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REVIEW

Bacterial infections in cirrhosis: A critical review and practical guidance

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Abstract

Bacterial infection is common and accounts for major morbidity and mortality in cirrhosis. Patients with cirrhosis are immunocompromised and increased susceptibility to develop spontaneous bacterial infections, hospital-acquired infections, and a variety of infections from uncommon pathogens. Once infection develops, the excessive response of pro-inflammatory cytokines on a pre-existing hemodynamic dysfunction in cirrhosis further predispose the development of serious complications such as shock, acute-on-chronic liver failure, renal failure, and death. Spontaneous bacterial peritonitis and bacteremia are common in patients with advanced cirrhosis, and are important prognostic landmarks in the natural history of cirrhosis. Notably, the incidence of infections from resistant bacteria has increased significantly in healthcare-associated settings. Serum biomarkers such as procalcitonin may help to improve the diagnosis of bacterial infection. Preventive measures (*e.g.*, avoidance, antibiotic prophylaxis, and vaccination), early recognition, and proper management are required in order to minimize morbidity and mortality of infections in cirrhosis.

Key words: Bacteria; Infection; Sepsis; Bacteremia; Liver cirrhosis; Vaccination; Spontaneous peritonitis; Immune dysfunction

Core tip: Bacterial infection is common and accounts for major morbidity and mortality in cirrhosis. Patients with cirrhosis are immunocompromised and increased susceptibility to develop spontaneous bacterial infec-

tions, hospital-acquired infections, and a variety of infections from uncommon pathogens. Once infection develops, the excessive response of pro-inflammatory cytokines on a pre-existing hemodynamic derangement in cirrhosis further predispose the development of serious complications such as shock, acute-on-chronic liver failure, renal failure, and death. The incidence of resistant bacteria has continually increased, especially in healthcare-associated settings. Preventive measures, early recognition and proper management are necessary to minimize morbidity and mortality of infections in cirrhosis.

Bunchorntavakul C, Chamroonkul N, Chavalitdhamrong D. Bacterial infections in cirrhosis: A critical review and practical guidance. *World J Hepatol* 2016; 8(6): 307-321 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i6/307.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i6.307

INTRODUCTION

In the past decades, there have been several improvements in the management of cirrhotic patients, such as antiviral therapy and management of portal hypertension and liver transplantation (LT). However, the mortality of infection in cirrhosis is still high and has not changed substantially. Cirrhosis is an immunocompromised state that predisposes patients to spontaneous bacterial infections, hospital-acquired infections, and a variety of infections from uncommon pathogens. Once infection develops, the excessive response of pro-inflammatory cytokines on a pre-existing hemodynamic derangement in cirrhosis further facilitate the development of severe complications such as septic shock, acute-on-chronic liver failure (ACLF), multiple organ failure, and death. Accordingly, bacterial infection in patients with cirrhosis is very common in clinical practice and sepsis is the main reason of intensive care unit admission and death among such patients. The incidence of resistant bacteria has been increasing, especially in healthcare-associated settings. Preventive measures, early recognition, and proper management are necessary to minimize morbidity and mortality of infections in cirrhosis.

MECHANISM OF INCREASED SUSCEPTIBILITY AND VULNERABILITY TO INFECTION IN PATIENTS WITH CIRRHOSIS

Immune dysfunction in cirrhosis

Patients with cirrhosis are in a state of immune dysfunction, in parallel with a state of excessive activation of pro-inflammatory cytokines, referred to as cirrhosis-associated immune dysfunction syndrome, which predisposes the patient for infections[1,2]. Portosystemic shunting allows less gut-derived bacteria and their products to be cleared from portal circulation by the liver, which contains about 90% of the reticuloendothelial cells in the body[1-5]. Nearly all components of systemic immune response are significantly impaired in cirrhosis, including a decrease in phagocytic activity, a reduction in serum albumin, complement and protein C activities, and an impaired opsonic activity both in serum and ascitic fluid[1-4,6-10]. Genetic polymorphisms of toll-like receptor (*TLR*) and nucleotide-binding oligomerisation domain 2 (*NOD2*) genes could be responsible for bacterial translocation (BT) and increase infection risk in cirrhosis by altering the TLR's ability to bind to lipopolysaccharide or endotoxins[11,12]. Further, cirrhosis-associated immune dysfunction may further complicate by additional factors such as malnourishment[13] and alcohol drinking[14] (Table 1).

