated with increased oxidative stress *via* viral proteins or chronic inflammation and lipids, controlling oxidative stress should be effective against progressive liver disease. Agents that can control oxidative stress might represent a more effective approach than reactive oxygen species-scavenging agents.

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INTRODUCTION

The risk and pathogenesis of hepatocellular carcinoma (HCC) has been investigated in detail because about 80% of this type of cancer is due to chronic infection with hepatitis B (HBV) and C (HCV) viruses[1]. Hepatocellular carcinoma differentiates from low- and high-grade dysplastic nodules and sequentially advances into well-, moderately- and poorly differentiated HCC. Such multistep carcinogenesis associated with chronic inflammation suggests that numerous complex pathogeneses are involved in hepatocarcinogenesis. Whole exome sequencing has revealed that HCC contains many oncogene and tumor suppressor gene mutations[2]. The most common pathways can be p53-, Wnt- and RB1-dependent[3]. Poor outcomes of HCC include p53 signaling-related genes such as the protein kinase TTK[4]. Activation of the Wnt-catenin pathway is frequently anomalous in HCC and high expression levels correlate with poor outcomes[5]. Mutations in RB1 are associated with cancer-specific and recurrence-free survival after resection[6]. Such changes could be induced *via* HBV, HCV and lipid-induced cellular stress and chronic inflammation.

The pathogenic mechanisms of HBV- and HCV-related chronic liver disease (CLD) and hepatocarcinogenesis include viral protein-related immune function interference, tumor initiation or suppression interference and CLD-related environmental changes[1]. Tumor-related gene methylation is induced *via* HBV and HCV infection without inflammation and it is increased stepwise under conditions of chronic hepatitis, dysplastic nodules and HCC[7,8]. Oxidative stress is included in this process *via* the direct effects of viral proteins or secondarily to chronic inflammation[1].

Recent advances in HBV- and HCV-targeted anti-viral therapy have enabled control of HBV and HCV[9,10]. So far, the HBV load can only be decreased by nucleos(t)ide analogues that cannot eradicate the virus[11]. Pegylated interferon (IFN) combined with ribavirin and HCV NS3 protease inhibitor, can eradicate HCV in nearly 80% of therapy recipients. However, patients with liver cirrhosis have difficulties enduring the many side-effects of these drugs such as anemia, leukocytopenia, and skin rash[12]. New treatment modalities with IFN-free oral direct-acting antiviral agents combined with other types of therapy do not generate many of the side effects that are associated with IFN, but they are not indicated for decompensated liver cirrhosis because metabolism is decreased in patients with defective liver function[13]. Therefore, the direct hepatocarcinogenic activities of HBV and type C liver cirrhosis remain a major threat in endemic areas.

The incidence of non-alcoholic steatohepatitis (NASH) has recently increased and it progresses to HCC. Non-alcoholic steatohepatitis is an advanced stage of non-alcoholic fatty liver disease (NAFLD) that is involved in metabolic syndrome, a condition that becoming increasingly prevalent[14]. Both NASH and related HCC require urgent investigation because obesity is widespread in first-world countries. Oxidative stress is a key factor in NASH progression and NASH-related hepatocarcinogenesis[15,16]. The standard treatment for NASH is supplementation with the representative antioxidant, vitamin E[17].

The administration of antioxidant therapies for diseases involving oxidative stress is controversial because reactive oxygen species (ROS) are essential for maintaining defense by anti-pathogenic microorganisms, or by anti-carcinogenic mechanisms. Although many antioxidants are already on the market, their proven antioxidant activities *in vitro* have not been confirmed *in vivo*[18]. Many studies of cerebrovascular diseases and mortality have associated vitamin E with unfavorable outcomes[14]. Therefore, the notion of controlling oxidative stress in this manner requires re-evaluation. This article reviews current understanding of oxidative stress in viral hepatitis- and NASH-related HCC and the controversy surrounding antioxidant therapy for these diseases.

OXIDATIVE STRESS INVOLVEMENT IN HEPATOCARCINOGENESIS

The mechanisms of hepatocarcinogenesis include several common functions such as oncogene activation, oxidative stress and tumor suppressor function attenuation, but the upstream functions differ. HBV related HCC can be found in non-cirrhotic carriers, whereas HCV-related HCC is found mainly in patients with cirrhosis[19]. The incidence of NASH related HCC is increasing and more clinical evidence is needed.

Oxidative stress could be induced *via* chronic hepatic inflammation regardless of etiology. Acute liver injury and hepatic inflammation induce ROS *via* neutrophils and Kupffer cell activation. As these cells invade liver parenchyma, hepatocytes could also be affected by the induced ROS. Although the free diffusion of superoxide is blocked by the plasma membrane, superoxide dismutase in the membrane can convert superoxide anions to H_2O_2 after internalization into hepatocytes[20]. The ROS in mitochondria are by-products of the beta oxidation pathway for fatty acid metabolism and they are generated *via* electron leakage from mitochondrial electron transport resulting in the activation of oncogenic pathways[21].

Mechanisms of HBV-related HCC and oxidative stress involvement

Enveloped HBV is a DNA virus containing a relaxed circular DNA genome enclosed by envelope protein[22,23]. After envelopment and the release of mature virions, HBV converts into a covalently closed circular DNA

that persists in the nucleus of infected cells as mini-chromosomes that are difficult to eradicate[24]. After initial infection, HBV persists in the liver for life, even if a patient achieves a clinical cure with seroclearance of HBV envelope antigen (HBsAg) and the emergence of anti-HBs antibody[25]. Chronic inflammation and liver fibrosis caused by chronic HBV infection contributes to the development of HCC[26]. However, in addition to these host factors, HBV itself plays a direct role in the development of HCC[27,28]. A significant proportion of HBV-related HCC arises in otherwise normal livers[19,29], and animal models transfected with this virus genome develop HCC, which confirms the oncogenic potential of HBV in the liver[27,30]. Gene expression profiles of HBV-related HCC indicate that several genes related to signal transduction, transcription and metastasis play direct hepatocarcinogenic roles[31]. Despite a considerable amount of research, the molecular basis of HBV-related hepatocarcinogenesis remains unclear[32,33].

Chronic inflammation is a common feature of chronic hepatitis B and C that results in induction of oxidative stress. The HBV evades immune surveillance resulting in altered viral-specific and non-specific immune responses[34]. Kupffer cells or macrophages exert both immunostimulatory and immunoregulatory activities upon exposure to HBV. The addition of HBV particles and HBsAg induces production of the proinflammatory cytokines interleukin (IL)-1β, IL-6, CXCL-8 and tumor necrosis factor (TNF)-α by human $CD68^+$ macrophage-enriched cells *via* nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation[34]. However, another study did not find cytokine production with immunoregulatory cytokine transforming growth factor-β production[35]. The immune system activates Kupffer cells to eradicate HBV, while HBV evades the Kupffer cell-related pathway to reduce the inflammatory pathway and render the environment favorable for survival. Such persistent atypical cytokine production and the resulting ROS affect hepatocarcinogenesis.

The HBV genome encodes several gene products such as DNA polymerase (Pol), capsid protein (core), envelope proteins L, M and S, and the multifunctional protein HBx. Among these products, the oncogenic potential of HBx protein has been analyzed in detail. Transactivating HBx protein stimulates viral gene expression and replication, and also protects virally-infected cells against immune-mediated destruction[36]. High and low levels of HBx protein are expressed in the cytoplasm and nucleus, respectively, of hepatocytes infected with HBV[37]. HBx protein regulates some oncogenes and affects several apoptosis-related signaling pathways[36]. Through binding to transcription factors such as CREB, RPB5, TFIIB, XAP-1, C/EBPalpha and XAP3, HBx can upregulate oncogenes such as Rab18 or Yes-associated protein[38,39]. HBx induces apoptosis by upregulating FasL protein through activating MLK3/MKK7/JNKs signaling and interacting with Bcl-2/CED-9 signaling[40,41].

Concentrated cytoplasmic HBx co-localizes with mitochondria that are sources of ROS[42]. The C-terminal region of HBx produced by HBx truncation is required for ROS production[37] and it is found in 46% of HCC, but not in non-tumor tissues[43]. The C-terminal truncated HBx found in HCC suggests that ROS are involved and that it is significantly associated with increased venous invasion and metastasis. The stable expression of C-terminal truncated HBx *in vitro* results in increased C-Jun kinase transcriptional activity and the enhanced invasiveness of hepatoma cell lines.

Integration of the *HBV* gene into the house genome is an important mechanism that is responsible for HCC development. The frequency of integration is reportedly higher in tumors than in adjacent liver tissues (86.4% *vs* 30.7%). Several cancer-related genes such as *TERT*, *MLL4*, and *CCNE1* are integrated by HBV, especially in tumors[44] and among these, HBx is most frequently integrated into the human genome.

Several studies have analyzed mutations within the HBV genome that might be associated with HCC. Genotypic diversity is related to differences in clinical and virological characteristics. For example, patients infected with genotype C have more severe chronic liver disease, including cirrhosis and HCC[29,45]. Deletions and point mutations, which are more subtle genetic variations than genotypes, have been identified, such as codon 38 in the *X* gene, the core promoter region, G1613A and C1653T, basal core promoter region *A1762T/G1764A* mutations and deletions of pre-S and X protein[46-49]. The analytical findings of HCC-related *HBV* mutations emphasized the importance of HBx and suggested the involvement of oxidative stress. The envelope protein pre-S is also involved in HCC development. The pre-S region might affect the pathway of hepatocarcinogenesis *via* oxidative stress.

The HCC-associated HBV variant pre-S has been identified in the pre-S2 start codon and/or in deletions of the 5′-terminal of the pre-S2 region and pre-S1 mutation with deletions of the 3′-terminal of pre-S1[50,51]. These pre-S region mutant products induce the accumulation of mutated L protein in the endoplasmic reticulum (ER) of hepatocytes. The ER plays a major role in the synthesis, folding and trafficking of secretory and membrane proteins that correlate with the cellular response known as ER stress. A considerable amount of experimental data have confirmed the potential pro-oncogenic role of pre-S mutated gene products *via* accumulation in the ER with enhanced ER stress and ROS[51]. The pre-S mutated proteins accumulating in the ER can trigger c-Raf-1/Erk2 signaling, which results in AP-1 and NF-κB activation, enhanced proliferative activity of hepatocytes and an increased incidence of liver tumors in transgenics[52]. Pre-S2 mutated proteins also have non-ER related functions such as interacting with Jun activation domain-binding protein 1, which results in cyclin-dependent kinase inhibitor p27 degradation and cell cycle progression[53].

Oxidative stress must be involved to some degree in HBV-related hepatocarcinogenesis, possibly *via* HBx and pre-S-related functions. Relatively weak oxidative stress has been defined in HBV-related hepatocar-

cinogenesis *in vitro*. HBV with HBx protein expressed in mitochondria, binds to voltage-dependent anion channels 3 and alters the mitochondrial transmembrane potential resulting in ROS generation and the activation of several transcription factors[54]. Analyses of serum from patients have shown that oxidative stress-related markers are significantly increased in HCV-, but not in HBV-related HCC[55]. One reason for this might be that HBV has other prevailing features such as the induction of mutations in oncogenic and tumor suppressor genes by viral proteins. Anti-oxidant therapy is seemingly logical for HBV-related HCC as HBx and pre-S proteins have promising effects on oxidative stress. However the inferior outcomes of oxidative stress in human serum and *in vitro* indicate that expectations should not be too high.

Mechanisms of HCV-related HCC and involvement of oxidative stress

Since HCV, like HBV, is not a cytopathic virus, immune reactions play a central role in the development of chronic hepatitis[56,57]. However, the clinical course and direct viral and hepatitis-related effects on hepatocarcinogenesis differ. The expression of several genes associated with detoxification and the immune response suggest that indirect and immune or detoxifying response-related hepatocarcinogenic roles are involved in HCV-related HCC[31].

Immune systems are disrupted by HCV proteins. Antigen-presenting cells such as Kupffer cells, macrophages, or dendritic cells exhibit both immunostimulatory and immunoregulatory activities upon exposure to HCV[58]. The HCV core and Kupffer cells affected by NS3 secrete pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α and the immunosuppressive cytokine, IL-10, *in vitro*[59]. The release of pro-inflammatory cytokines might explain the induction and persistent inflammation of patients with chronic hepatitis C, while immunosuppressive cytokine release explains the difficulty of eradicating HCV-infected hepatocytes. The direct effects of HCV on the inflammatory signal in Kupffer cells upregulates the immunoregulatory molecule PD-L1[60]. In addition to interference with Kupffer cell-related anti-viral activities, HCV induces a sufficient amount of inflammatory cytokines to result in chronic inflammation. Kupffer cells accumulate around inflammatory foci and express cytotoxic molecules such as granzyme B, perforin and ROS that induce inflammation and fibrosis[61]. Sustained inflammation results in hepatocyte apoptosis and repeated regeneration cycles followed by spontaneous DNA mutation and damage resulting in HCC[1].

HCV antigens, especially core protein, play major roles in chronic hepatitis C pathogenesis and hepatocarcinogenesis[62]. Because microarray analysis has not suggested a direct hepatocarcinogenic pathway in HCV-related HCC, the direct effects of viral proteins are seemingly less powerful than HBV. However, HCV core protein seems to play direct roles in TNFR, PKR and Stat3 pathways that are associated with cell proliferation, apoptosis, transformation and immortalization[63,64]. Transgenic mice expressing HCV core protein exhibit hepatocarcinogenesis *via* fatty metamorphosis and increased oxidative stress[65,66]. The glutathione pool is oxidized and NADPH content is decreased *via* the direct interaction of HCV core and mitochondria from the livers of transgenic mice expressing HCV proteins[67]. Patients with HCV-related HCC have more oxidative stress in the liver and higher levels of serum oxidative stress markers such as 8-hydroxy-2′-deoxyguanosine or reactive oxygen metabolites than those with HBV-related HCC[55,68,69]. A weak, direct carcinogenic effect of HCV core protein might coordinate with oxidative stress, chronic inflammation and damage to apoptosis regeneration cycle DNA to result in hepatocarcinogenesis at a more advanced age than patients with HBV-related infection[70]. Oxidative stress is associated with aging that also drives hepatocarcinogenesis in patients with HCV-related HCC[71].

Serum markers and hepatocyte deposition associated with iron especially in lysosomes are frequently elevated in patients with chronic hepatitis C infection[72]. Reticuloendothelial systems including Kupffer cells are also targets of iron deposition that might affect chronic inflammation[73]. Excess divalent iron atoms are highly toxic, as they induce the Fenton reaction and produce highly toxic ROS, hydroxyl radicals. Because some investigators have reported that phlebotomy and a low-iron diet lowers the risk of HCC developing in patients with chronic hepatitis C infection, iron toxicity is thought to be involved in hepatocarcinogenesis[74,75]. Oxidative stress induced by HCV reduces hepcidin transcription followed by ferroportin expression in enterocytes and increases duodenal iron uptake[72,76]. An excess of dietary iron fed to HCV transgenic mice induces excess hepatocarcinogenesis[77]. Iron and resultant oxidative stress closely correlate with the progress of chronic hepatitis C and related HCC development that should be incorporated as treatment options. As described above, the lipid-related, direct pro-oxidant functions of HCV proteins, especially the core and iron accumulating functions, indicate the importance of the relationship between oxidative stress and hepatocarcinogenesis in patient with chronic hepatitis C.

Mechanisms of NASH-related HCC

The pathophysiology of NASH has been considered to comprise a "two hit" theory[78]. The first hit is hepatocyte steatosis, which is characterized by the accumulation of triglyceride in hepatocytes. The second hit consists of various types of cellular stresses, such as apoptosis, oxidative stress, ER stress, and intestinal circumstances. The recent genome-wide association study discovered that the patatin-like phospholipase 3 (*PNPLA3*) gene correlates with NASH progression[79]. This genetic mechanism might be involved in the first hit and fatty deposition could be the second hit; nonetheless, this two-hit theory is too simple to explain all aspects of

NASH[15,80]. Other studies have found that inflammation induces fatty deposition in hepatocytes**.** Such findings have recently led to a "multiple hit" theory to explain the fact that inflammation promotes steatosis or that genetic factors such as PNPLA3 correlate with disease progression[80]. Lipid droplets were originally thought to function simply as cellular energy-storage structures. However, they now considered to be complicated organelles that are involved in many processes such as metabolic, inflammatory and immunological responses. Lipid toxicity induces multiple hits such as oxidative stress, ER stress, and immune reactions[14]. These cellular stresses are also involved in carcinogenesis.

Obesity-related symptoms such as hypertriglyceridemia and hypertension are established risk factors for NAFLD[81]. Central obesity presenting as visceral fat accumulation is associated with various pathologies such as cerebrovascular diseases, type 2 diabetes, NASH and gastrointestinal cancers. Visceral obesity induces several cytokines including the inflammatory cytokine IL-17[82], which induces neutrophil chemokine expression *via* IL-17 receptor A which is extensively expressed in the liver. Controlling the IL-17-related pathway effectively prevents NASH progression in mouse models[83]. Elevated pre-therapy serum IL-17 levels in patients with HCC correlate with risk of early recurrence after curative hepatectomy[84]. Co-cultured HCC cell lines and T cells producing IL-17 *in vitro* augment the proliferation of HCC cells, suggesting the importance of IL-17 for HCC pathogenesis. Neutrophil infiltration might be involved in NAFLD progression in human NASH. Therefore, visceral adipocytes that accumulate in patients with central obesity are related to such cytokines and should be involved in the pathogenesis of NAFLD.