BT

BT is the migration of viable native bacteria from gut lumen through systemic circulation *via* mesenteric lymph nodes (MLN) and portal vein. Although this can be a healthy phenomenon, BT has increased pathologically compromising effects in cirrhosis[15-17]. The diagnosis of BT relies on the isolation of viable bacteria in MLN, while the detection of bacterial DNA in serum or ascitic fluid is proposed as a useful surrogated marker[15-18]. It has been shown that oral administration of radio-labeled *Escherichia coli* (*E. coli*) to cirrhotic rats revealed the detection of these bacteria not only in the gut lumen but also in the MLN and ascites[19]. Several experimental and clinical studies have suggested that small intestinal overgrowth, increased intestinal permeability, impaired intestinal motility, lack of bile acids, sympathetic overactivity, and local innate and adaptive immunological alterations (*e.g.*, impaired leukocyte recruitment, altered T-cell activation, *TLR* and *NOD2* mutation) are important factors involved in the pathogenesis of BT[11,12,17,20,21].

BT is pathogenetically linked to the development of infections, particularly spontaneous bacterial infections, and other serious complications in cirrhosis[15-17]. Apart from infections, bacterial DNA and bacterial products, such as endotoxin, can translocate to extra-intestinal sites and promote host immunological and hemodynamic responses, which is associated with the development of systemic pro-inflammatory and hyperdynamic circulatory state in cirrhosis[16,18]. The pathological translocation of viable bacteria occurs in the decompensated stage, while the rate and degree of translocating bacterial products also increases in the earlier stages of cirrhosis[15]. Notably, treatment with non-selective beta-blockers has been shown to ameliorate intestinal permeability and reduce BT[22].

Systemic inflammatory response syndrome and circulatory dysfunction in cirrhosis

Patients with cirrhosis are susceptible to the development of severe infection, septic shock, and organ

Table 1 State of immune dysfunction in patients with cirrhosis

Natural barriers	Fragile, thin and/or edematous skin
	Alteration of GI motility and mucosal permeability
	Alteration of GI bacterial flora, bacterial overgrowth
	↑ GI mucosal ulcerations
Hepatic RES activity	Portosystemic shunting
	Kupffer cells - ↓ number, impaired function
Cellular defense mechanisms	RES - ↓ activation, ↓ chemotaxis, ↓ phagocytosis, ↓ production of pro-inflammatory cytokines (IL-1, IL-6, IL-18, TNF-α)
	PMN - ↓ lifespan, ↓ intracellular killing activity, ↓ phagocytosis, ↓ chemotaxis
Serum factors	↓ Complement levels (C3, C4, CH50)
	↓ Opsonic activity
	↓ Protein C activity
Iatrogenic and treatment-related factors	↑ Invasive procedure and catheters
	Frequent hospitalization
	Immunosuppressive agents (autoimmune hepatitis, post-transplantation)
	Interferon therapy (viral hepatitis)
	Proton pump inhibitors
Other compelling factors	Malnutrition
	Alcohol drinking

Adapted from Bunchorntavakul C, Chavalitdhamrong D. *World J Hepatol* 2012; 4: 158-168. RES: Reticuloendothelial system; GI: Gastrointestinal; IL: Interleukins; TNF: Tumor necrosis factors; PMN: Polymorphonuclear cells.

failure[1,2,23]. In cirrhosis, bacterial infection is associated with a dysregulated cytokine response, which transforms helpful responses against infections into excessive, damaging inflammation[1,2,23]. Nitric oxide is strikingly released in cirrhotic patients with sepsis and is a key driver of circulation dysfunction in this setting[23,24]. A pre-existing hyperdynamic circulatory state in patients with advanced cirrhosis predisposes detrimental complications from a sepsis-induced nitric oxide and cytokine storm which subsequently leads to intractable hypotension, insufficient tissue perfusion, multiple organ failure and death[1-3,23].