Visceral fat accumulation correlates with increased adipokine levels, significant risks for HCC and recurrence after curative treatment[85,86]. Adiponectin is a "good" adipokine that modulates many metabolic processes including glucose regulation and fatty acid oxidation. Adiponectin is the most abundant adipokine mainly secreted from mature, white adipose tissue, and levels of expression and secretion increase during adipocyte differentiation. Adiponectin levels inversely correlate with BMI, visceral obesity contents and insulin resistance. Serum adiponectin is significantly higher in females than in males, in whom serum androgens become more evident during puberty[87]. Adiponectin has anti-inflammatory, anti-diabetic and anti-lipid storage effects. Furthermore, weight-loss induces adiponectin synthesis, whereas proinflammatory adipokines such as TNF-α and IL-6 suppress adiponectin[88]. Adiponectin increases the expression of CXCL-8 in primary hepatocytes that functions in cell survival and in anti-apoptotic activities to guard cells; however, this also induces uncontrolled cell survival resulting in malignant transformation[89]. High levels of serum adiponectin are associated with reduced risk for several cancers such that prostate, breast, colorectal and pancreatic cancers[90,91]. However, higher levels of low- and medium-molecular weight adiponectin are also associated with a higher risk of HCC through a relationship with the inflammatory response[92]. Increased levels of adiponectin might be induced *via* a compensatory mechanism to dampen inflammation. However, several studies have also found that adiponectin has direct proinflammatory activities. More evidence is needed to confirm this.

The relationship between hepatic iron deposition and disease progression in NASH remains controversial[93]. Iron accumulation in hepatocytes correlates with more severe damage[94]. The iron metabolic pathway is implicated in the insulin resistance and hepatic cholesterol synthesis pathways. Hepatic lipid-induced ER stress results in an unfolded protein response and hepatic iron accumulation[95]. Several reports describe the risk of iron overload in NASH[96].

TREATMENTS FOR HCC THAT TARGET OXIDATIVE STRESS

Oxidative stress in HCC

Oxidative stress closely correlates with HCV- and NASH-related hepatocarcinogenesis, but relatively weakly with HBV-related HCC. Thus, anti-oxidant therapy would seem reasonable for controlling HCV- and NASH-related hepatocarcinogenesis. Liver inflammation is definitely associated because hepatocarcinogenesis arises mostly in patients with chronic hepatitis. The major inflammatory cytokine TNF-α alters mitochondrial integrity by mimicking a mild uncoupling effect in liver cells, as indicated by a reduction in membrane potential and ATP depletion[97]. The TNF-α induced ROS activation of NF-κB and downstream target genes such as *CXCL1*, *IκBα* and A20 results in enhanced migration activity of hepatoma cell lines. Reduced hepatic inflammation *via* nucleos(t)ide analogues in hepatitis B and interferon in hepatitis C correlates with reduced oxidative stress. However, preventing or controlling HCC using antioxidants is a matter of debate.

Oxidative stress is increased through the generation of ROS and defects in redox defense mechanisms with glutathione (GSH), catalase or superoxide dismutase (SOD)[98]. Mitochondria comprise the most important and abundant source of intracellular ROS. Mitochondrial dysfunction therefore plays a central role in the pathological mechanisms of chronic hepatic inflammation and subsequent hepatocarcinogenesis (Figure 1). An imbalance in the mitochondrial respiratory chain is the main source of ROS, O_2^-, H_2O_2 and hydroxyl radicals (•OH). The transport of high-energy electrons through the mitochondrial electron transport chain (ETC; complexes Ⅰ, Ⅲ and Ⅳ) is an important step for ATP production. This energy-producing pathway also produces ROS. High-energy electrons in ETC complexes Ⅰ-Ⅲ react with O_2 and produce superoxide (O_2^-) which accounts for up to 4%-5% of the consumed O_2. The amount of resulting O_2^- increases in damaged mitochondria. The tricarboxylic acid cycle and the β-oxidation of fatty acids generate reduced

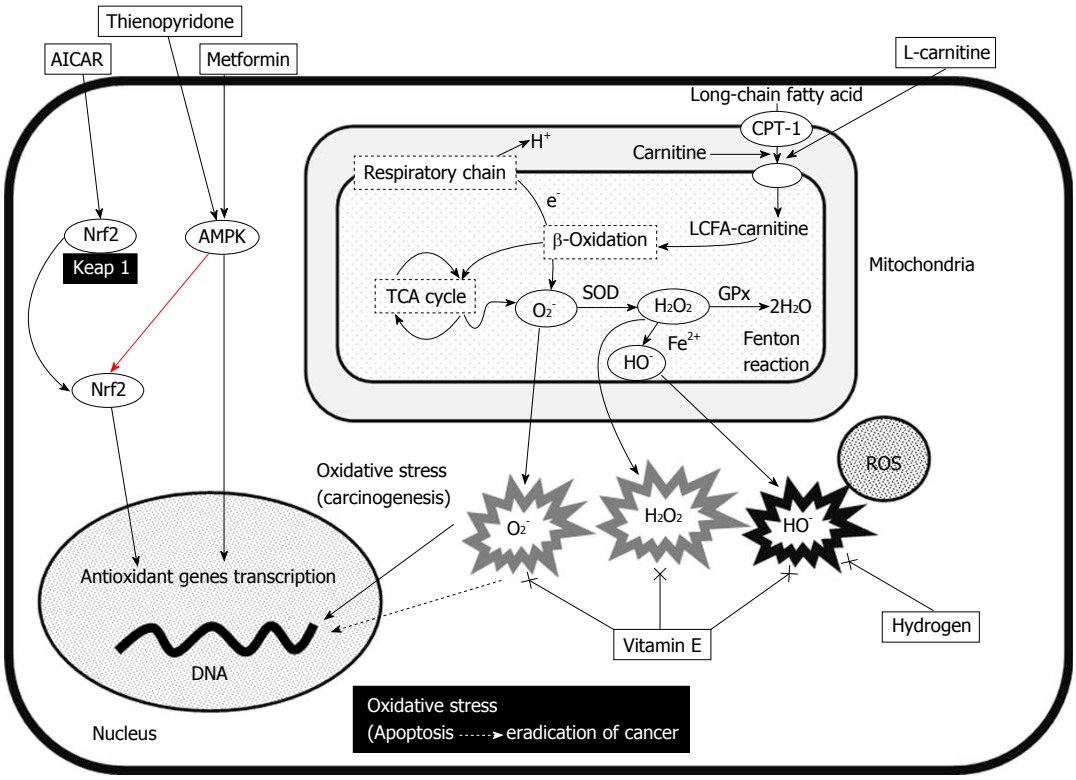

Figure 1 Oxidative stress production and treatment targets in hepatocytes. High levels of plasma free fatty acids increase levels of hepatic free fatty acids. Long-chain fatty acids taken up by mitochondria as complexes with L-carnitine are subsequently metabolized in β-oxidation pathway. Under oxidative stress, oxidative reactions convert oxidized cofactors (NAD^+ and FAD) into reduced cofactors (NADH and FADH2) and deliver electrons to respiratory chain. Imbalance between increased delivery of electrons to, and decreased outflow from respiratory chain causes electrons and ROS products to accumulate. Antioxidant defenses, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) or catalase can metabolize O_2^- and H_2O_2 to non-toxic H_2O. However, Fenton and/or Haber-Weiss reactions generate highly reactive, toxic, hydroxyl radicals (•OH). Vitamin E and hydrogen as general and selective cytotoxic ROS scavengers erase oxidative stress. L-carnitine supports mitochondrial function to increase long-chain fatty acid uptake. Metformin or thienopyridone activates AMPK and induces antioxidant gene transcription and AICAR activates Nrf2, possibly like metformin. AICAR: 5-aminoimidazole-4-carboxamide-1-b-D-ribofuranoside; AMPK: AMP-activated protein kinase; Keap1: Kelch-like ECH associating protein; Nrf2: Nuclear factor erythroid 2-related factor.

(NADH and FADH2) cofactors from oxidized (NAD^+ and FAD) cofactors. The NADH uses two electrons from the oxidation pathway in the inner membrane to supply the rest of the ETC for the reduction of O_2 to water. As the substrates and cofactors of complex I have the lowest reduction potential in the mitochondrial respiratory chain, side reactions could occur such as nonspecific electron transfer to species in solution. Under physiological conditions, reactive and incompletely reduced forms of oxygen such as superoxide (O_2^-) are detoxified into water by anti-oxidant defenses and repair enzymes to maintain a low steady state of toxic oxidants[14].

The mitochondrial capacity to control the oxidative balance would be destroyed under conditions of continuous oxidative stress. Excess O_2^- could be produced within damaged mitochondria through electron leakage and SOD would convert the resulting excess O_2^- into H_2O_2. Glutathione plays an important role in the balance between pro- and anti-oxidants correlating with the detoxification of H_2O_2 and other ROS. Glutathione-dependent enzymes as glutathione peroxidase, glutathione reductase (GR), and glutathione S-transferase (GST) play important roles in the stimulation of glutathione function. Glutathione peroxidase catalyzes H_2O_2 to non-toxic H_2O, GR regenerates the pool of reduced glutathione and GST catalyzes glutathione reactions. Despite such anti-oxidant mechanisms, the Fenton and/or Haber-Weiss reactions generate the highly reactive toxic ROS, •OH from H_2O_2 and oxidative stress produced by Fenton reactions is mediated by iron.

Iron loading of the liver causes further hepatic oxidative stress that is a main cause of HCC[99]. Iron overload can induce hepatocarcinogenesis in animals and is associated with the high incidence of HCC among African and Taiwanese individuals[100,101]. Iron depletion in HCV- and NASH-related hepatocarcinogenesis should play a more important role in the prevention of HCC.

Preferential antioxidative drugs to treat HCC

Several antioxidant agents and foods are widely available and the effects of several of them *in vitro* and *in vivo* are under investigation. The optimal choice of agents to control chronic hepatitis or related hepatocarcinogenesis without suppressing the physiological roles of oxidative stress is difficult to determine.

Phlebotomy is a method of reducing iron overload that has been effective against chronic hepatitis C and NASH in selected patients[102,103]. Iron depletion improves not only iron overload but also insulin resistance, suggesting that iron toxicity is involved in several metabolic pathways[104].

Iron depletion for NASH remains a matter of debate but it could nevertheless function as an antioxidant treatment strategy.

Candidate antioxidant treatments for HCC include antioxidant genes, inducible transcriptional factors such as AMP-activated protein kinase (AMPK) or nuclear factor erythroid 2-related factor (Nrf2) activators, ROS scavengers and agents that support mitochondrial uptake. The following are representative drugs that are associated with reducing oxidative stress.

Metformin: Metformin is an anti-diabetic drug, but it also has an anti-oxidative function. Metformin increases intracellular levels of AMP after activating AMPK, which is a highly conserved heterodimeric serine-threonine kinase that serves as an energy sensor in eukaryotic cells and bridges metabolism to carcinogenesis[105]. It is activated by an increase in the cellular AMP/ATP ratio under hypoglycemia, hypoxia, ischemia and heat shock[106]. The activation of AMPK suppresses cell proliferation in non-malignant and malignant cells *via* regulation of the cell cycle, apoptosis, autophagy and the inhibition of fatty acid synthesis[107]. Phospho (p)-AMPK is down-regulated in HCC tissues from patients and low levels of p-AMPK expression correlates with a poor prognosis, indicating the importance of AMPK signaling in HCC[108]. Adding metformin to hepatoma cell lines results in AMPK activation as well as dose- and time-dependent growth inhibition. Metformin also induces cell-cycle block, apoptosis, STAT3-induced IL-6 production[109] and the antioxidant enzyme heme oxygenase-1 (HO-1) in human endothelial cells *via* the Nrf2 signaling pathway[110]. The recently-discovered direct AMPK activator thienopyridone also activates AMPK through distinct mechanisms with metformin[111]. This might be a future approach to activate AMPK.

A meta-analysis of anti-diabetic drugs found that metformin, sulfonylurea and insulin induce a 50% reduction, and 62% and 161% increases, respectively in HCC incidence[112]. However, randomized controlled trials have not shown significant effects. Metformin reduced the occurrence of HCC and liver-related death, and increased survival rates in patients with diabetes and HCC who underwent radiofrequency ablation without any severe side effects[113]. The standard treatment for NASH according to the guidelines of the American Association for the Study of Liver Disease (AASLD) is vitamin E. This was derived from the findings of a clinical trial that has shown improvements in the clinical profile and histological findings of NASH activity within two years[114]. Metformin improves histological activity in the livers of mouse models of non-diabetic NASH, but not in human NASH[115]. Thus, metformin is preferential for treating NASH-related HCC in mouse models[116], but clinical trials are needed to confirm the effects of metformin on HCC management.

Nrf2-acting agents: Under basal conditions, Nrf2 binds to Kelch-like ECH associating protein 1 (Keap 1) which exists in the cytoplasm in an inactive form[117]. The AMPK activator 5-aminoimidazole-4-carboxamide-1-b-D-ribofuranoside (AICAR) induces an increase in Nrf2 protein and antioxidant enzyme expression in endothelial cells, whereas AICAR activates Nrf2 in hepatoma cell lines resulting in antioxidant enzyme expression[118]. A combination of metformin and AICAR might activate AMPK and Nrf2 to control HCC. A more detailed analysis in a basic approach with clinical trials might be needed to determine the value of this new type of drug.

Vitamin E: Controversy surrounds the value of ROS-scavenging agents because ROS have essential functions for life. Scavengers of ROS consistently exert effective chemical activities *in vitro*, but not often *in vivo*[18]. Scavenging ROS is considered effective in preventing the development of cancer and cancer stemness, yet ROS also contribute to the prevention of cancer[119]. Stem cell-like cancer cells that express the CD44 variant have an antioxidative phenotype. The CD44 variant protects cancer stem-like cells from oxidative stress and prevents their apoptosis[120]. Oxidative stress upon normal cells might induce transition to a cancer cell phenotype that is highly resistant to further oxidative stress. Several clinical trials are presently investigating the induction of oxidative stress under these conditions as a cancer treatment[121]. The AASLD recommends treating NASH with vitamin E at a dose of 800 IU/d, which is higher than that usually administered to treat NASH[17]. This recommendation is based on a two-year randomized study of NASH that demonstrated improved alanine transaminase and histological activity[114]. However, that trial did not find an improvement in liver fibrosis[122]. Further investigations of longer duration are required to determine the effects of vitamin E on hepatocarcinogenesis.

Peroxisome proliferator-activator-γ agonist (pioglitazone): The transcription factor peroxisome proliferator-activator (PPAR)γ regulates lipid metabolism and its activation suppresses hepatic lipoperoxide and reduces the production of the hepatic pro-inflammatory cytokines, TNF-α and IL-6[123]. The effects of PPARγ on a model of diethylnitrosamine (DEN)-induced HCC *via* cyclin-dependent kinase inhibitor p27 expression are favorable[124]. Pioglitazone serve as an anti-oxidant to treat NASH. A large clinical study has shown fair results of using pioglitazone to treat NASH with respect to serum alanine aminotransferase levels[114]. Although an improvement in histological activity was not proven, this drug will be assessed for patients with NASH-related diabetes. A long observational assessment of hepatocarcinogenesis will be needed later.

Hydrogen: Molecular hydrogen (H_2) has powerful and selective antioxidant effects with unique features[125]. Hydrogen scavenges toxic hydroxyl radicals, but not O_2^-, H_2O_2 or nitric oxide in cultured cells. This selective reduction of ROS has been explained by the strong

oxidative activity of hydroxyl radicals reacting with mild anti-oxidative function of hydrogen. The easy distribution of hydrogen is one characteristic of the effects. Most hydrophilic compounds are retained at membranes and cannot pass into the cytoplasm, whereas hydrophobic compounds such as vitamin E need specific carriers or receptors to penetrate biomembranes. However, H_2 can penetrate biomembranes, diffuse into the cytosol and easily reach the nucleus where it can protect nuclear DNA and prevent mitochondrial damage. Hydrogen is effective against DEN-induced HCC and HCC associated with type 1 diabetes and NASH[126,127]. Data from patients with NASH are not yet available and thus clinical trials are required to further evaluate the effects of molecular hydrogen on HCC.

L-carnitine: L-carnitine is an essential nutrient that converts fat into energy in mitochondria and ameliorates liver damage. It acts as a fatty acid carrier across the mitochondrial membrane and it also exists as free or acyl forms in plasma[128]. L-carnitine plays an important role in lipid metabolism as it is an essential cofactor for the β-oxidation of fatty acids through facilitating the transport of long-chain fatty acids and its ability to activate carnitine palmitoyltransferase, the key enzyme in fatty acid oxidation[129]. L-carnitine has recently been proposed as treatment for various diseases, including liver damage. Several studies have shown that L-carnitine can ameliorate or prevent liver damage with various etiologies. Animal studies have shown that dietary supplementation with L-carnitine prevents chemically induced hepatitis and subsequent HCC, as well as NASH-related HCC[130,131]. L-carnitine supplementation greatly improves plasma glucose levels in patients with NASH, lipid profiles and histological manifestations[132]. The results of these clinical trials were fair, and more clinical trials of larger populations are required to further evaluate the effects of L-carnitine on hepatocarcinogenesis.