Epidemiology and types of infection

Bacterial infection accounts for about 30%-50% death in patients with cirrhosis[3,24,25]. Infections present in 32%-34% of hospitalized patients with cirrhosis, which is 4-5 folds higher than hospitalized patients in general, and is especially higher in those with gastrointestinal bleeding (45%-60%)[26-28].

Common types of infections in patients with cirrhosis include spontaneous bacterial peritonitis (SBP) (25%-31%), urinary tract infection (UTI) (20%-25%), pneumonia (15%-21%), bacteremia (12%), and soft tissue infection (11%)[2,27,29]. The major causative organisms are gram-negative bacteria, *e.g.*, *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., whereas gram positive bacteria, especially *Enterococci* and *staphylococcus aureus*, comprise about 20% and anaerobes only 3%[2]. Risk factors of infection by gram positive bacteria are recent or current hospitalization, receiving quinolones prophylaxis, and invasive procedures[27,28,30].

Healthcare-associated is defined as infections diagnosed within 48 h of hospital admission in patients with any prior 90-d healthcare contact and nosocomial is defined as infections diagnosed after 48 h of admission. These infections are increasingly common in cirrhosis, frequently resistant to antibiotics (up to 64%) and are associated with bad outcomes[30]. In a large prospective study of cirrhotic patients with infections (> 650 infectious episodes)[31], multi-resistant bacteria (18%) were isolated in 4%, 14%, and 35% of community-acquired, healthcare-associated, and nosocomial infections, respectively ($P < 0.001$). The main resistant organism was extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, followed by *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Enterococcus faecium*[31]. There was a significantly higher incidence of septic shock and death from infections caused by resistant bacteria. Notably, the efficacy of empirical antibiotic treatment was decreased in nosocomial infections (40%), compared to community-acquired and healthcare-associated episodes (83% and 73%, respectively; $P < 0.0001$), especially in SBP, UTI, and pneumonia (26%, 29% and 44%, respectively)[31]. Due to an increasingly use of broad spectrum antibiotics (ATB), it is speculated that infections with multi-resistant gram-negative organisms and *Enterococci* will be largely more common and more problematic in the near future.

The common types of infections in cirrhosis and suggested empiric therapy are summarized in Table 2[32]. In addition, the common clinical features and risk factors of less common pathogens are summarized in Table 3[2]. It should be noted that the data regarding these less common pathogens derived from case reports and series from various regions of the world, in which the patterns of infection and ATB usage varies among reports. In real-life practice, empirical ATB should be selected based upon types of infection, individual risk factors, and the local epidemiological pattern of resistant bacteria, then narrow-downed according to the culture and ATB susceptibility testing.

Table 2 Types of infection and suggested empirical antibiotic therapy in patients with cirrhosis

Types of infection	Common responsible bacteria	Suggested empirical antibiotic
SBP, spontaneous bacteremia, SBE	*Enterobacteriaceae* *S. pneumoniae* *S. viridans*	1st line: Cefotaxime or ceftriaxone or BL-BI *IV* Options: Ciprofloxacin PO for uncomplicated SBP[1]; carbapenems *IV* for nosocomial infections in areas with a high prevalence of ESBL BL-BI may prefer in those with suspicious for enterococcal infection[2]
Pneumonia	*Enterococci* *S. pneumoniae* *H. infuenzae* *M. pneumoniae* *Legionella* spp. *Enterobacteriaceae* *P. aeruginosa* *S. aureus*	Community-acquired: ceftriaxone or BL-BI *IV* + macrolide or levofloxacin *IV*/PO Nosocomial and health care-associated infections: Meropenem or cetazidime *IV* + ciprofloxacin *IV* (*IV* vancomycin or linezolid should be added in patients with risk factors for MRSA[3])
Urinary tract infection	*Enterobacteriaceae* *E. faecalis* *E. faecium*	1st line: Ceftriaxone or BL-BI *IV* in patients with sepsis. Ciprofloxacin or cotrimoxazole PO in uncomplicated infections Options: In areas with a high prevalence of ESBL, *IV* carbapenems for nosocomial infections and sepsis (+ *IV* glycopeptides for severe sepsis); and nitrofurantoin PO for uncomplicated cases
Skin and soft tissue infections	*S. aureus* *S. pyogenes* *Enterobacteriaceae* *P. aeruginosa* *Vibrio vulnificus* *Aeromonas* spp.	Community-acquired: Ceftriaxone + cloxacillin *IV* or BL-BI *IV* Nosocomial: Meropenem or cetazidime *IV* + glycopeptides *IV*
Meningitis	*S. pneumoniae* *Enterobacteriaceae* *L. monocytogenes* *N. meningitidis*	Community-acquired: Cefotaxime or ceftriaxone *IV* + vancomycin *IV* Ampicillin *IV* should be added if *L. monocytogenes* is suspected[4] Nosocomial: Meropenem + vancomycin *IV*

Adapted from Fernandez J, Gustot T. *J Hepatol* 2012; 56 (Suppl 1): S1-12. [1]Quinolones should not be used in patients submitted to long-term norfloxacin prophylaxis or in geographical areas with a high prevalence of quinolone-resistant Enterobacteriaceae; [2]Risk factors for *Enterococci*: Quinolone prophylaxis, hospital-acquired infection; [3]Risk factors for MRSA: Ventilator-associated pneumonia, previous antibiotic therapy, nasal MRSA carriage; [4]Risk factors for *L monocytogenes*: Hemochromatosis, detection of gram-positive bacilli/coccobacilli in cerebrospinal fluid. BL-BI: Beta-lactam/beta-lactamase inhibitors (*e.g.*, amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin/tazobactam); MRSA: Methicillin-resistant *Staphylococcus aureus*; ESBL: Extended spectrum beta-lactamases; SBP: Spontaneous bacterial peritonitis; SBE: Spontaneous bacterial empyema; *IV*: Intravenous; *S. pneumoniae*: *Streptococcus pneumoniae*; *S. viridans*: *Streptococcus viridans*; *H. infuenzae*: *Haemophilus influenzae*; *M. pneumoniae*: *Mycoplasma pneumoniae*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *E. faecalis*: *Enterococcus faecalis*; *E. faecium*: *Enterococcus faecium*; *S. pyogenes*: *Streptococcus pyogenes*; *L. monocytogenes*: *Listeria monocytogenes*; *N. meningitidis*: *Neisseria meningitidis*.

Biomarkers of bacterial infection in cirrhosis

It is crucial, but often difficult to make an early diagnosis of bacterial infections in cirrhosis due to non-specific manifestations, which are indistinguishable from other non-infectious causes of systemic inflammatory response syndrome (SIRS) and the symptoms of liver deterioration. Therefore, serum biomarkers that are sensitive, reliable and inexpensive are being pursued in order to improve the diagnosis of bacterial infection in the setting of cirrhosis. General inflammatory markers, such as C-reactive protein (CRP, synthesized by the liver), ferritin (synthesized by the liver) or white blood cells (WBC), lack specificity for bacterial infections. Procalcitonin (PCT) is potentially a more specific marker for bacterial infection. PCT is produced by nearly all tissues in response to endotoxin or mediators released in response to bacterial infections [interleukin (IL)-1b, tumor necrosis factor-alfa, and IL-6]. It highly correlates with the severity of bacterial infections and may be helpful to distinguish bacterial infections from viral infection or other non-infectious causes[33].

In the meta-analysis included 10 diagnostic studies (1144 cirrhotic patients and 435 bacterial infection episodes), PCT displayed an area under the curve of 0.92, a sensitivity of 0.79, and a specificity of 0.89 in diagnosing bacterial infection[34]. The pooled sensitivity estimates were 79% for PCT and 77% for CRP tests, whereas the pooled specificity were higher for both PCT (89%) and CRP tests (85%)[34]. The results were consistent when stratified to patients with SBP or patients with systemic infection. The authors suggested that the PCT test can be used as a rule-in diagnostic tool (positive likelihood ratio 7.38), CRP test can be used as a rule-out diagnostic tool (negative likelihood ratio 0.23) in patients without signs of infection[34]. However, the diagnostic accuracy of CRP in the detection of bacterial infections decreased in setting of advanced liver disease. The combination of CRP and PCT may slightly improve the diagnostic accuracy of bacterial infection[35].