CONCLUSION

Because oxidative stress is an essential survival mechanism, erasing it is not a feasible approach to disease control. Rather, controlling oxidative stress should be effective because hepatocarcinogenesis is closely associated with increased oxidative stress *via* viral proteins or chronic inflammation and lipids. Agents that can control oxidative stress such as the AMPK activator metformin or the mitochondrial support agent L-carnitine probably comprise a more effective approach than ROS scavengers such as vitamin E.

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REVIEW

Immunotherapy for hepatocellular carcinoma: From basic research to clinical use

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Abstract

Hepatocellular carcinoma (HCC) is a common cancer worldwide with a poor prognosis. Few strategies have been proven efficient in HCC treatment, particularly for those patients not indicated for curative resection or transplantation. Immunotherapy has been developed for decades for cancer control and is attaining more attention as a result of encouraging outcomes of new strategies such as chimeric antigen receptor T cells and immune checkpoint blockade. Right at the front of the new era of immunotherapy, we review the immunotherapy in HCC treatment, from basic research to clinical trials, covering anything from immunomodulators, tumor vaccines and adoptive immunotherapy. The mechanisms, efficacy and safety as well as the approach particulars are unveiled to assist readers to gain a concise but extensive understanding of immunotherapy of HCC.

Key words: Interferon; Chemokine; Tumor vaccine; Adoptive immunotherapy; Chimeric antigen receptor; Checkpoint blockade

Core tip: This paper supplies a comprehensive review of immunotherapy for hepatocellular carcinoma from basic experiments to clinical trials. The development of interferon, chemokines, tumor vaccines, adoptive immunotherapy, including natural killer, natural killer T and T cells armed with chimeric antigen receptor, as well as regulatory T cell is summarized.

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INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for 95%

of primary liver cancer[1], the second most common cause of cancer-associated death worldwide and estimated to be responsible for around 746000 deaths in 2012[2]. Only liver resection and liver transplantation are considered curative, with poor efficiency of other modalities such as radiofrequency ablation (RFA) and transarterial chemoembolization (TACE). However, very few patients are indicated for liver resection and donors of liver are usually exceptional. Postoperative recurrence frequently occurs, resulting in a dismal prognosis in most affected individuals[3].

Immunotherapy has been explored in HCC for decades[4] and carries high expectations due to the recent progress in other malignancies such as melanoma. Different from other organs, liver shows its distinguished characteristics, such as an "immune organ", and patients with HCC present with unique anti- or pro-tumor responses during the development and progression of HCC[5]. Immunotherapy can be categorized into several types according to their distinct strategies. For instance, immunomodulators and tumor vaccines are used to enhance the immune response to HCC in an indirect way; adoptive immunotherapy introduces a great amount of effective immune cells to directly remove tumor cells. In this review, we summarize the critical immune characteristics of liver and cover the immunotherapy of HCC in animal models as well as clinical trials (Figure 1).

ROLES OF THE IMMUNE SYSTEM IN CARCINOGENESIS AND PROGRESSION OF HCC

The inherent immune tolerance of liver hinders immune surveillance and therefore makes the carcinogenesis of HCC possible. Liver confronts abundant xenogenous antigens within blood from the gut *via* the portal vein. Specific mechanisms with regards to immune tolerance are activated to inhibit unneeded immune responses. Unfortunately, these mechanisms, such as recruitment of regulatory T cells (Tregs) and myeloid-derived suppressor cells, as well as overexpression of inhibitory ligands such as programmed death ligand 1 (PD-L1), contribute to weaken anti-tumor immune responses[6].

As an "immune privileged" organ, multiple pathways exist within liver to maintain its function. Not only can hepatocytes cause T cell anergy in certain conditions, but many other nonparenchymal cells, including stellate cells, hepatic dendritic cells and liver sinusoidal endothelial cells, also induce tolerance or apoptosis of effective or naive T cells. In particular, HCC lacks major histocompatibility complex (MHC) class Ⅱ, the activator of $CD4^+$ T helper (Th) cells[6].

Clinically, various cytokines dysregulate and contribute to HCC progression[7]. Increased immunosuppressed cells in patients are in parallel with a poorer prognosis. Th17 and its secretory product interleukin 17 (IL-17) promote angiogenesis of HCC and recruit neutrophils to enhance angiogenesis[8,9]. The effector function of $CD8^+$ T cells is prone to be impaired by increased Tregs, which predicts a poor prognosis of HCC patients[10]. In addition, the functional impairment of other cells like natural killer (NK) cells also contributes to tumor progression[11].

IMMUNOMODULATORS

Cytokines

Various cytokines are involved in immune responses. Certain cytokines can directly inhibit tumor cell growth or enhance the capacity of relevant immune cells to delay tumor development.

Interferon is well known for immunomodulation, antiproliferation and anti-angiogenesis. They were found to be decreased in serum of patients with HCC. All three types of interferon (IFN-α, IFN-β, IFN-γ) have been proved to be effective in inhibiting HCC by inducing tumor cell apoptosis or autophagy[12-14]. However, the efficiencies among different types of interferon are under debate. Although some investigators have addressed that IFN-β showed better anti-HCC effects[15], the use of IFN-α in HCC treatment have been more frequently reported. However, IFN-α alone did not show satisfactory survival benefit in patients with unresectable HCC, confirmed by randomized controlled trials (RCTs)[16,17]. IFN-α-2b also failed to decrease the risk of postoperative recurrence[18]. In contrast, interferon showed some benefits when combined with other modalities such as chemotherapy, curative resection and TACE[19-22]. Two meta-analyses revealed that adjuvant interferon therapy after curative therapy for HCC could improve both overall survival and recurrence-free survival[23,24]. Combination of IFN-α with sorafenib was also reported to be efficient in a mouse model[25] but this has not been tested in humans. In addition, interferon treatment may gain further benefits for HCC patients with hepatitis B or C virus infection from removing the viruses[26,27]. Currently, two registered clinical trials regarding IFN-α are still recruiting participants. One multicenter RCT is planning to test IFN-α as an adjuvant therapy in HCC with low miR-26 expression (NCT011681446) and the other phase Ⅱ trial is trying to combine IFN-α with fluorouracil to treat HCC patients who underwent liver resection (NCT01834963). Generally, IFN-α showed demonstrated equivocal effects and should only be applied to selected patients as supportive or adjuvant therapy within the assumption of the current evidence.

ILs

ILs have been applied to enhance anti-tumor responses of the immune system. However, few studies concerning these ILs have been performed in humans. Small scale clinical studies evaluated the efficacies of IL-2 or IL-12 alone or combined together in HCC treatment but the results were inconclusive[28,29]. Other sorts of ILs were

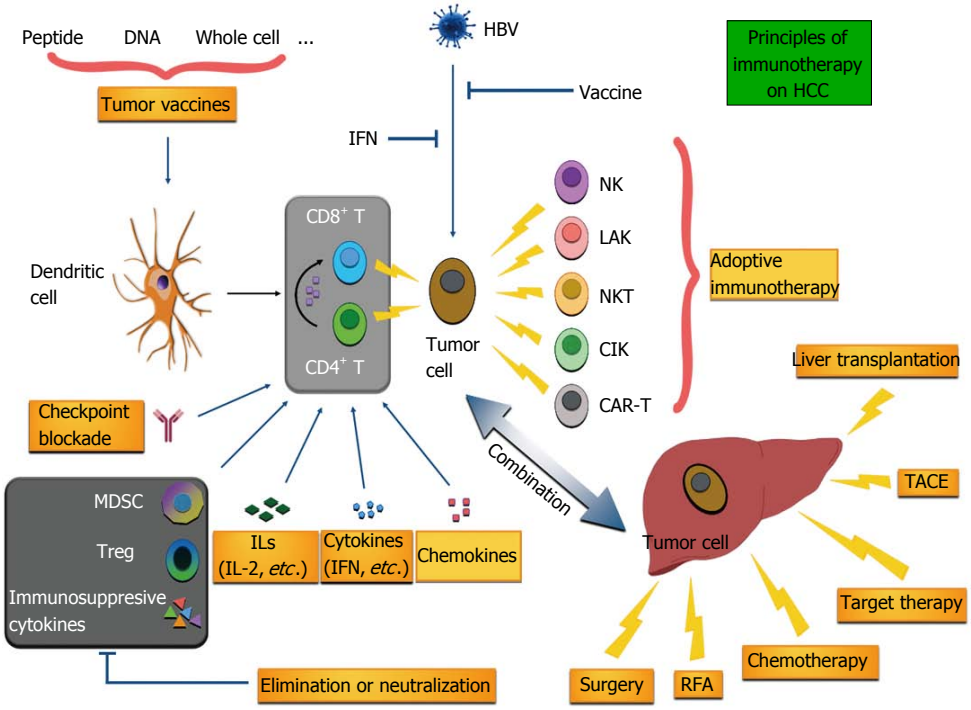

Figure 1 Principles of immunotherapy on hepatocellular carcinoma. NK: Natural killer; LAK: Lymphokine-activated killer; NKT: Natural killer T; CIK: Chemokine-induced killer; CAR-T: Chimeric antigen receptor-T; HBV: Hepatitis B virus; MDSC: Myeloid-derived suppressor cells; Treg: Regulatory T; IL-2: Interleukin 2; IFN: Interferon; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; HCC: Hepatocellular carcinoma.

also studied. For instance, IL-37 was found to selectively recruit NK cells to conduct anti-tumor activity in HCC patients[30]. IL-24 was reported to show *in vivo* anti-tumor activity in the presence of apoptin[31]. However, clinical trials are lacking to determine the therapeutic effects of ILs in human.

Chemokines

Chemokines regulate activities and behavior of cells, including hepatocytes, immune cells and the tumor micro-environment. By mediating pro- and anti-inflammatory responses, chemokines can regulate leukocyte recruitment, angiogenesis and tumor progression[32]. Several chemokine-associated signaling, including CXCR4/CXCL12, CCR6/CCL20 axes, were evident in promoting HCC[33,34]. Blockade of these signalings by relevant receptors seems logical to control HCC.

Chemokines can regulate the function of immune cells by interacting with the receptors on the membrane of these cells. Tumor infiltrating anti-tumor cells, including T cells, NK cells and natural killer T (NKT) cells, showed enhanced expression of certain chemokine receptors[35]. Consistently, overexpression of certain chemokine genes, such as CXCL10, CCL5 and CCL2, in HCC tissue correlated with Th1, cytolytic T lymphocyte (CTL) and NK cells and predicted a better prognosis[36]. Regulated release of chemokines or genetic modification of chemokine receptors in immune cells may enhance anti-tumor immune response. Unfortunately, only preclinical data can be found in this field.

IMMUNE CHECKPOINT BLOCKADE THERAPY

Due to the great achievements in melanoma, immune checkpoint blockade therapy sheds light on other solid tumors, including HCC. Co-inhibitory signals diminish the intensity of anti-tumor response even although HCC specific antigen has presented with MHC receptors. To overcome these, immune checkpoints should be a promising approach to restore anti-tumor function of immune cells. Many immune checkpoints have been identified in the lab. In addition to PD-1 and CTLA-4 which have been intensively studied, there are also other potential checkpoints, like TIM-3, OX40, VISTA, LAG-3 and BTLA[37].

Anti-CTLA-4 antibody blocks the binding of CTLA-4 and CD80/86, which is a defunct antigen-presenting cell (APC) and results in suppressed anti-tumor immune responses mediated by T cells[38,39]. Basically, the efficacy of CTLA-4 blockade correlates with the immunogenicity of the tumor. A phase Ⅰ trial of tremelimumab (anti-CTLA-4 monoclonal antibody) in HCC patients was reported in 2013[40]. The study enrolled 21 patients with advanced HCC not amenable to percutaneous ablation or TACE and showed that tremelimumab was well tolerated. Partial response was found in 17.6% of the patients and 45% of the cases had stable disease for more than 6 mo. Another phase Ⅰ clinical trial of tremelimumab combined with RFA or TACE is now ongoing (NCT01853618; Table 1).

Table 1 Information of clinical trials of checkpoint blockade on hepatocellular carcinoma after 2008

Interventions	Design	Start year	Main inclusion criteria	Primary outcomes	Registered No.	Status
CP-675,206: anti-CTLA-4 antibody	Phase Ⅱ	2008	Unresectable disease not amenable to loco regional treatment, HCV chronic infection	Tumor response	NCT01008358	Completed
CT-011 (Pidilizumab): anti-PD-1 antibody	Phase Ⅰ/Ⅱ	2009	HCC not eligible for surgery, TACE, or other systematic treatments	Safety and tolerability	NCT00966251	Terminated because of slow accrual
Nivolumab: anti-PD-1 antibody	Phase Ⅰ	2012	Advanced HCC, failed in previous one line therapy	Adverse events	NCT01658878	Recruiting
Tremelimumab: anti-CTLA-4 antibody, combined with TACE or RFA	Phase Ⅰ	2013	Not amenable to curative resection, transplantation or ablation	Safety and feasibility	NCT01853618	Recruiting

HCV: Hepatitis C virus; RFA: Radiofrequency ablation; TACE: Transarterial chemoembolization; HCC: Hepatocellular carcinoma; PD-1: Programmed death 1.

Anti-PD-1 and anti-PD-L1 antibodies interfere with the binding of PD-1 and PD-L1/2 which inhibits T cell proliferation and cytokine release[41]. Although CTLA-4 and PD-1 were found predominantly expressed in T cells with anti-tumor function[42,43] and showed similar effects when used alone, different mechanisms and indicated patients regarding the two pathways were suggested by clinical observations[44]. Unfortunately, the phase Ⅰ/Ⅱ trial (NCT00966251) of a new PD-1 blockade CT-011 was terminated because of slow accrual. Another phase Ⅰ trial of nivolumab (anti-PD-1 monoclonal antibody) is ongoing. This trial plans to recruit three cohorts of patients stratified by viral infection hepatitis C virus, hepatitis B virus (HBV) and no viral infection.

With the satisfactory effects in animal models, more and more trials are being conducted to investigate the role of immune checkpoint blockade therapy in HCC treatment (Table 1). Immune checkpoint blockade therapy is considered to be a strategy with a bright future and is cast into the limelight by oncologists worldwide.

TUMOR VACCINES

Although prophylactic vaccines for HCC such as HBV vaccine contributed to the decrease of HCC patients[45], a therapeutic vaccine for HCC is still awaited. Numerous approaches have been investigated, seeking to trigger the host immune system to remove cancer cells. The most important constraint for progression of a tumor vaccine is the lack of tumor specific antigens or tumor associated antigens (TAA). With all the evolving understanding of tumor heterogeneity, it appears to be unprecedentedly challenging to exterminate cancer cells by a tumor vaccine alone. On the other hand, it could possibly be an effective approach to exhibit positive aspects in certain patients and play important roles in regimens.

Peptide-based vaccine

Alpha-fetoprotein (AFP) and glypican-3 (GPC3) are two frequently used TAAs in HCC vaccines. GPC3 can be overexpressed in more than 80% of HCC and AFP can be positive in 60%-80% of HCC. Other HCC biomarkers that might be candidates for a vaccine involving antigens include squamous cell carcinoma antigen, heat shock protein 70, NY-ESO-1b, *etc*[46,47].

AFP is rare in healthy adults but can be highly expressed in HCC, making it an ideal target for anti-HCC immunotherapy. Actually, AFP is currently the most well studied target antigen for HCC immunotherapy. The earliest relevant clinical trial was more than a decade ago, testing AFP-specific T cell response to an AFP-derived peptide in six patients[48]. GPC3 (144-152) (FVGEFFTDV) and GPC3 (298-306) (EYILSLEEL) peptides were proven to induce specific $CD8^+$ CTLs in HCC patients with HLA-A2 and HLA-A24 restriction, respectively[49]. Encouraged by this result, a phase Ⅱ trial of a GPC3-based vaccine as adjuvant therapy for patients after surgery or RFA was registered and is now ongoing (UMIN-CTR: 000002614).

Proper design of epitopes with cross-recognition of wild-type antigens can enhance immune responses. To overcome the limitation of weak immune responses induced by native TAAs, Hong *et al*[50] created a highly immunogenic AFP *via* computer-guided methodical epitope optimization. This genetic modified AFP vaccine showed amazing anti-tumor effects in xenograft and diethylnitrosamine-induced mouse model of HCC by means of activating $CD8^+$ T cells[50]. Polypeptide or fusion peptide was another method to amplify anti-tumor immune responses. A combination of full-length HBV core protein, HBV-X protein $(HBx)_{52\text{-}60}$, $HBx_{140\text{-}148}$, $AFP_{158\text{-}166}$ and melanoma antigen gene-$A_{271\text{-}279}$ is an example for a HCC vaccine[51]. Innovative creation of a fusion peptide containing different epitopes that involve multiple steps of the immune response was also proved to inhibit HCC in animals[52].

For a peptide-based tumor vaccine, the choice of peptide is critical for clinical response. Not all proteins that contribute to tumor progression are suitable for vaccine development. For instance, although expression and activity of telomerase was found up-regulated in most HCCs[53,54], telomerase peptide did not lead to any complete or partial responses in a phase Ⅱ study on advanced HCC[55]. Additionally, the origin of the peptide

affects induction efficiency of CLTs and consequent anti-tumor effects. Peptides originated from endogenously presented antigen are thought to be sparse on tumor cells and inefficient in inducing CTLs[56]. Intratumoral peptide injection was thus developed to enhance tumor cell antigenicity[56]; however, this needs further investigation.

DNA-based vaccine

A DNA-based vaccine assumes that DNA directly injected into the body undergoes transcription and translation in host cells and that the expressed peptide induces immune responses. Theoretically, all peptide vaccines can be transformed into DNA vaccines. AFP and GPC3 DNA vaccines were both developed and tested in the lab and showed tumor growth inhibition and survival improvement in mouse models[57-59]. In a recent small scale clinical observation, two HCC (stage Ⅱ) patients after locoregional therapy underwent AFP DNA vaccine and adenovirus boost immunization. This approach was confirmed to be safe and well tolerated; however, both patients experienced HCC recurrence after a mere nine and eighteen months, respectively[60]. To our best information, no clinical trial regarding DNA vaccines on HCC has been reported or is currently ongoing.

Tumor vaccine using antigen-presenting cells

APCs play a key role in anti-tumor function of immune responses. Dendritic cells (DCs) are the most potent APC and are closely related to HCC. Numerous studies have proved that DCs from peripheral blood and lymph nodes of HCC patients were decreased, with an immature phenotype and an impaired function[61-63]. A study revealed that the more DCs that were detected in HCC nodules, the better the prognosis would be. Infiltration of DCs in HCC nodules was strongly associated with the prognosis of HCC patients after surgical resection[64]. The composition of DCs in the hepatic lymph nodes of HCC patients was aberrant which may be one of the causes of the inadequate T cell response against HCC in these patients[65]. In addition, some tumor-derived factors, such as vascular endothelial growth factor, granulocyte macrophage colony-stimulating factor, IL-6 and IL-10, influence the differentiation, number and phenotype of DCs[66].

Given the importance in cancer development, DCs are increasingly applied to vaccination in various cancers, including HCC. A DC-based vaccine was reported to not only induce tumor antigen-specific CTLs[67], but also to activate NK cells and inhibit Tregs in HCC patients[51,68]. Logically, DCs pulsed with tumor tissue of an individual patient should be used. However, the tumor tissue is not always available. Therefore, peptides or cell line lysate was commonly used to substitute tumor tissue by many investigators. At least in HCC, this replacement strategy proved to be feasible and safe[69]. DCs infused with cancer cells, transfected with total RNA of cancer cells or transfected with designed plasmids were all able to mature and prime Th1 cells and CTLs[69-71].

Based upon these characteristics and success in preclinical studies, many clinical trials were carried out to evaluate the efficacy of DC-based immunotherapy to treat HCC patients. Two phase Ⅰ studies showed immunization by tumor lysate pulsed DCs was feasible for end stage HCC patients[72,73]. Another clinical trial of a DC vaccine pulsed with autologous tumor lysate addressed that 12.9% of advanced HCC patients had partial response (PR) and 54.8% had SD[74]. Notably, a monthly boost vaccination resulted in a significantly better 1 year survival[74]. In another RCT on advanced HCC, DCs pulsed with HepG2 cell lysate resulted in 13.3% patients with PR and 60% with SD after 6 mo of treatment[75]. However, the proportion of clinical response with this therapy is relatively low. As an illustration, one phase Ⅱ study using DCs pulsed with tumor lysate in HCC revealed only one out of 39 patients exhibited PR[76]. Furthermore, a phase Ⅰ/Ⅱ study using a multiple TAA-pulsed DC vaccine showed clinical response in only one out of five patients with advanced HCC[77]. Some studies also evaluated the efficacy of DC immunotherapy combined with local radiation[78] or TACE[79,80] but the results showed that DC infusion could not prevent HCC recurrence. Therefore, further studies are needed to increase the efficacy of this therapeutic approach. A new phase Ⅰ trial on DC vaccine for HCC was registered last year and is now recruiting participants (NCT 01974661; Table 2).

ADOPTIVE IMMUNOTHERAPY

Adoptive immunotherapy is now promising in the scenario of potential approaches for the treatment of solid tumors which are refractory to conventional therapies. An increasing amount of the literature discusses the efficacy of adoptive immunotherapy to control tumors. Meanwhile, many clinical trials have demonstrated that adoptive immunotherapy showed potentially promising anti-tumor effects on various cancers, including HCC.

Lymphokine-activated killer cells

First reported in the early 1980s, lymphokine-activated killer (LAK) cells are cytotoxic effector lymphocytes whose cytolytic activities are not restricted by MHC and are capable of killing tumor cells as well as NK-resistant tumor cell lines. There are actually constrained studies concerning the effectiveness of LAK cells for HCC treatment. A report stated that dealing with LAK cells cultivated by IL-2 reduced the recurrence rate in postoperative HCC patients[81]. Another study found that LAK cell-based immunotherapy was not an ideal adjuvant strategy after hepatic resection[82]. Results from a clinical trial indicated that tumor-specific CTL therapy is more effective than LAK cell therapy in advanced HCC patients[83]. The enthusiasm of study on

Table 2 Information of clinical trials of tumor vaccine on hepatocellular carcinoma after 2008

Interventions	Design	Start year	Main inclusion criteria	Primary outcomes	Registered No.	Status
AFP + GM-CSF plasmid prime and AFP adenoviral vector boost	Phase Ⅰ/Ⅱ	2008	Locoregionally treated HCC	Dose, toxicity, and immunological response rate	NCT00669136	Terminated because of poor accrual
DC loaded with autologous tumor	Phase Ⅱ	2008	Metastatic HCC, available of tumor tissue	2-mo response rate	NCT00610389	Unknown
DC loaded with specific peptides of AFP	Phase Ⅰ/Ⅱ	2009	Patients with previous treatment, AFP ⩾ 40 ng/mL, HLA A 0201 group	Adverse events	NCT01128803	Terminated
DEC-205-NY-ESO-1 fusion protein vaccine	Phase Ⅰ	2012	After resection and TACE for HCC	Adverse events	NCT01522820	Recruiting
COMBIG-DC: allogeneic DC cancer vaccine	Phase Ⅰ	2013	Not eligible for curative treatment or TACE, BCLC stage B and C	Adverse events	NCT01974661	Recruiting
In-situ therapeutic cancer vaccine	Phase Ⅰ	2013	Refractory HCC, not eligible for or failed any treatment, AFP > 30	Safety	NCT01923233	Recruiting
V5 therapeutic vaccine	Phase Ⅲ	2014	Advanced HCC	Changes in plasma AFP	NCT02232490	Not yet recruiting

AFP: α-fetoprotein; DC: Dendritic cell; GM-CSF: Granulocyte macrophage colony-stimulating factor; HCC: Hepatocellular carcinoma; HLA: Human leukocyte antigen; TACE: Transarterial chemoembolization; BCLC: Barcelona clinic liver cancer classification.

LAK for HCC treatment has significantly declined over the last decade.

Cytokine-induced killer cells

Cytokine-induced killer (CIK) cells exhibit potent, non-MHC restricted cytolytic activities against susceptible tumor cells of both autologous and allogeneic origins[84]. CIK cells are characterized by expression of both T cell biomarker CD3 and NK cell biomarker CD56[85] and can be generated from the human peripheral blood mononuclear cells (PBMC) induced by IFN-γ, anti-CD3 antibody and IL-2[84,86,87].

Compared with other immune cells, CIK cells possess some advantages. Firstly, CIK cells have a higher proliferation rate and can be obtained from cancer patients by *in vitro* culture[88]. Secondly, CIK cells have strong cytolytic activities and cover a broad spectrum of targeted tumors, including those that are insusceptible to LAK cells or NK cells[89]. Finally, CIK cells show minimal toxicity and do not cause graft-*vs*-host disease[84,90]. These merits make CIK cells a preferential adoptive immunotherapy for selected cancer patients[91].

Recently, escalating proof from clinical trials demonstrated that CIK cells adoptive transfer demonstrated a substantial anti-tumor effect in patients with solid tumors and hematological malignancies[92-94]. Some reports showed that CIK adjuvant immunotherapy significantly improved the outcomes of HCC patients[95-101]. In these studies, CIK cell transfusion reduced the relapse rate of HCC in patients after TACE and RFA therapy and prolonged the disease-free survival and OS for HCC patients after radical resection or TACE. A meta-analysis including 13 RCTs evaluated the efficacy of CIK immunotherapy in the treatment of HCC and revealed a significant superiority in prolonging the OS and progressive-free survival of patients[102]. Recently, another meta-analysis assessed the efficacy of CIK therapy after TACE or TACE plus RFA and showed that CIK therapy combined with TACE plus RFA treatment was associated with a higher 1 year recurrent free survival rate and 1, 2 year OS rates[103]. However, due to the limited number of patients in this field, the efficacy of CIK immunotherapy for HCC is still not convincing.

CIK cells are an encouraging tool within cancer adoptive immunotherapy but more basic research and clinical trials with high quality are urgently desired. To date, we have witnessed at least four ongoing registered clinical trials regarding CIK therapy for HCC (Table 3).

NK cells

NK cells belong to the innate immune system, predominantly reside in the liver and play a critical role in the host defense against tumorigenesis[104,105]. Carcinogenesis is under close surveillance of NK cells and other members of immune system. In addition to the capability of killing tumor cells directly, NK cells are able to release immunomodulatory cytokines which can activate leukocytes of both the innate and adaptive immune system[105]. Unfortunately, cytotoxicity of NK cells was significantly inhibited in patients with advanced HCC[104]. In line with this, NK cells derived from HCC patients displayed a reduced cytotoxicity against HCC cell lines after stimulation with IL-2 *in vivo*[106]. The authors suggested that functional defects of NK cells might be responsible for the failure of anti-tumor immune responses. The NK cells from HCC patients were also impaired in their IFN-γ production and cytotoxicity and this functional impairment was found to be associated with increased Tregs[107]. Meanwhile, myeloid-derived suppressor cells inhibited NK cell cytotoxicity and cytokine secretion[108]. This evidence suggested that HCC patients could benefit from reactivation of NK cells. In a mouse model, administration of IL-12 and IL-18 increased NK cells in the liver and resulted in

Table 3 Information of clinical trials of adoptive therapy on hepatocellular carcinoma after 2008

Interventions	Design	Start year	Main inclusion criteria	Primary outcomes	Registered No.	Status
Immuncell-LC: activated T lymphocyte	Phase Ⅲ	2008	Stage Ⅰ and Ⅱ, complete resection within 12 wk	Efficacy and safety	NCT00699816	Completed
CIK	Phase Ⅲ	2008	After radical resection of HCC, no prior anti-cancer therapy	Time to recurrence	NCT01749865	Recruiting
CIK	Phase Ⅲ	2008	After radical resection, no prior anti-cancer treatment	Time to recurrence	NCT00769106	Recruiting
Ex vivo expanded autologous immune killer cell, combined with TACE	Phase Ⅱ/Ⅲ	2009	Never receive TACE treatment, BCLC stage B and C	2-yr reduction of tumor cells	NCT01024530	Unknown
NK cells, combined with liver transplantation	Phase Ⅰ	2010	After liver transplantation for HCC	Side effect	NCT01147380	Ongoing, but not recruiting
Young TIL	Phase Ⅱ	2010	Metastatic HCC with at least one lesion resectable	Tumor regression rate	NCT01174121	Recruiting
Autologous tumor infiltrating lymphocytes, combined with IL-2	Phase Ⅰ	2011	Metastatic HCC	Safety and tolerability	NCT01462903	Unknown
CIK, combined with Licartin	Phase Ⅳ	2012	Postoperative patients	1-yr PFS	NCT01758679	Recruiting
Dendritic and cytokine-induced killer cells	Phase Ⅱ	2013	After complete resection or TACE	PFS	NCT01821482	Not yet recruiting
CTL induced by DC loaded with multiple antigens	Phase Ⅰ	2013	Complete tumor resection within 8 wk	2-yr PFS and adverse events	NCT02026362	Recruiting
DC incubated with irradiated autologous tumor stem cells + GM-CSF	Phase Ⅰ	2013	Candidates for HCC resection	Vital signs, physical examinations and adverse events	NCT01828762	Completed
Cord blood-derived CIK	Phase Ⅰ	2013	After radical resection	Adverse events	NCT01914263	Not yet recruiting
Autologous NKT cells	Phase Ⅰ	2013	Advanced HCC, refractory to standard treatments	Adverse events	NCT01801852	Recruiting
Immuncell-LC, combined with Nexavar	Phase Ⅱ	2013	Stage Ⅲ and Ⅳ, receiving or ready for Nexavar treatment	2-yr PFS	NCT01897610	Recruiting
MG4101: *ex vivo* expanded allogeneic NK cell	Phase Ⅱ	2013	Stage Ⅲ, after curative resection	1-yr DFS	NCT02008929	Recruiting

CIK: Cytokine-induced killer; CTL: Cytolytic T lymphocyte; DC: Dendritic cell; DFS: Disease-free survival; NK: Natural killer; NKT: Natural killer T; PFS: Progression-free survival; TACE: Transarterial chemoembolization; TIL: Tumor infiltrating lymphocyte; HCC: Hepatocellular carcinoma; GM-CSF: Granulocyte macrophage colony-stimulating factor; IL-2: Interleukin 2; BCLC: Barcelona clinic liver cancer classification.

reduction of intrahepatic tumor nodules[109]. A similar outcome was obtained in additional research which established that activation of NK cells increased survival in a xenograft mouse model[110]. Thus, the approach of enhancing the function of NK cells could possibly be accomplished in human HCC treatment. Although much solid evidence showed the role of NK cells in an anti-tumor reaction, there are, however, insufficient clinical studies to corroborate the efficacy of NK cell immunotherapy in HCC. Recently, a study demonstrated that RFA could activate the peripheral blood circulating NK cells in HCC patients[111]. Two ongoing clinical trials are trying to assess NK cell therapy combined with liver resection (NCT02008929) or liver transplantation for HCC (NCT01147380; Table 3). In the future, it would be of great interest to investigate the efficacy of NK cells combined with other strategies to improve immunotherapy in HCC.

NK T cells

NK T (NKT) cells are a heterogeneous group of T cells with a range of characteristics different from conventional T cells. Human NKT cells are found in small numbers in healthy liver (0.5%) and blood (0.02%)[112,113]; however, they are critical players in the regulation of anti-tumor immunity[114-116]. NKT cells are best known for their immunosuppressive functions; however, they can interact with many other immune cells, such as DCs, macrophages as well as NK cells, and the outcome of NKT cell stimulation depends on these interactions and the cytokine milieu[115]. NKT cells can manifest anti-tumor effects mediated by their reactivation with exogenous cytokines or ligands but recently the natural role of NKT cells in anti-tumor immunity was reported[112,115]. A phase Ⅰ trial using autologous NKT cells to treat advanced HCC is now ongoing (NCT01801852; Table 3). Further investigation is needed to elucidate the role of NKT cells in human HCC.

Chimeric antigen receptor-T cells

One of the most important aims of T cell engineering is to generate tumor-targeted T cells through the genetic transfer of antigen specific receptors. T cell engineering consists of either physiological, MHC-restricted T cell receptors (TCRs) or non-MHC-restricted chimeric antigen receptors. The conception of the chimeric antigen receptor (CAR) originally generated from the

growing understanding of the barriers to effective immune therapy of various types of cancers. T cells armed with CARs (CAR-T cells) are able to recognize the cell surface antigens directly and are not blunted by tumor variations possessing lower surface expression of major MHC antigens which are considered a common mechanism of tumor immune escape[117,118]. The three basic elements of CAR are an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain.

To date, three generations of CARs have been developed. The first generation CAR only contains a T cell signaling domain that transmits the activation signal. It owns a feature of a single signaling domain most commonly derived from the CD3ζ component of the TCR/CD3 complex. The full activation of T cells needs multiple signals and it is obvious that the signaling from these first-generation CARs only provided the so-called "signal one" that could drive T cell effector functions; however, due to the absence of further signals, the T cells are unable to fully engage their effector machinery[119]. Considering this modular nature of the CAR, later designs aimed to add additional signaling domains which would increase the potency of the CARs and the consequent effector function of T cells. The second generation CARs incorporate a single co-stimulatory molecule endodomain, such as the endodomain of CD28 or 4-1BB[120]. The third generation CARs incorporate at least two co-stimulatory molecule endodomains, such as the endodomains of CD28 and 4-1BB[121]. Ligation of CD28 on CAR-T cells through the expression of B7 co-stimulatory ligands on target cells or co-expression of the CD28 molecule together with the scFv (specific monoclonal antibody) and CD3ζ domain of the CAR was shown to promote the proliferation of CAR-modified T cells and anti-tumor activity[122-124].