SBP

Epidemiology and clinical features of SBP

SBP is common and quite unique in patients with cirrhosis. The prevalence of SBP in cirrhotic patients with ascites admitted to the hospital ranges from 10%-30%;

Table 3 Common manifestations and risk factors of bacterial pathogens in patients with cirrhosis

Pathogens	Common clinical syndrome	Risk factors	Remarks
Aeromonas spp. (*A. hydrophila*, *A. sobria*, *A. aquariorum*)[120-126]	SBP, bacteremia, SSTI, enterocolitis	Contaminated food and water Diabetes Most reports were from East Asia	Increased incidence High mortality (20%-60%), especially when presence of hypotension on admission
Campylobacter spp.[127,128]	Bacteremia, SBP	Alcoholic	Increased incidence High mortality (10% in bacteremia)
Clostridium spp. (*C. perfringens*, *C. bifermentans*, *C. septicum*)[4,129,130]	SSTI	Diabetes	Increased incidence Very high mortality (54%-65%)
Clostridium difficile[108,131-133]	ATB-associated diarrhea and colitis	Broad-spectrum ATB Hospitalization PPIs	Increased incidence Higher mortality (14%) when compare to non-cirrhotics Increased cost and length of hospital stay
Enterococcus spp. (*E. faecium*, *E. faecalis*, *E. galinarum*)[134-136]	SBP, bacteremia, UTI, endocarditis, biliary tract infection	Healthcare-associated infection Quinolone prophylaxis	Increased incidence High mortality (30% in bacteremia; 60% in SBP) Increased incidence of VRE colonization and infection in liver transplant setting
Listeria monocytogenes[137,138]	SBP, bacteremia, meningitis	Hemochromatosis Alcoholic	Increased incidence
Mycobacterium TB[2,139,140]	Pulmonary TB, TB peritonitis, TB lymphadenitis, disseminated TB	Developing countries Exposed to TB case	Increased incidence, especially extrapulmonary forms (> 50% of TB peritonitis cases in the United States had underlying cirrhosis) High mortality (22%-48%) Increased risk for multi-drug resistant TB Increased risk for anti-TB-induced hepatotoxicity
Pasteurella multocida[141-143]	SBP, bacteremia septic arthritis, meningitis	Presence of ascites (TB peritonitis) Domestic animal (cats or dogs) bites or scratches	Increased incidence High mortality (10%-40% in bacteremia)
Staphylococcus aureus[45,144,145]	SSTI, UTI, SBP, bacteremia, endocarditis	Alcoholic Invasive procedures Hospitalization	Increased incidence of MRSA carriage and infection High mortality (30% in bacteremia) Removal of the eradicable focus was associated with decreased mortality
Streptococcus bovis[146,147]	Bacteremia, SBP meningitis, endocarditis, septic arthritis	Quinolone prophylaxis Colonic lesion(s): Adenoma or adenocarcinoma (presence in 18%-40% of cases)	Increased incidence High mortality (up to 40% in bacteremia with advanced cirrhosis) Colonic lesion(s) was present in 18%-40% of cases
Streptococcus group B[148-150]	SSTI, bacteremia, SBP, meningitis, pneumonia	Alcoholic Post endoscopic sclerotherapy and banding ligation	Increased incidence High mortality (10%-25% in SBP and bacteremia; 45% in meningitis)
Streptococcus pneumoniae[89-92]	Pneumonia, SBP bacteremia, SSTI, meningitis	Alcoholic Post-splenectomy Not vaccinated	Increased incidence of invasive pneumococcal disease High mortality (10%-20%)
Vibrio spp. (*V. vulnificus*, *non-o1 V. cholera*, *V. parahemolyticus*)[151-153]	SSTI, bacteremia, gastroenteritis, diarrhea, SBP	Hemochromatosis Exposed to seawater and undercooked seafoods Most reports were from East Asia	Increased incidence Very high mortality (50%-60% in bacteremia; 24% in SSTI)
Yersinia spp. (*Y. enterocolitica*, *Y. pseudotuberculosis*)[154,155]	Bacteremia, SBP, hepatosplenic abscesses	Hemochromatosis Exposed to animals and contaminated foods	Increased incidence (in hemochromatosis) High mortality (50% in bacteremia)

SBP: Spontaneous bacterial peritonitis; SSTI: Skin and soft tissue infection; UTI: Urinary tract infection; ATB: Antibiotics; PPIs: Proton-pump inhibitors; TB: Tuberculosis; MRSA: Methicillin-resistant *Staphylococcus aureus*; *A. hydrophila*: *Aeromonas hydrophila*; *A. sobria*: *Aeromonas sobria*; *A. aquariorum*: *Aeromonas aquariorum*; *C. perfringens*: *Clostridium perfringens*; *C. bifermentans*: *Clostridium bifermentans*; *C. septicum*: *Clostridium septicum*; *E. faecium*: *Enterococcus faecium*; *E. faecalis*: *Enterococcus faecalis*; *E. galinarum*: *Enterococcus galinarum*; *Mycobacterium TB*: *Mycobacterium tuberculosis*; *V. vulnificus*: *Vibrio vulnificus*; *V. cholera*: *Vibrio cholera*; *V. parahemolyticus*: *Vibrio parahemolyticus*; *Y. enterocolitica*: *Yersinia enterocolitica*; *Y. pseudotuberculosis*: *Yersinia pseudotuberculosis*; VRE: Vancomycin-resistant *Enterococci*.

about 50% of cases are present at the time of hospitalization and 50% develop during the hospitalization[1,29,36]. BT, systemic, and local immune dysfunction, particularly a decreased opsonic activity in ascitic fluid, are the main elements in the pathogenesis of SBP[1,15,17,37] (Figure 1). Accordingly, gut microflora including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Enterococci*, and *Streptococci* are common causative organisms[1,15,17,37]. The classical symptoms of SBP include fever, abdominal pain, and worsening of pre-existing ascites, although these symptoms may be absent in up to one-third of cases[38]. Therefore, diagnostic paracentesis is recommended to perform in all cirrhotic patients with ascites at the time of admission and/or in case of gastrointestinal (GI) bleeding, shock, signs of inflammation, hepatic encephalopathy, worsening of liver or renal function[37,39-41]. The hospital mortality for SBP ranges from 10%-50% depending on various factors[37]. Predictors for poor prognosis in SBP include older age, higher Child-Pugh scores, nosocomial origin, encephalopathy, elevated serum creatinine