Plenty of studies have evaluated the efficacy of CAR-T cell treatment in hematological malignancies such as lymphoid leukemia[125,126] and acute myeloid leukemia[127,128]. Nonetheless, the clinical efficacy of CAR-T cells continues to be marginal in solid tumors compared to leukemia. So far, as we have acknowledged, there are no reported clinical trials evaluating the efficacy of CAR-T in HCC. Only one research stated that genetically modified T cells could be used to reconstitute virus-specific T cell immunity in chronic HBV patients and target tumors in HBV-related HCC. They found TCR re-directed HBV-specific T cells produced from PBMC of hepatitis B related HCC patients were capable of recognizing HCC tumor cells[129].

Besides the failed clinical trials of CAR-T cells in renal cell cancer and ovarian cancer[130-132], the safety and tolerance of CAR-T cells likewise need further assessment since the trial ceased and the demise of patients was often documented[124,133,134]. In a clinical trial of renal cell cancer, a maximum of 10 infusions of a total 0.2 to 2.1 $\times 10^9$ CAR-T cells administered to patients manifested in liver enzyme disturbances and the treatment was finally stopped[133]. A patient receiving CAR-T cells based on a Her2/neu-specific CARs died soon after the treatment[124]. These data from renal carcinoma seemed to indicate a dim future for CAR-T applications in solid tumors. To achieve a better clinical response, CAR-T cells need to overcome two major barriers, which are insufficient T-cell migration into the lesions and highly immunosuppressive microenvironments within the tumors[118,135,136].

Considered collectively, the original studies highlighted two important lessons. To begin with, clinical anti-tumor responses of T cells seem to be proportional to T cell persistence. Consequently, we ought to enhance the T cell persistence and then try to efficiently traffic adequate quantities of CAR-T cells from the peripheral blood towards tumor tissues. Once there, these CAR-T cells must functionally respond against tumor cells within such an immunosuppressive context. Secondly, understanding and defining the specific target is crucial for the safety of CAR-T treatment. Increasing evidence demonstrates that a careful choice of target antigen, including an understanding of accessibility and expression level, must be under consideration for future CAR-T clinical trials.

Treg-based immunotherapy

Treg mediated immunosuppression is the essential mechanism accountable for tumor immune evasion and could be the primary hurdle of tumor immunotherapy[137]. Tregs, characterized by $CD4^+CD25^+FoxP3^+$, play a critical role in immune homeostasis and suppress function of immune cells, such as $CD8^+$ T cells, $CD4^+$ T cells and NKT cells. Many reports have demonstrated that the amount of Tregs in solid tumor decreased and was inversely related to the prognosis of HCC patients[10,138-141].

Targeting Tregs has been of great interest to change the immune suppression milieu and enhance tumor specific immune responses. Depletion of Tregs using anti-CD25 monoclonal antibody has been shown to have substantial anti-tumor effects in murine tumor models[142]. Nonspecific approach of $CD25^+$ T cell depletion by injection of PC61 antibody was also tested on an orthotropic HCC model and led to a significant protection against tumor development[143]. No such strategy has been performed on humans yet.

CONCLUSION

An array of translational research and pilot clinical trials have revealed that immunotherapy is safe and tolerated by patients with cancers. The efficacies are also offered in some types of immunotherapy in selected patients. In HCC, more studies, including basic and clinical research, are urgently required to improve the outcomes of immunotherapy with best cost performance. Currently, immune checkpoint blockade and CAR-T strategies are specifically expected. In addition, it is become obvious that incorporating standard anti-tumor therapies with immunotherapy is the most likely effective alternative. Ultimately, more substantial randomized, controlled trials are required to authenticate the efficacy of

immunotherapy for HCC patients.

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MINIREVIEWS

Acute kidney injury in patients with chronic liver disease

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Abstract

Acute kidney injury (AKI) is a frequent clinical event in patients with liver disease, compounding their prognosis. Furthermore, it is likely that the occurrence of AKI has a detrimental impact on the subsequent renal function and the long-term survival of these patients. Recently, some authors advocated the use of new diagnostic criteria for detecting acute kidney injury in patients with cirrhosis. These criteria are based on the rapidity and extent of the creatinine increase comparing to the basal creatinine and also on the kinetics of diuresis decrease. Although their validity in this population requires further studies to be clearly established, these new criteria could have two advantages: (1) to allow earlier diagnosis of AKI and, thus, hepatorenal syndrome for which earlier intervention could improve patients' survival; and (2) to promote more intensive monitoring of renal function in these patients with high risk of AKI. Finally, recent practice guidelines about the prevention and treatment of general AKI have been published which should be useful in optimising the management of AKI in cirrhotic patients.

Key words: Acute kidney injury; Cirrhosis; Hepatorenal syndrome; Serum creatinine; RIFLE/AKIN classification

Core tip: Acute kidney injury (AKI) is associated with detrimental effect on early survival in hospitalised cirrhotic patients. Due to several hemodynamic modifications, both at the systemic and renal level, induced by cirrhosis, these patients are at increased risk to present acute kidney injury. Recently, new diagnostic criteria have been developed to insure early detection and allow severity assessment of AKI and to optimize the patient's treatment. The studies available so far, showed that these criteria could have a clinical interest in the management of renal dysfunction but additional data are needed to ascertain their clinical benefit.

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INTRODUCTION

The acute kidney injury (AKI) occurring in patients with liver disease (LD) is due to an external causative agent, with little or no influence of concomitant LD, or is related to the disorders induced by the underlying LD. Because of the elevated frequency of liver cirrhosis, cirrhotic patients are, by far, the largest proportion

of patients suffering from LD who experience AKI. Indeed, portal hypertension (PH) is associated with hemodynamic systemic disorders that favour AKI[1]. In this context, the occurrence of AKI negatively impacts the patient's prognosis. This review will focus on conventional diagnostic criteria of AKI and on new criteria that have been recently proposed in order to diagnose and assess the severity of AKI. It will also address the pathophysiology, prevention and treatment of AKI in patients with cirrhosis.

DIAGNOSTIC CRITERIA OF AKI

AKI is defined as a brutal reduction in the renal function, which is generally reversible. Until recently, no standardised criteria existed to define this disease and its severity. This led to some heterogeneity in the clinical situations described as acute renal insufficiency or acute renal failure. This in turn caused difficulty in the interpretation of results, particularly concerning the frequency and the impact of AKI on the patients' outcomes[2]. During the last decade, an attempt to establish a standard definition, based on evidences arising from the literature was undertaken, leading to the development of the RIFLE classification, published in 2004[3]. The main goal was to gather under the same name (AKI) some clinical situations, which present a quick and potentially reversible decrease of the renal function. Another goal was to stratify AKI by level of severity[2]. Therefore this classification proposes diagnostic criteria for AKI, and a staging system of its severity, based on changes in the levels of three renal markers: the relative increase in serum creatinine (SCr) and/or the relative decrease of the glomerular filtration rate (GFR) in the previous 7 d and the decrease of the diuresis. These RIFLE criteria were then slightly modified to produce the classification RIFLE/AKIN: in this staging system, a new criterion defined as an absolute increase in the SCr (0.3 mg/dL or 26.5 μmol/L) within a period of 48 h was introduced for diagnosing stage 1 AKI and the criterion based on relative decrease of GFR was dropped[4] (Table 1). In a study on patients hospitalised in intensive care units (ICU), the use of this additional criterion allowed to detect patients previously undetected by the RIFLE classification (especially stage 1 AKI). These newly detected patients exhibited double the early mortality rate compared to patients without AKI according to the AKIN classification[5].

Concerning the clinical relevance of these classifications, various studies on hospitalised patients found an independent link between the occurrence of AKI and a higher patient early mortality, with increasing severity stages of AKI associated with decreased survival. In 2008, a systematic review, including approximately 71000 patients (mostly hospitalised in ICU) showed a relative risk (RR) of mortality of 2.4 in patients diagnosed with stage 1 AKI (also called Risk or R stage) compared to non-AKI patients. Moreover, there was a regular increase in the RR for the more severe stages of AKI: RR = 4.15 for stage 2 (also called Injury or 1 stage) and RR = 6.37 for stage 3 (or Failure or F stage)[6].

Among patients hospitalised for at least 24 h in non-ICU department, Uchino *et al*[7] showed that the risk of mortality was multiplied by 2.5, 5.1 and 10.4 for patients presenting stages 1, 2 and 3 of the RIFLE classification respectively, compared to patients without AKI. Lastly, by using the classification RIFLE/AKIN in patients hospitalised in ICU, AKI stages 1, 2 and 3 were independently associated with a relative risk of early mortality of 2.07, 1.93 and 2.99 respectively, compared to the patients without AKI[5].

Apart from the impact on early mortality, some studies suggest that AKI is responsible for the increased risk of chronic kidney disease (CKD) incidence or the increased development of an initial CKD[8,9]. Also, it is not excluded that AKI could have a detrimental effect on longer-term mortality[2].

In 2012, the KDIGO international initiative published guidelines concerning the diagnosis, prevention and treatment of AKI in the general population based on an exhaustive review of the literature and the opinion of some experts in the field. The results of the studies cited above (showing the clinical interest of the RIFLE/AKIN classification to predict increased mortality risk in patients with AKI) led the authors to advocate the use of the RIFLE/AKIN staging system, notwithstanding a couple of minor changes in the criteria (Table 1). However, the authors underline some limitations regarding these criteria, in particular the need to confirm their clinical relevance among patients hospitalised in non-ICU departments[2]. In these guidelines, the use of biomarkers other than the SCr is not recommended because the results of studies did not clearly prove their clinical relevance so far.

Apart from the relevance of these criteria in the early diagnosis of AKI, these guidelines stress the importance of the initial evaluation of the risk of developing AKI based on the detection of AKI susceptibility factors (that include the presence of chronic hepatic disease). Finally, they also underline the importance of determining the cause(s) of AKI in order to start specific treatment when this is possible[2].

AKI AND LDS

As the presence of a chronic hepatic disease is a factor of susceptibility for AKI, the patients suffering from such a disease constitute a population with increased risk of developing AKI. Therefore, these patients require reinforced clinical and biological monitoring, particularly during hospitalisation for one or several complications, some of which are well known as triggering factors for AKI[10].

The AKI occurring in patients with cirrhosis or acute liver failure has been the most extensively studied due to the high frequency of this LD[1]. It is estimated that AKI occurs in around 20% of hospitalised cirrhotic patients[11,12]. In the context of cirrhosis or acute liver

Table 1 Diagnostic criteria and assessment of the severity of acute kidney injury according to the criterion of RIFLE, RIFLE/AKIN and KDIGO clinical practice guidelines classifications

Definition of AKI	Increased SCr × 1.5 the basal value presumably to have occurred within the prior 7 d or GFR decrease > 25% or urine output < 0.5 mL/kg per hour for 6 h	Increased SCr × 1.5 the basal value or ≥ 0.3 mg/dL within 48 h or urine output < 0.5 mL/kg per hour for > 6 h	Increased SCr ≥ × 1.5 the basal value presumably to have occurred within the prior 7 d or ≥ 0.3 mg/dL (26.5 μmol/L) within 48 h or urine output < 0.5 mL/kg per hour for 6 h
Stage 1 (risk)	Increased SCr × 1.5 the basal value or GFR decrease > 25% or urine output< 0.5 mL/kg per hour for 6 h	Increased SCr × 1.5 to 2 the basal value or ≥ 0.3 mg/dL within 48 h or urine output < 0.5 mL/kg per hour for > 6 h	Increased SCr × 1.5 to 1.9 the basal value or ≥ 0.3 mg/dL or urine output < 0.5 mL/kg per hour for 6-12 h
Stage 2 (injury)	Increased SCr × 2 the basal value or GFR decrease > 50% or urine output < 0.5 mL/kg per hour for 12 h	Increased SCr × 2 to 3 the basal value or urine output < 0.5 mL/kg per hour for > 12 h	Increased SCr × 2 to 2.9 the basal value or urine output < 0.5 mL/kg per hour for ≥ 12 h
Stage 3 (failure)	Increased SCr × 3 the basal value or GFR decrease > 75% or SCr > 4 mg/dL with acute rise of ≥ 0.5 mg/dL or urine output < 0.3 mL/kg per hour for 24 h or anuria for 12 h	Increased SCr × 3 the basal value or ≥ 4 mg/dL (353.6 μmoL/L) with acute rise ≥ 0.5 mg/dL or initiation of RRT or urine output < 0.3 mL/kg per hour for ≥ 24 h or anuria for ≥ 12 h	Increased SCr × 3 the basal value or ≥ 4 mg/dL (353.6 μmol/L) or initiation of RRT or urine output < 0.3 mL/kg per hour for ≥ 24 h or anuria for ≥ 12 h

SCr: Serum Creatinine; GFR: Glomerular filtration rate; RRT: Renal replacement therapy; AKI: Acute kidney injury.

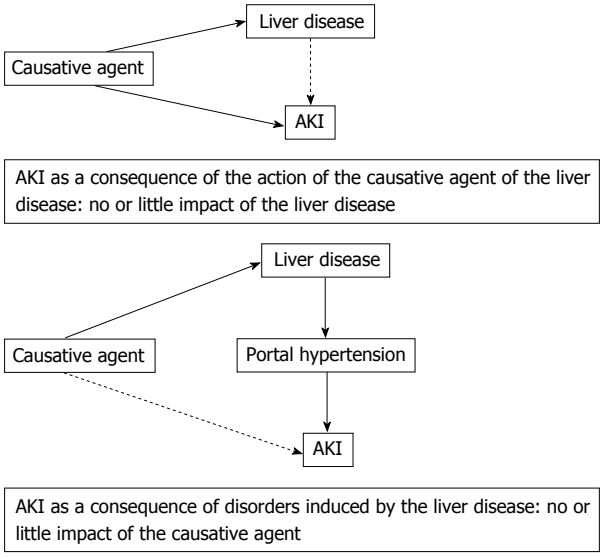

Figure 1 Mechanism of acute kidney injury in liver disease. AKI: Acute kidney injury.

failure, several modifications of the renal perfusion, induced by PH, occur which considerably increase the sensitivity of the renal perfusion and the GFR to the changes in volemia. These are responsible for a decline in the GFR, even if only minor variations of extra-cellular fluid take place[11,13]. Moreover, these hemodynamic modifications may be accompanied by renal tissue injury related to the renal toxicity of a very high concentration of serum bilirubin, which can promote the development of AKI[14,15]. Consequently, the AKI occurring in the case of PH is often of functional origin. However, the increasing susceptibility to develop AKI is also applicable in the case of renal ischaemic or/and toxic injury, which can cause acute tubular necrosis (ATN). Finally, AKI of obstructive or vascular origin can be found as well, though this is a rare occurrence[10].

Besides these cases in which the underlying LD largely supports the development of AKI, other clinical conditions are associated with both acute hepatic and renal dysfunction, such as the infection by a leptospira[16]. In this case, the dysfunction of both organs shares the same etiologic agent with little or no influence of the LD on the development of AKI (Figure 1). Thus, this is a very different context and, in this review, we will focus on AKI arising in the context of cirrhosis.

DIAGNOSTIC CRITERIA OF AKI IN PATIENTS WITH CIRRHOSIS

In 2011, a working group proposed a revised classification system for diagnosing and assessing the renal dysfunction in cirrhotic patients. This meeting resulted in a publication that proposes to use the term "hepato-renal disorders" in the case of AKI or CKD arising in conjunction with advanced LD[17]. The authors advocate the use of the RIFLE/AKIN classification in order to enhance detection and management of AKI in cirrhotic patients and improve studies regarding pathophysiology, treatment and clinical consequences of AKI. The authors propose a paradigm shift regarding acute renal dysfunction in patients with LD, switching from a classification based on the triad pre-renal azotemia, ATN and hepatorenal syndrome (HRS) to a classification based on the severity of the renal dysfunction quantified with the RIFLE/AKIN criteria (Table 1). The aim of these proposals is to make the clinicians caring for cirrhotic patients aware of the broad spectrum of renal injuries that may arise in these patients because they are often focused on the occurrence of HRS. Indeed, this leads to probable under-recognition of mild AKI with possible detrimental effect on patient's outcomes. This publication also emphasizes the interest to assess the severity of AKI in order to optimize and initiate promptly the treatment. Finally, besides the potential benefits brought by the RIFLE/AKIN classification, some authors have underlined its possible limitations in patients with cirrhosis[18]. One of these limitations is related to the value of SCr in patients with advanced cirrhosis, which is

abnormally decreased compared to the level of GFR, and is a poor marker of the actual renal function. Furthermore, the ability of standardised changes in SCr to account for the actual amplitude of the renal function variations is questionable in these patients.

STUDIES THAT TESTED THE RIFLE/ AKIN CLASSIFICATION IN CIRRHOTIC PATIENTS

Several studies assessed the link between AKI and its severity, as diagnosed by the RIFLE or RIFLE/AKIN criteria, and the outcomes of hospitalised cirrhotic patients. In 2009, a study by Cholongitas *et al*[19] of 412 cirrhotic patients hospitalised in ICU found that the presence of AKI, as diagnosed by the RIFLE criteria, was independently associated with an increase in early mortality (OR = 2.1). Furthermore, in this study, the RIFLE score exhibited the best sensitivity (90%) to predict death in the patients. More recently, Wong *et al*[20] found a higher mortality in cirrhotic patients hospitalised for infection that developed AKI (according to the RIFLE criteria), compared to infected patients without AKI (34% *vs* 7%). In addition, the incidence of complications (transfer to ICU, need for mechanical ventilation, *etc.*) was also increased in patients with AKI. However, in a logistic regression model to determine factors affecting 30-d mortality of these patients, only the outcome of AKI (defined as partial or complete recovery or persistence of renal dysfunction) had an independent effect, with a worse prognosis for patients without recovery of renal function[20]. In another recent study, Belcher *et al*[21] included cirrhotic patients with AKI to determine the link between severity of AKI and early mortality. Interestingly, these authors have shown that instead of the increased level of severity per se (assessed by the AKIN criteria), the worsening of AKI during hospitalisation was associated with an increased early mortality (adjusted OR = 3.8), suggesting that early detection and treatment of AKI could have a beneficial impact on cirrhotic patients prognosis.

The results of two other studies suggested that a change in the AKIN classification could help to refine the prognosis of cirrhotic patients. Fagundes *et al*[22] tested the clinical effectiveness of the AKIN classification in hospitalised cirrhotic patients and found a higher 90-d mortality rate in patients with AKI compared to non-AKI patients (most of them presenting stage 1 AKI). However, their results showed that mortality rate was lower in stage 1 patients when it was compared to those with stage 2 or 3 AKI (whereas no difference was seen between patients with stage 2 and those with stage 3). More remarkably, among patients with stage 1 AKI, the mortality rate was significantly different according to peak SCr, with higher mortality rate occurring only when peak SCr > 1.5 mg/dL, while patients with stage 1 AKI and peak SCr below 1.5 mg/dL and non-AKI patients had similar mortality rate[22]. A study by Piano *et al*[23] compared the use of "conventional" criteria of AKI diagnosis (*i.e.,* incease of SCr by 50% to a level above 1.5 mg/dL) to the new AKIN criteria in predicting hospital mortality in cirrhotic patients. These authors found that patients with stage 1 AKI did not have higher mortality rate (compared to non-AKI patients), while stage 2 or 3 AKI patients did (however with no differences in mortality between stage 2 and stage 3 patients). More importantly, in a multiple logistic regression model to predict the in-hospital mortality, they found that inclusion of AKIN criteria did not improve prediction capacity of the model whereas inclusion of conventional criteria did. Furthermore, the model that presented the best prediction ability included both AKIN criteria and the "progression" status of the AKI (defined as the change to a more severe AKI stage during hospitalisation) with "progressor" patients demonstrating lowest survival. Finally, a peak SCr higher than 1.5 mg/dL was associated with an increase of mortality rate but it was strongly linked with the progression of AKI as well. When this criterion was added to the multivariate model of death prediction, no improvement of the model predictive capacity was seen[23].

Taken together, these studies suggest that using the AKIN classification to detect and assess AKI might have a clinical interest, for example by favouring early recognition of HRS (whereas current diagnostic criteria require a SCr above 1.5 mg/dL)[13]. However, they also suggest that other parameters could be taken into account, in order to strengthen the clinical relevance of this classification, such as peak SCr level or the progression of the AKI severity during hospitalisation. Whether cirrhotic patients with stage 1 AKI and peak SCr below 1.5 mg/dL exhibit increased mortality rate compared to non-AKI patients remains to be determined by further studies. Moreover, whether less severe AKI could impact renal prognosis in these patients has to be established as well. Finally, the true impact on the patients' prognosis of the cause of AKI should be assessed to avoid its potential confounding role, as suggested by Fagundes *et al*[22] and also by a study in liver transplant recipients by Nadim *et al*[24] (in which the cause of AKI was the single factor with independent impact on one-year post-transplantation mortality rate).

FACTORS FAVOURING THE OCCURRENCE OF AKI IN CIRRHOSIS

The mechanisms involved in the occurrence of AKI in cirrhosis have been extensively studied and are described in several review papers over the past 10 years[10,11,13,16]. Briefly, the *primum* movens of this disorder is related to the PH, which causes a decrease of effective arterial blood volume by inducing a large splanchnic vasodilation. This is followed by an increase in systemic vasodilation, related to the increased production of vasodilators in splanchnic circulation that reach the systemic circulation through the re-opening

Table 2 Diagnostic criteria of type 1 hepatorenal syndrome porposed by the international ascites club[25]

Cirrhosis with ascites
SCr > 1.5 mg/dL (133 μmol/L)
Doubling of basal SCr to a level greater than 2.5 mg/dL (226 μmol/L) in less than 2 wk
No improvement in SCr (decrease to 1.5 mg/dL or less) after at least 2 d of diuretic withdrawal and expansion of plasma volume with albumin (1 g/kg body weight/d up to a maximum of 100 g/d)
Absence of shock
No current or recent treatment with nephrotoxic drugs or vasodilators
Absence of parenchymal kidney disease as indicated by proteinuria > 0.5 g/d, hematuria (> 50 red blood cells per high-power field), or abnormal renal ultrasonography

SCr: Serum creatinine.

of portal-systemic shunts. This systemic vasodilation is responsible of an increase in cardiac output to maintain organs perfusion. However, the systemic and splanchnic vasodilations lead to the activation of sympathetic nervous system and the production of vasoconstrictors that decrease the renal blood flow (RBF). At some stage of the LD, this decline in RBF by renal vasoconstriction is reversible with the improvement of PH. Otherwise, the renal vasoconstriction tends to worsen due to the persistent secretion of several vasoconstrictors and also to an increased sensitivity of the kidney to these vasoconstrictors. Finally, a disturbance of the renal autoregulation also takes place in this context[13]. All these factors explain the extreme sensitivity of the renal function to the changes of volemia. In some instances, a triggering factor may elicit HRS, which can be roughly presented as a functional AKI that does not recover after adequate volume expansion. These modifications of renal hemodynamic also favour intrinsic AKI, caused by nephrotoxic compounds and/or ischemia.

Besides the renal hemodynamic changes induced by PH, the nephrotoxicity of chronic high plasma bilirubin and also some renal histological changes (like IgA glomerular deposits seen in patients with alcohol related LD) are possible worsening factors which could increase the risk of AKI[10].

HRS

Type 1 HRS (HRS-1) is an AKI occurring in patients with advanced cirrhosis, which differs from HRS-2 by its rapid occurrence. HRS-1 affects about 7% of hospitalised cirrhotic patients, both HRS-1 and HRS-2 affecting approximately 10% of cirrhotic patients with ascites[11]. A triggering factor can be found in around 50% cases (mostly, a bacterial infection)[10].

The diagnostic criteria of HRS-1 were defined in 2007 (Table 2)[25]. The criteria of HRS-1 include a two-fold increase in initial SCr, which must exceed 2.5 mg/dl (226 μmol/L)[11]. The publication, previously cited, by Wong *et al*[17] in 2011 suggested a possible interest to amend the HRS diagnostic criteria, particularly concerning the threshold of SCr (set at 1.5 mg/dL), by using the RIFLE/AKIN criteria. In this paper, the authors suggest that the use of these criteria could be clinically relevant because HRS is a progressive disease, that the SCr threshold doesn't take into account. This leads to ignore patients with less increased SCr though they are already presenting HRS and could receive early treatment and benefit from improved outcomes. In 2011, a study by Boyer *et al*[26] found that in cirrhotic patients with HRS, initial SCr was an independent predictor of the probability to get a response to terlipressin, and also to survive at 3 mo. In this study, every 1 mg/dL of increase of SCr was associated with an increase of 50% of the risk of death. The usual triggering factors of HRS are the incidence of a bacterial infection or a syndrome of systemic inflammation that, in the context of a hyperdynamic circulatory state due to systemic vasodilation, aggravates the systemic vasodilation and the renal vasoconstriction, leading to a further decrease of the renal perfusion. All causes of effective arterial blood volume decline may also trigger HRS, with the usual causes being the excessive use of diuretics, the occurrence of diarrhoea and/or vomiting and the implementation of a large-volume paracentesis[10]. Therefore, a proper clinical (weight, diuresis and blood pressure) and biological (SCr, urine sodium) monitoring can be particularly useful in these patients.

FUNCTIONAL AKI: PRERENAL AZOTEMIA

The presentation of prerenal azotemia is very close to HRS, the main difference is the absence of reversibility of AKI after an adequate expansion of plasma volume. Urinary biochemical abnormalities that can be found are similar, with, unlike ATN, the absence of epithelial cellular cast in the urine. However, in patients with advanced LD and high level of serum bilirubin, bile-stained granular casts may be found in both prerenal azotemia and HRS[11,18]. Regarding the response to volume expansion in prerenal azotemia, the improvement of the renal function may happen at variable speed depending on the initial state of effective volemia (which is difficult to assess correctly in patients with severe cirrhosis). Thus this improvement can be slower in some cases, which can be misleading for the clinician.

ACUTE TUBULAR NECROSIS

It accounts for about one-third to 40% of AKI in cirrhotic patients [10,11]. Toxic injury is the most frequent cause of ATN, but ischemic injury (related to shock or sepsis) is not rare. The differential diagnosis with prerenal azotemia and HRS is based on clinical examination and the analysis of plasma and urine biochemistry. Among the remarkable biochemical differences, there is the level of urine sodium, classically below 10 to 20 mEq/L in HRS and prerenal azotemia, and above 30 mEq/L in ATN. Other changes regard the value of the ratio urine on plasma

for creatinine (below 20 for ATN, while above 30-40 for HRS and prerenal azotemia) and osmolarity[13,16]. These differences may however not be present or may be trivial. Furthermore, like HRS, the renal function does not respond to volume expansion in ATN. In this context, the usefulness of the new biomarkers of renal injury is not clearly established and further studies are needed, even if one study found that it could help to distinguish between ATN and other causes of AKI[27]. About the pathophysiology of ATN, ischemic ATN is secondary to extended and critical renal hypoperfusion, whether it is due to a brutal (acute bleeding, for example) or to a gradual further decrease of previously low RBF. The persistence of critical hypoperfusion causes the necrosis and loss of tubular cells with some detaching from the tubular basement membrane. The detached cells can form cellular casts able to obstruct the tubules. The ATN of toxic origin is linked to the administration of potentially nephrotoxic anti-infective agents arising in a context of extreme fragility of the renal perfusion: aminoglycosides, some penicillins and amphotericin B are frequently involved[28]. Contrast induced nephropathy (CIN) is a toxic ATN secondary to iodinated contrast agent's administration and also a common cause of ATN in cirrhotic patients. The contrast agents, especially when a large volume of a high osmolarity agent is injected, are responsible for a decrease of the RBF, leading to an additional worsening of the renal hypoperfusion. The risk of CIN is increased in patients with CKD and/or diabetes mellitus. However, at least in the general population, the CIN can be effectively prevented by the preliminary correction of an effective hypovolemia. In addition, the administration of N-Acetylcysteine could also help the prevention, as does the use of small volumes of contrast agent and the preferential use of low or iso-osmolar contrast agents[2]. Therefore, this prevention should be implemented in cirrhotic patients because of their high risk to develop CIN.

PREVENTION OF AKI IN PATIENTS WITH CIRRHOSIS

The main preventive measure of AKI involving low effective blood volume as a mechanism is a careful monitoring of the volemia of hospitalised patients, especially those receiving diuretic therapy and/or treated with large-volume paracentesis[11]. Monitoring should be based on both clinical examination (regular assessment of weight, diuresis and perfusion) and systematic monitoring of renal function and blood and urinary biochemical parameters. In case of significant effective blood volume depletion, treatment with albumin solution (and possibly with crystalloid solution) must be undertaken promptly. Any excessive depletion of the extracellular fluid should be avoided.

About the prevention of ATN, it is based on appropriate prescription of potential nephrotoxic agents. Aminoglycosides are well-known nephrotoxic antibiotics frequently prescribed to treat gram-negative bacilli and are thus largely utilised in cirrhotic patients. KDIGO practice guidelines recommend to select an alternative agent to aminoglycosides whenever possible. If the use of aminoglycosides is required, a "once a day" injection scheme should be preferred whenever possible. From our own experience, we can also suggest that, in the presence of renal dysfunction, monitoring the aminoglycoside blood level in order to adjust the dose is helpful to prevent nephrotoxicity. Finally, the use of NSAIDs in cirrhotic patients, which is known to aggravate renal vasoconstriction, should be avoided whenever possible[2,11].

Concerning the prevention of CIN, several measures have been already cited, including the use of minimal volume of contrast agents and the importance to avoid decreased effective blood volume before the injection[2].

Finally, the prevention of HRS is based on avoiding the events able to trigger it, and also on their rapid treatment when they occur. Spontaneous bacterial peritonitis is a common circumstance that initiates HRS, and some studies suggested that the rapid introduction of a treatment by albumin infusion and antibiotic is effective to prevent HRS[29,30]. The EASL guidelines from 2010 recommend the use of intra-venous albumin in this situation and suggest that the use of norfloxacin might have an interest[31].

TREATMENT

Recent clinical practice guidelines made recommendations regarding the treatment of AKI[2] and HRS[31].

For functional AKI, therapeutic measures include discontinuation of diuretics and/or restoration of adequate effective blood volume, which leads to recovery of renal function. Regarding the ATN, there are no specific recommendations for its management in cirrhotic patients. Nonetheless, the recommendations and suggestions from the KDIGO can be used in these patients. Before the stage of dialysis, these recommendations do not advocate to use, for the purpose of renal protection, fenoldopam, nor low-dose dopamine neither diuretic therapy (except in cases of renal sodium overload and non-oliguric AKI). When threatening electrolytes disorders arise or volume overload cannot be adequately controlled by non-dialytic treatment, renal replacement therapy (RRT) should be started rapidly. However, it can be started out of emergency, depending on the overall clinical context. The combination of several factors including, for example, multiple electrolyte disturbances, progressive deterioration of renal function with high level of blood urea nitrogen or increasing difficulty to prevent adequately volume overload can justify to start RRT. According to the KDIGO guidelines, this therapy should be continue for the patients demonstrating hemodynamic instability. Moreover, a bicarbonate-buffered dialysate instead of a lactate-buffered one should be used, particularly in hemodynamically instable patients or in

patients with liver failure. Indeed, these two conditions favour the accumulation of lactate (hyperlactatemia), which may worsen both acidosis and hemodynamic instability. Dialysis should be performed without systemic anticoagulation in most of these patients owing to their high risk of bleeding. The preferred vascular access to perform RRT should be a non-tunneled catheter, primarily inserted in the right jugular vein. Finally, it is not recommended to use diuretic therapy in this setting because it does not promote renal recovery[2].

Regarding the management of HRS the recommendations from the EASL assert that furosemide and paracentesis can be used to treat ascites in this setting. However, it contraindicates the use of spironolactone because of a high risk of hyperkalemia. The first-line treatment of HRS-1 should include both the use of a vasoconstrictor and the perfusion of albumin. Among vasoconstrictors, terlipressin is the first choice agent. It must be administered with increasing dose, depending on the evolution of SCr under treatment. Alternatives to terlipressin are norepinephrine or the association midodrine-octreotide. In case of treatment failure, RRT should be started if a worthwhile indication exists. Others treatment options, such as transjugular intrahepatic portosystemic shunt, that improves the effective hypovolemia by increasing the return of blood to the heart, or systems of artificial liver support, based on the adsorption of blood toxic molecules with albumin, are not recommended. Indeed, there is a lack of evidences as regards their clinical benefits. Finally, the standard of care in the case of type HRS-1 remains orthotopic liver transplantation. This should be undertaken after initiation of the treatment of HRS. In case of prolonged renal dysfunction requiring dialysis treatment, a combined transplantation of liver and kidney should be undertaken[31].

CONCLUSION

The occurrence of AKI in patients with severe cirrhosis is a common event associated with a worsening of the prognosis. This warrants special attention in the monitoring of the renal function in these patients. Using RIFLE/AKIN classification to detect AKI and determine its severity could allow earlier diagnosis of AKI and adaptation of its treatment according to the level of severity. However, the independent association between the gravity of AKI and the early mortality rate still remains to establish in cirrhotic patients, particularly regarding the potential confounding effect of the cause of AKI. In addition, the results of recent studies suggest that this classification could be optimised in cirrhotic patients, by taking into account some other criteria like the increasing severity of AKI during hospitalisation. Lastly, regular monitoring of the renal status in cirrhotic patients is also warranted because it seems likely that the occurrence of AKI is associated with an increased risk of long term CKD development, as in the general population.

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ORIGINAL ARTICLE

Observational Study

Mean platelet volume as a novel predictor of systemic inflammatory response in cirrhotic patients with culture-negative neutrocytic ascites

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Conflict-of-interest: The authors have not conflict of interest to declare. None of the researchers involved in the present study have received fees for serving as a speaker, consultant or as advisory board member for any organization.
Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at fatimahiguera@yahoo.com.mx. Consent for data sharing was not obtained but the present data are anonymized and risk of identification is low. No additional data are available.

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Abstract

AIM: To identify a mean platelet volume (MPV) cutoff value which should be able to predict the presence of bacterial infection.

METHODS: An observational, analytic, retrospective study. We evaluated medical records of cirrhotic patients who were hospitalized from January 2012 to January 2014 at the Gastroenterology Department of "Hospital General de México Dr. Eduardo Liceaga", we included 51 cirrhotic patients with ascites fluid infection (AFI), and 50 non-infected cirrhotic patients as control group. Receiver operator characteristic curves were used to identify the best cutoff value of several parameters from hematic cytometry, including MPV, to predict the presence of ascites fluid infection.