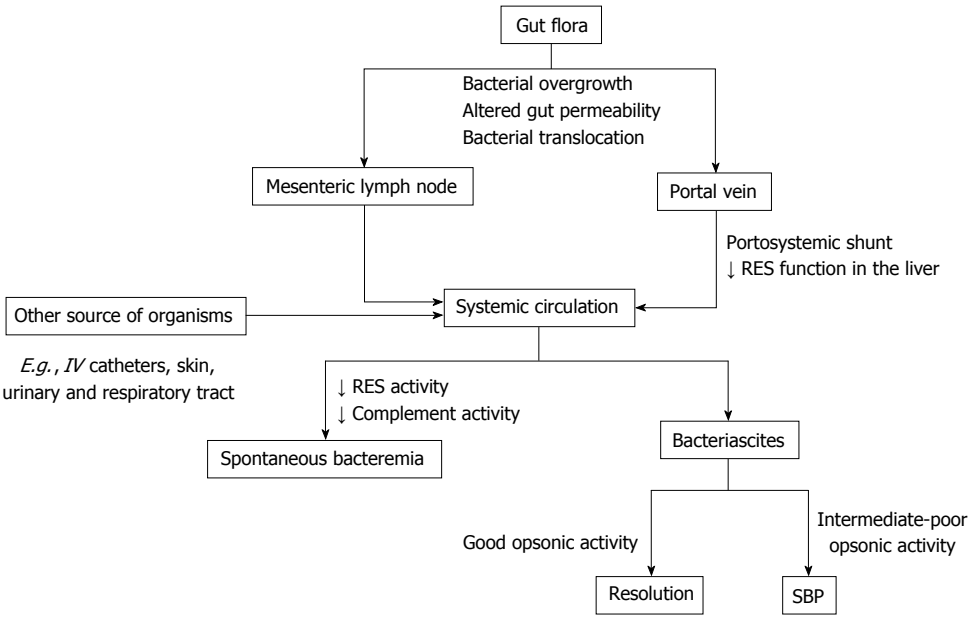

Figure 1 Pathogenesis of spontaneous bacterial peritonitis and bacteremia (reproduced from Bonnel *et al*[1]. *Clin Gastroenterol Hepatol* 2011; 9: 729. With permission). SBP: Spontaneous bacterial peritonitis; RES: Reticuloendothelial system; *IV*: Intravenous.

and bilirubin, ascites culture positivity, presence of bacteremia, and infections with resistant organisms[42-45]. Notably, the modifiable factors to reduce morbidity and mortality in SBP include prompt diagnosis, proper first-line ATB treatment and prevention of subsequent renal failure[37]. SBP is one of the important prognostic landmark in the natural course of cirrhosis as the overall one-year mortality rate after a first episode of SBP are 30%-93% regardless of its recurrence[37,46,47].

Diagnosis of SBP

The diagnosis of SBP is relied on the cell count of the ascitic fluid, determined either by microscope or appropriate automated cell counters, and bacterial culture[40,41,48]. Ascitic fluid culture is important and should be performed before initiating ATB therapy by bedside inoculation of ascites ⩾ 10 mL into blood culture bottles[49]. Reagent strips to assess leucocyte esterase activity of activated polymorphonuclear cells (PMN) are not recommended for rapid diagnosis of SBP due to unacceptable false-negative rates[50]. To date, most of reagent strips (LERS) that had been evaluated were developed for UTI with a threshold of > 50 PMN/mm^3[37]. More recently, ascites-calibrated reagent strips (cut-off of > 250 PMN/mm^3) have been introduced for SBP with promising preliminarily results[51]. Based on available evidences, LERS seem to have low sensitivity for SBP, but have reliably given a high negative predictive value (> 95% in most studies), which supports the potential role of LERS as a screening tool for SBP[52]. In addition, neutrophil gelatinase-associated lipocalin (NGAL), a protein involved in iron metabolism and links to the inflammation, and bacterial DNA in ascitic fluid have the potential to improve the diagnosis of SBP. The pivot study of using NGAL to differentiate bacterial peritonitis (30% were SBP) from nonbacterial peritonitis reported that AUC were 0.89 for NGAL and 0.94 for combination of NGAL and lactate dehydrogenase[53]. Detection of bacterial DNA by real-time polymerase chain reaction and sequencing of *16S rDNA* gene demonstrated poor results with negative results in almost half the culture-negative SBP episodes[54]. In contrast, another study using newly *in situ* hybridization method to detect global bacterial DNA demonstrated high sensitivity (91%) and specificity (100%) for detecting phagocytized bacterial DNA in the WBC of SBP ascites, with all test results obtained within one day[55].

Management of SBP

Empirical ATB should be given promptly to all cirrhotic patients with ascites PMN counts > 250 cells/mm^3 in clinical settings that suggestive for ascitic fluid infection (culture results are often unavailable at this time)[40,41] (Figure 2). The choice of empirical ATB should be based on the origin of infection, individual risk factors for resistant organism and local microbial epidemiology. In general, the suggested initial treatments of community-acquired SBP are third-generation cephalosporins (mostly preferred), amoxicillin-clavulanate or quinolones (Table 2). These empirical ATB should be given intravenously for a duration of 5-10 d[40,41]. In countries with low rate of quinolone-resistant *Enterobacteriaceae*, oral quinolones may be used for uncomplicated SBP, as defined by cases without shock, ileus, GI bleeding, hepatic encephalopathy (⩾ grade Ⅱ) or renal impairment (creatinine > 3 mg/dL)[56]. In nosocomial SBP, use of the antibiotics recommended above can be associated with unacceptable failure rates because resistance to third-generation cephalosporins (23%-44%) and quinolones (38%-50%) are increasingly reported[37,57,58].