RESULTS: Of the 51 cases with AFI, 48 patients (94.1%) had culture-negative neutrocytic ascites (CNNA), 2 (3.9%) had bacterial ascites, and one (2%)

had spontaneous bacterial peritonitis. Infected patients had greater count of leucocytes and polymorphonuclear cells, greater levels of MPV and cardiac frequency (P < 0.0001), and lower mean arterial pressure compared with non-infected patients (P = 0.009). Leucocytes, polymorphonuclear count, MPV and cardiac frequency resulted to be good or very good predictive variables of presence of AFI in cirrhotic patients (area under the receiving operating characteristic > 0.80). A cutoff MPV value of 8.3 fl was the best to discriminate between cirrhotic patients with AFI and those without infection.

CONCLUSION: Our results support that MPV can be an useful predictor of systemic inflammatory response syndrome in cirrhotic patients with AFI, particularly CNNA.

Key words: Mean platelet volume; Cirrhosis; Ascites fluid infection; Culture negative neutrocytic ascites; Systemic inflammatory response

Core tip: To suspect and recognize promptly those patients with ascites fluid infection (AFI) is crucial. Systemic inflammatory response syndrome and clinical signs are not always present in cirrhotic patients with AFI, and gold standard tests for diagnosis, such as neutrophils count and ascites cultures are not always quickly available in many clinical settings. The mean platelet volume (MPV) is a very good predictor of systemic inflammatory response. A MPV cutoff value equal or greater than 8.3 fl predicts the presence of AFI particularl culture-negative neutrocytic ascites.

Gálvez-Martínez M, Servín-Caamaño AI, Pérez-Torres E, Salas-Gordillo F, Rivera-Gutiérrez X, Higuera-de la Tijera F. Mean platelet volume as a novel predictor of systemic inflammatory response in cirrhotic patients with culture-negative neutrocytic ascites. *World J Hepatol* 2015; 7(7): 1001-1006 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i7/1001.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i7.1001

INTRODUCTION

Bacterial infections are considered one of the main cause of death in decompensated cirrhotic patients, they are also related to the development of other complications, such as hepatic encephalopathy, hepatorenal syndrome, and recurrence of variceal bleeding[1,2].

Spontaneous bacterial peritonitis (SBP) is the most common infection in patients with cirrhosis, counting for 10% to 30% of all bacterial infections, and representing around 10% to 50% off in-hospital mortality, meanwhile mortality at 1 year has been reported around 31% to 93%[3,4].

According to the definition of ascites fluid infection (AFI) in the cirrhotic patient there are three main categories: Culture-negative neutrocytic ascites (CNNA) is defined by greater or equal count of polymorphonuclear (PMN) to 250 cells/mm^3 but without a positive culture in the appropriate setting, which includes: The fluid must be cultured in blood culture bottles, no previous antibiotic therapy, and no other explanation for an elevated PMN count.

Bacterial ascites is defined as a positive result of culture, without increment of polymorphonuclear cell count and it has been reported in 2%-3% of ambulatory patients and 11% in hospitalized cirrhotic patients.

Spontaneous bacterial peritonitis is found in patients where besides the increase of polymorphonuclear counting, they present a positive result in culture.

CNNA has the same mortality rate as SBP and must be treated promptly[5-10]. The PMN count is not always quickly available in clinical practice and the culture result usually takes 72 h or more, these situations could delay the start of antibiotic therapy, therefore it is important to search biomarkers wide and quickly available that help to predict ascites infection[11-15].

Platelet size is a determinant factor of platelet pro-inflammatory functions[16]. Several studies have found relationship between the mean platelet volume (MPV) and pro-inflammatory conditions, particularly acute infections, such as, pyelonephritis[17] and endocarditis[18,19]. Thus they may predict the severity of sepsis[20]. Recently, two different studies have found increase in MPV levels in cirrhotic patients with AFI and proposed it as an accurate diagnostic test to predict AFI, nevertheless, these two studies differ in their propose cutoff values, and found different sensitivity, specificity and predictive values[21,22]. Therefore, our objective was to determine if there is difference between MPV value in cirrhotic patients without infection and cirrhotic patients with AFI and to identify a MPV cutoff value which could be able to predict the presence of bacterial infection in cirrhotic patients.

MATERIALS AND METHODS

Study design

Observational, analytic, retrospective study. We evaluated medical records of cirrhotic patients who were hospitalized from January 2012 to January 2014 at the Gastroenterology Department of "Hospital General de México Dr. Eduardo Liceaga", México City, we included 51 cirrhotic patients who met the diagnosis of SBP, CNNA and bacterial ascites according to the latest guidelines of the American Association for the Study of Liver Disease[10], and we included 50 non-infected cirrhotic patients as control group. We excluded patients with conditions that could affect the MPV value, such as other bacterial infections, diabetes, hypertension, history of cerebrovascular event, cardiac failure, dyslipidemia, recent hemorrhage, or antibiotic use in the previous month.

We also collected the following data: age, gender, cause of cirrhosis, Child-Pugh class, parameters which are part of the systemic inflammatory response syndrome

Table 1 Demographic characteristics of the patients

Variable	Group A *n* = 50	Group B *n* = 51	*P*	95%CI
Age (yr)	57 ± 9.8	55 ± 10.8	0.30	-1.9 to 6.2
Gender (female), *n* (%)	35 (70)	27 (53)	0.08	
Child-Pugh A/B/C	9/26/15	0/7/44	< 0.0001	
Cause of cirrhosis:				
Alcohol	18	27	0.34	
NASH	9	4		
CHC	13	9		
Autoimmune	2	2		
Cryptogenic	8	9		

Group A (controls: cirrhotic patients without infection); Group B (cases: cirrhotic patients with ascites infection); NASH: Non-alcoholic steatohepatitis; CHC: Chronic hepatitis C. $P < 0.01$ was considered statistical significant.

Table 3 Clinical and hematological predictors of acute pro-inflammatory response in cirrhotic patients with ascites infection

Variable	AUROC	95%CI	*P*
Leukocytes	0.89	0.82-0.95	< 0.0001
PMN	0.94	0.90-0.99	< 0.0001
MPV	0.90	0.84-0.96	< 0.0001
MCV	0.73	0.63-0.83	< 0.0001
MCH	0.72	0.61-0.82	< 0.0001
CF	0.86	0.78-0.94	< 0.0001

PMN: Polymorphonuclear cells; MPV: Mean platelet volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; CF: Cardiac frequency; AUROC: Area under the receiving operating characteristic.

(SIRS); such as, cardiac frequency, respiratory frequency, temperature, leukocytes count. Additionally we addressed the mean arterial pressure (MAP) and we collected other relevant data from the hematic cytometry report: PMN count, MPV, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH).

Statistical analysis

The distribution of numerical variables was analyzed trough kurtosis, asymmetry and Kolmogorov-Smirnov test, in case of non-normal distribution we performed base 10 logarithmic transformation. Numerical variables were expressed as median and standard deviation, and categorical variables were expressed as proportion and percentages. To compare between groups, student's *t* test, chi square test or Fisher's exact test were used as appropriate. Receiver operator characteristic (ROC) curves were used to identify the best cutoff value of several parameters from hematic cytometry, including MPV, to predict the presence of AFI.

The statistical methods of this study were reviewed by Fatima Higuera de la Tijera, MD, MSc. from "Hospital General de Mexico, Dr. Eduardo Liceaga".

RESULTS

We included 101 cirrhotic patients, 50 of them were used as controls without bacterial infection (group A), 51 of them were cases with AFI (group B), of them, 48 patients (94.1%) had CNNA, 2 (3.9%) had bacterial ascites, and one (2%) had SBP. Eighty eight patients were women who corresponded to 87.1% of our study population.

Table 2 Comparison between groups regard to different clinical and cellular parameters as predictors of pro-inflammatory acute response in presence of ascites infection in cirrhotic patients

Variable	Group A *n* = 50	Group B *n* = 51	*P*	95%CI
Leukocytes (10^3/mcl)	4.9 ± 2.1	12.4 ± 7.2	< 0.0001	-9.6 to -5.4
PMN (10^3/mcl)	3.0 ± 1.6	10.7 ± 6.8	< 0.0001	-9.6 to -5.7
Platelets (10^3/mcl)	106.7 ± 56.0	110.0 ± 69.0	0.80	-28.1 to 21.6
MPV (fl)	7.7 ± 0.5	9.0 ± 0.8	< 0.0001	-1.5 to -0.9
Hemoglobin (g/dL)	11.6 ± 2.5	11.1 ± 2.2	0.33	-0.5 to 1.4
MCV(fl)	89.7 ± 13.3	98.5 ± 9.4	< 0.0001	-13.3 to -4.3
MCH (pg)	30.1 ± 4.3	33.0 ± 3.7	< 0.0001	-4.5 to -1.3
RDW (%)	17.7 ± 3.1	18.3 ± 4.6	0.47	-2.1 to 1.0
MAP (mmHg)	81.9 ± 10.3	74.7 ± 16.1	0.009	1.8 to 12.5
CF (beats/min)	73 ± 16	96 ± 18	< 0.0001	-30.0 to -16.0
RF (breaths/min)	20 ± 2	24 ± 14	0.04	-8.4 to -0.2
Temperature (℃)	36.4 ± 0.5	36.7 ± 0.8	0.12	-0.5 to 0.6
SIRS, *n* (%)	0 (0)	38 (74.5)	< 0.0001	NA

Group A (controls: cirrhotic patients without infection); Group B (cases: cirrhotic patients with ascites infection); CF: Cardiac frequency; MAP: Mean arterial pressure; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume; MPV: Mean platelet volume; PMN: Polymorphonuclear cells; RDW: Red cell distribution width; RF: Respiratory frequency; SIRS: Systemic inflammatory response syndrome. $P < 0.01$ was considered statistical significant.

The media and standard deviation of age was 55.7 ± 10.3 years. According to Child-Pugh class, 59 patients (58.4%) were class C, 33 patients (32.7%) were class B, and 9 patients (8.9%) were class A. The most frequent cause of cirrhosis was alcohol intake, other etiologies are summarized in Table 1.

When we compared between groups the different parameters of the SRIS and parameters from the hematic cyotmetry, patients in the group B had greater count of leucocytes and PMN, also greater levels of MPV, MCV, MCH and cardiac frequency ($P < 0.0001$). Furthermore, patients in the group B had lower MAP compared with patients in the group A ($P = 0.009$). Other hematological and clinical variables which are part of the SRIS, such as, temperature and respiratory frequency were not different between groups (Table 2).

The variables that were different between groups: leucocytes and PMN count, MPV, MCV, MCH, and cardiac frequency ($P < 0.0001$) were tested trough ROC curves. From all of them, leucocytes and PMN count, MPV and cardiac frequency resulted to be good or very good predictive factors of presence of AFI in cirrhotic patients [area under the receiving operating characteristic (AUROC) > 0.80]. MCV and MCH were regular predictors of presence of AFI in cirrhotic patients (AUROC > 0.60 and < 0.75) (Figure 1 and Table 3).

We determined sensitivity, specificity, positive predictive value, negative predictive value and accuracy

Table 4 Different cutoff values for mean platelet volume as indicators of inflammatory response in cirrhotic patients with ascites infection

Cutoff value (fl)	Sensibility % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)	Accuracy
8.0	92 (84-100)	62 (48-76)	71 (60-83)	89 (77-100)	77 (69-86)
8.1	88 (78-98)	78 (66-90)	80 (69-92)	87 (76-98)	83 (75-91)
8.2	84 (73-95)	80 (68-92)	81 (70-93)	83 (72-95)	82 (74-90)
8.3	84 (73-95)	82 (70-94)	83 (72-94)	84 (72-95)	83 (75-91)
8.4	78 (66-91)	88 (78-98)	87 (76-98)	80 (69-91)	83 (75-98)
8.5	76 (64-89)	88 (78-98)	87 (76-98)	78 (67-90)	82 (74-90)

NPV: Negative predictive value; PPV: Positive predictive value.

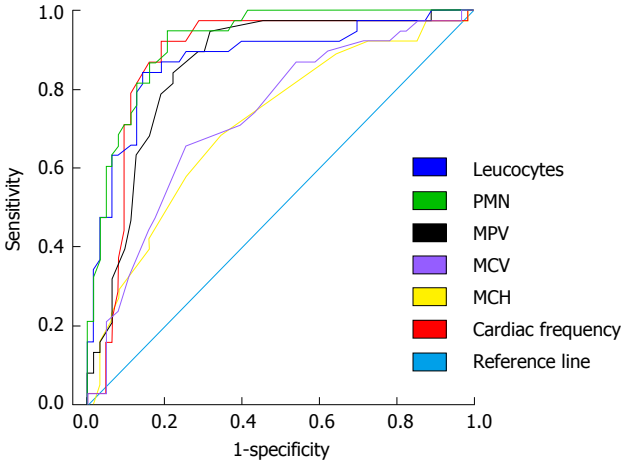

Figure 1 Area under de receiver operator characteristic curves from different clinical and hematological parameters as predictors of presence of ascites infection in cirrhotic patients. MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume; MPV: Mean platelet volume; PMN: Polymorphonuclear cells count.

for different cutoff MPV values. A cutoff MPV value of 8.3 fl was the best to discriminate between cirrhotic patients with AFI and those without infection (Table 4).

DISCUSSION

AFI is a severe condition with a high mortality rate if is not diagnosed and treated promptly. Although, gold standard for diagnosis is based on the determination of PMN cells count equal or greater than 250 cells/mm^3 of ascites, with or without a positive culture[9,10], in many settings the results from these gold standard tests are not quickly available, causing the delay in the diagnosis and early treatment. For this reason, other methods, more rapid and widely available have been proposed; nevertheless, some of them, such as the use of reagent strips have a low diagnostic accuracy for the diagnosis of AFI[23]. In addition, many patients with AFI may have not classic symptoms or signs of peritonitis[6,23] nor develop classic manifestations of sepsis. Several characteristics of the cirrhotic patients may difficult the diagnosis of SIRS and sepsis, for example: Baseline reduced PMN count due to hypersplenism, baseline elevated heart rate because of the hyperdynamic circulatory syndrome, baseline hyperventilation due to hepatic encephalopathy or blunted elevation of body temperature that is often observed in cirrhotic patients[24]. In our study we found that although temperature and respiratory frequency are variables which integrate the SIRS, there were not differences between them in patients with and without AFI. On the other hand, patients with AFI had greater count of leucocytes and PMN, but also greater levels of MPV, MCV, MCH and cardiac frequency, and they had also lower MAP compared with patients without AFI.

In our study, leucocytes and PMN count, MPV and cardiac frequency resulted to be good or very good predictive variables of presence of AFI in cirrhotic patients (AUROC > 0.80). Leucocytes and PMN count and cardiac frequency are variables already well known predictors of systemic inflammatory response, however, the novelty of our study is that the MPV resulted on being an additional variable of great utility to predicting systemic inflammatory response. Several conditions, such as, thrombotic events, endothelial dysfunction and inflammation are associated with platelet activation and consumption[25,26]. Besides their hemostatic function, platelets also play a role in recruiting neutrophils to sites of injury and infection; platelets also help to the activation of neutrophils *via* engagement of neutrophil and endothelial cell receptors and release of chemokines[27].

There are two previous studies conducted in patients with cirrhosis and AFI, which demonstrated that MPV could be a predictor of SIRS. In the first study by Suvak *et al*[21], the authors found that MPV increased the response to AFI in patients with cirrhosis, compared with those patients without AFI and with healthy controls. These authors proposed a cutoff value of 8.45, with a sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of 70.7%, 67.5%, 75.4% and 62.1% respectively (area under the curve: 0.768). A recently study by Abdel-Razik *et al*[22], also found a greater level of MPV in cirrhotic patients with AFI compared with cirrhotic patients without AFI and healthy controls. These authors found that MPV had 95.9% sensitivity and 91.7% specificity for detecting AFI (area under the curve: 0.964) at a cutoff value of 8.77. In our study, we tested different cutoff values, and we found that the best was the cutoff value of 8.3 fl, with sensitivity, specificity, PPV, NPV and accuracy of 84%, 82%, 83%, 84% and 83% respectively.

Our results support that MPV can be an useful

predictor of SIRS in cirrhotic patients with AFI, particularly CNNA, better than other variables such as temperature or respiratory frequency.

COMMENTS

Background

Bacterial infections are considered one of the main causes of death in decompensated cirrhotic patients, being the spontaneous bacterial peritonitis the most common infection, clinical data and laboratories, such as polymorphonuclear count, or ascites culture, are not always available and close to 30% of patients remain asymptomatic. A diagnosis and timely treatment of the infection of ascites fluid is able to reduce the mortality rate of 80% to 20%. Hence the reason for using non invasive markers, that are fast and easy to apply, which can help to predict the development of ascites infection.

Research frontiers

Platelet size is a determinant factor of platelet pro-inflammatory functions. Several studies have found relationship between the mean platelet volume (MPV) and pro-inflammatory conditions, particularly acute infections, such as, pyelonephritis and endocarditis.

Innovations and breakthroughs

Recently, two different studies have found increase in MPV levels in cirrhotic patients with ascites fluid infection (AFI) and proposed it as an accurate diagnostic test to predict AFI, nevertheless, these two studies differ in their propose cutoff values, and found different sensitivity, specificity and predictive values, being that the main reason why the authors attempted to determine if there is any difference between MPV value in cirrhotic patients without infection and cirrhotic patients with ascites fluid infection and to identify a MPV cutoff value, able to predict the presence of bacterial infection in cirrhotic patients.

Applications

The study results suggest that MPV can be an useful predictor of systemic inflammatory response syndrome in cirrhotic patients with AFI, particularly culture-negative neutrocytic ascites (CNNA), better than other variables such as temperature or respiratory frequency.

Terminology

According to the definition of spontaneous bacterial peritonitis there are three main categories: CNNA is defined by greater or equal count of polymorphonuclear to 250 cells/mm^3 but without a positive culture. Bacterial ascites is defined as a positive result of culture, without increment of polymorphonuclear cell count. Spontaneous bacterial peritonitis is found in patients where besides the increase of polymorphonuclear counting, they present a positive result in culture.

Peer-review

Interesting study.

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CASE REPORT

Hyperammonemia-induced encephalopathy: A rare devastating complication of bariatric surgery

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Author contributions: Kromas ML and Mousa OY participated in patient care and initial evaluation, diagnosis, and follow-up of patient as described in report; Kromas ML, Mousa OY and John S analyzed the laboratory and imaging findings and conducted review of previous publications and wrote the paper.
Ethics approval: The case report was deemed to meet criteria for exemption from review by SUNY Upstate Medical University Institutional Review Board.
Informed consent: The patient provided informed verbal consent for inclusion in case report at time of discharge from hospital.
Conflict-of-interest: The authors including Michelle L Kromas, Omar Y Mousa, Savio John have no conflicts of interest to disclose including but not limited to receipt of research funding, fees for service as speakers, or owning shares or stocks in any organizations.

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Abstract

The clinical manifestations of hyperammonemia are usually easily identifiable to the clinician when associated with liver disease and lead to prompt diagnosis and treatment. However, hyperammonemia-induced encephalopathy is rare in adults in the absence of overt liver disease, thus diagnosis is often delayed or missed leading to potentially life threatening complications. Without proper treatment, such patients can decompensate rapidly with poor outcomes including seizures, coma, and death. Early assessment of plasma ammonia levels in patients with normal hepatic function and characteristic symptoms of encephalopathy can lead to early intervention while investigating the underlying etiology. We describe a patient who presented with a 2-year progression of waxing and waning acute mental status changes after a Roux-en-Y gastric bypass surgery. He was found to have elevated ammonia level as well as orotic aciduria; results consistent with a urea cycle disorder. After consulting neurology as well as toxicology, he ultimately improved after dietary protein restriction, sodium benzoate and lactulose therapy. While rare, clinicians should have a high index of suspicion for late onset urea cycle disorders in symptomatic patients presenting with encephalopathy secondary to hyperammonemia.

Key words: Hyperammonemia; Urea cycle; Bariatric surgery; Encephalopathy; Hepatic

Core tip: Encephalopathy secondary to hyperammonemia in the absence of hepatic dysfunction presents a diagnostic dilemma to many clinicians. As such, early and accurate diagnosis can be easily missed, leading to increased morbidity and mortality. We describe a case of adult onset urea cycle disorder presenting with encephalopathy after gastric bypass surgery.

Although this challenging diagnosis is rare, treatment is inexpensive and readily available. Thus early recognition and intervention can prevent the rapid decline that may occur if the diagnosis is unrecognized.

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INTRODUCTION

Obesity is a prevalent and challenging issue in healthcare, affecting more than 60% of the United States population and contributing to a myriad of other comorbidities. Gastric bypass surgery (GBS) has been offered as an effective treatment for morbid obesity with reported success rates of weight loss up to 60%-70% of excess body weight[1,2]. However, GBS can be complicated by nutritional derangements and rare neurological manifestations, including encephalopathy[3,4]. Carnitine deficiency has been implicated in hyperammonemia-induced encephalopathy in the setting of valproic acid use as well as GBS[5-8]. Case reports have also unmasked ornithine transcarbamylase (OTC) deficiency in the setting of GBS[9]. Our case presents hyperammonemic encephalopathy following GBS related to an underlying late onset urea cycle disorder (UCD).

The disposal of nitrogen in the body is based on ammonia conversion to urea. Complete or partial enzyme deficiencies in the process of ammonia conversion leads to UCD. Classical presentation of such disorders occurs in neonates who can develop neurological manifestations and fail to thrive. Interestingly, some patients with similar manifestations presented in adulthood. The mechanism was either related to partially deficient enzymes of the urea cycle (UC) or enzyme mutation that is provoked by a certain stressor.

CASE REPORT

A 56-year-old male patient was hospitalized with worsening neurological symptoms including tremors, confusion, ataxia, and labile emotions. He reported his recurrent neurologic symptoms that progressed over a 2-year period to his primary care physician. His symptoms started mildly within 2 mo of a Roux-en-Y GBS, which the patient underwent for morbid obesity without comorbidities. He was otherwise healthy with no previous substance abuse or exposure to toxins. Of note he had lost 20 pounds prior to the surgery with proper nutritional guidance and 108 pounds post-operatively. He sought multiple medical subspecialties for advice and was diagnosed with Parkinson's disease. He was treated with levodopa and he followed with a neurologist without improvement. His disabling symptoms continued to progress, mandating referral to the emergency room. On physical examination he had tremors, ataxia, poor concentration and impaired memory. He developed general weakness that made him wheelchair-bound and dependent. Laboratory investigation and appropriate imaging studies reflected a UCD (Table 1). We managed our patient conservatively through the administration of lactulose and sodium benzoate as well as dietary modification, by providing foods low in protein. The symptoms of our patient improved gradually during his hospitalization. He specifically had resolution of confusion, ataxia, and tremors over a course of 1 wk following treatment initiation.

DISCUSSION

Ammonia is a usual component of the body fluids, which exists mainly as ammonium ion. Excess ammonia from the products of protein catabolism enters the UC in the liver for conversion into urea, prior to renal excretion[10,11]. Abnormalities in this process can lead to hyperammonemia, which increases the entry of ammonia to the brain and leads to neurological disorders. This can be due to impaired hepatic function and portal hypertension, where an excess nitrogen load over saturates the capacity of liver metabolism and bypasses it *via* portosystemic shunting[12]. Other causes of hyperammonemia include congenital UCD, Reye syndrome, as well as encephalopathies of metabolic or toxic natures. Hyperammonemia can be toxic with signs and symptoms that include: episodic irritability, vomiting, ataxia, mental retardation, and lethargy that can progress to alteration of consciousness and coma[13,14].

Both acute and chronic hyperammonemia alters the brain neurotransmitter system. Acute hyperammonemia causes accumulation of glutamate extracellularly in the brain, which activates the N-methyl D-aspartate receptor, causing seizures. Chronic hyperammonemia leads to an increase in inhibitory neurotransmission *via* down regulation of glutamate receptors and increased GABAergic tone, causing deterioration of cognitive function and coma. Most cases of hyperammonemia in the pediatric population are due to enzyme defects in the UC. This includes deficiencies of N-acetylglutamate synthetase, carbamoyl phosphate synthetase Ⅰ, ornithine transcarbamoylase, argininosuccinic acid synthetase, argininosuccinic lyase and arginase. Adults with partially deficient enzymes can have disease manifestations during stressful medical conditions such as postpartum stress, short bowel disease, parenteral nutrition with high nitrogen consumption, heart-lung transplantation, and gastrointestinal bleeding as discussed in previous reports[15-18]. Previous reports suggested that encephalopathy secondary to hyperammonemia in the setting of UCD can be unmasked by GBS[19,20]. Furthermore, the rapid weight loss that occurs in these patient's results in protein catabolism and a large nitrogen load, which can further contribute to the symptoms. The mechanism by which gastric bypass disturbs the function of UC and the disposal of ammonia in such patient populations is not

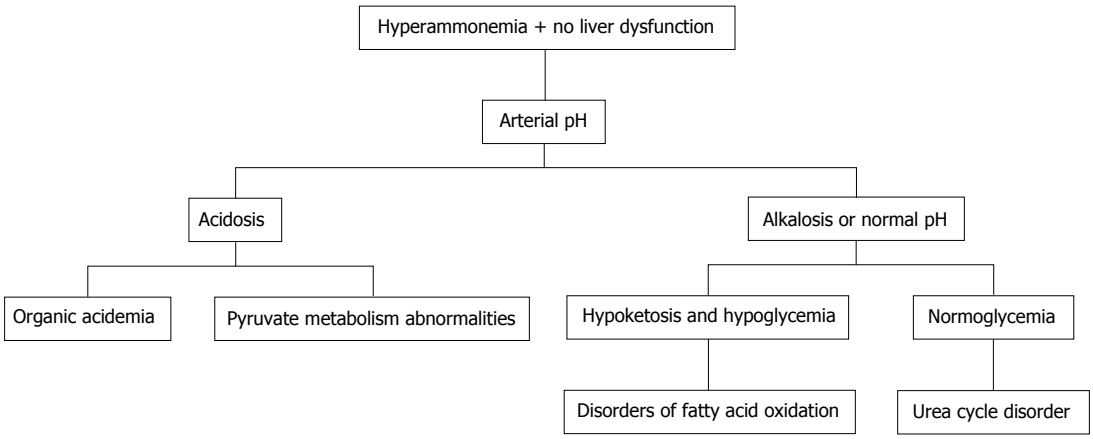

Figure 1 Algorithm for hyperammonemia workup in patients with normal hepatic panel. The arterial pH and blood glucose are among the initial tests to identify etiology of non-hepatic hyperammonemia.

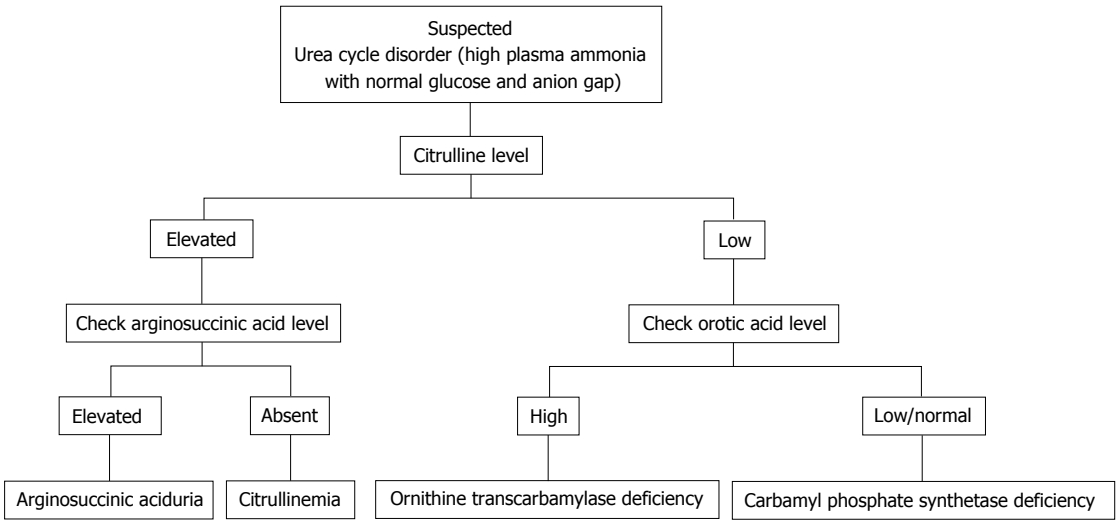

Figure 2 Algorithm of urea cycle disorder workup. Citrulline level, arginosuccinic acid level, and orotic acid level are the primary tests in the workup of urea cycle disorder.

Table 1 Laboratory investigations and imaging at time of hospitalization

Laboratory investigation/imaging	Values
BMP	Within normal limits (Cr 1.0 mg/dL)
CBC	Within normal limits (platelets 158 K/μL)
Iron panel	Within normal limits
Hepatic panel	AST 30 U/L ALT 9 U/L Alkaline phosphatase 52 U/L Total Bilirubin 0.9 mg/dL
Urine orotic acid	1.4 mmol/mol of creatinine
Ammonia level	155 mcg/dL
Carnitine level, zinc, manganese, vitamin B12, vitamin A, vitamin D	Within normal limits
Abdominal ultrasound	No evidence of liver disease or cirrhosis
MRI brain	No acute or chronic intracranial abnormalities

MRI: Magnetic resonance imaging; BMP: Basic metabolic panel; CBC: Complete blood count; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

fully understood. Table 2 shows the different etiologies of hyperammonemia of non-hepatic origin.

Distinguishing features of UCD in neonates comprises critically elevated ammonia levels (> 1000 mmol/L), whereas other etiologies seldom present with ammonia levels greater than 200-300 mmol/L. In addition, UCD is suggested by normal blood glucose and anion gap, as well as respiratory alkalosis from central hyperventilation. The initial workup of a UCD should include glucose, electrolytes, amino acids, serum ammonia, lactate, urine organic acids, orotic acid and arterial blood gases looking for arterial pH and carbon dioxide. OTC gene mutations cannot be revealed by DNA analysis in up to 20% of patients with OTC deficiency. Therefore other lab measures may give an indication as to the etiology of hyperammonemia without a history of liver disease[21]. The workup to identify the etiology of hyperammonemia of non-hepatic origin is outlined in Figure 1 and the workup to identify the UCD specifically is further outlined in Figure 2.

The management of hyperammonemia includes prevention of seizures and cerebral edema, medical

Table 2 Etiology of hyperammonemia of non-hepatic origin[21]

Age	Category	Examples
Adults	Stressful events	-
	Partial enzyme deficiency	-
	Infection	Urinary tract infections (urease-producing organism, such as proteus mirabilis)
Pediatrics	Medications	Valproate
		Topiramate
		Salicylates such as aspirin
		Acetazolamide
		Zonisamide
		High-dose chemotherapy (5-fluorouracil)
	Infection	Urinary tract infections (urease-producing organism, such as proteus mirabilis)
	Organic acidemias	Isovalericacidemia, propionic acidemia, methylmalonicacidemia, glutaricacidemia type Ⅱ, multiple carboxylase deficiency, beta-ketothiolase deficiency
	Congenital lactic acidosis	Pyruvate dehydrogenase deficiency
		Pyruvate carboxylase deficiency
		Mitochondrial disorders
	Fatty acid oxidation defects	Acyl CoA dehydrogenase deficiency
		Systemic carnitine deficiency
	Dibasic aminoacid transport defects	Lysinuric protein intolerance
		Hyperammonemia-hyperornithinemia-homocitrullinuria
	Miscellaneous	Transient hyperammonemia of the newborn asphyxia
		Reye syndrome
		Lactic acidosis

therapy to remove excess ammonia and dietary protein restriction. The medications used for treatment assist in offloading the UC by converting nitrogen to non-urea products that are easily excreted. These include sodium benzoate and arginine, followed by phenylacetate and phenylbutyrate. Our patient's cognitive impairment resolved after dietary protein withdrawal and implementation of medical therapy that included sodium benzoate and lactulose. He showed significant improvement over a period of one week following initiation of medical therapy.

A major proportion of the United States population is obese resulting in very high rates of annual bariatric surgeries. Even though it is rare for hyperammonemic encephalopathy to complicate GBS, it is yet more unusual for the etiology to be adult onset UCD. Such non-specific neurological presentation is a true diagnostic challenge especially when liver disease is absent, thus delaying the management plan, thus increasing associated morbidity and mortality.

Early diagnosis is crucial to prevent complications. Conservative management should include dietary modification with protein restriction. Patient satisfaction following GBS can be improved by educating these patients about the potential complications. Raising awareness among internists of this possible complication of GBS is also important and having a high index of suspicion for unmasked UCD post-GBS can prevent the debilitating consequences of unrecognized and untreated disease, improve its' outcomes as well as have a great impact on utilization of health care resources.

COMMENTS

Case characteristics

A 56-year-old male presents with progressive tremors, confusion, ataxia and emotional lability.

Clinical diagnosis

The authors' patient was not oriented to place or time, demonstrated tremor and ataxia.

Differential diagnosis

Manganese toxicity, Parkinson's disease, hepatic encephalopathy.

Laboratory diagnosis

Basic metabolic panel, hepatic function panel, complete blood count, iron panel, carnitine level, zinc level, manganese, vitamin B12, vitamin A, and vitamin D levels were within normal limits. Ammonia level 155 mcg/dL and urine orotic acid 1.4 mmol/mol of creatinine.

Imaging diagnosis

Imaging of the abdomen was unremarkable.

Treatment

The authors administered lactulose, sodium benzoate and modified his diet.

Related reports

The physiology of adult onset urea cycle disorder is poorly understood and several case reports of similar occurrences have been cited in the case report presented by the authors.

Term explanation

All terms used in this case report are common and do not require further explanation.

Experiences and lessons

Hyperammonemia encephalopathy in the absence of overt liver disease identified on imaging and blood work, can be the result of non-hepatic etiology and should be thoroughly evaluated as described in this case report.

Peer-review

The authors have described the rare complication of non-hepatic hyperammonemia-induced encephalopathy in the setting of gastric bypass surgery that has not been well described in the literature. Furthermore, the article highlights the appropriate workup, differential diagnosis, and treatment for this clinical scenario.

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