Notably, the incidence of SBP causing by with gram-positive and resistant bacteria (mainly ESBL-producing

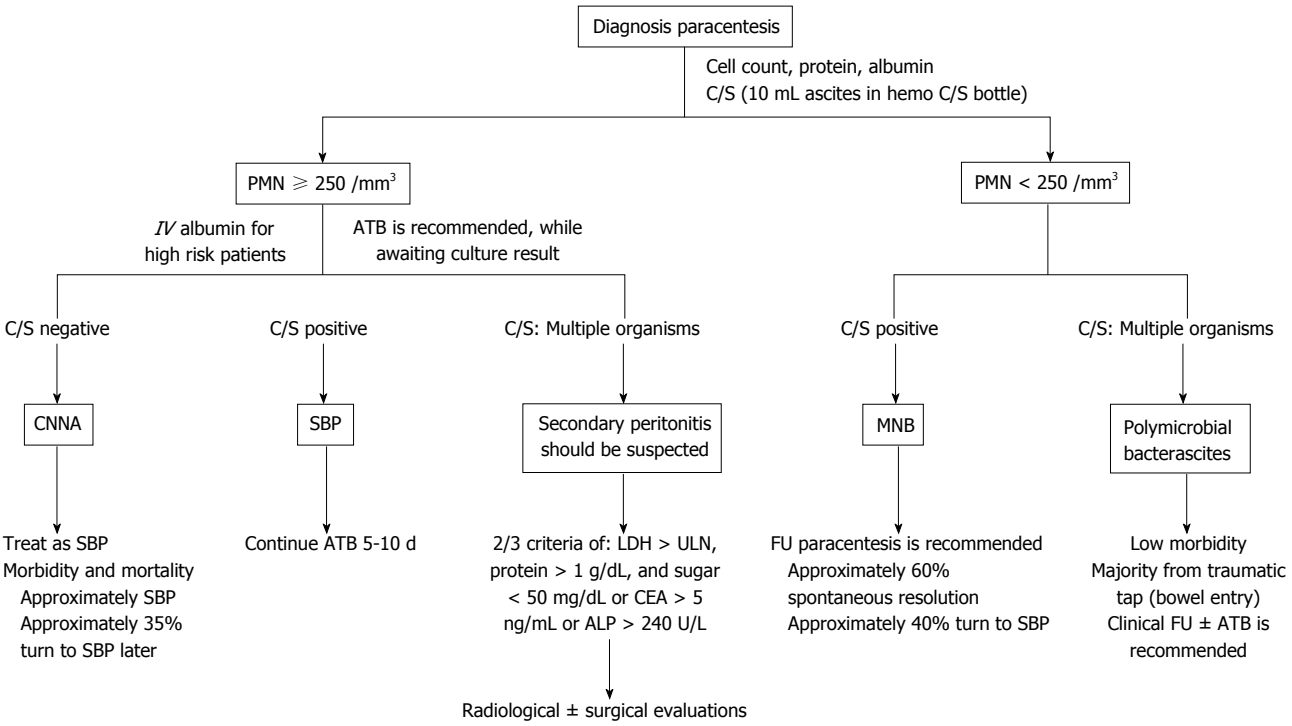

Figure 2 Algorithm for the management of cirrhotic patients with suspicious for ascitic fluid infection (adapted from Bonnel *et al*[1]. *Clin Gastroenterol Hepatol* 2011; 9: 732. With permission). PMN: Polymorphonuclear cells; SBP: Spontaneous bacterial peritonitis; ATB: Antibiotics; CNNA: Culture-negative neutrocytic ascites; MNB: Monobacterial non-neutrocytic bacterascites; LDH: Lactate dehydrogenase; CEA: Carcinoembryonic antigen; ALP: Alkaline phosphatase; ULN: Upper limit of normal; FU: Follow-up; C/S: Culture.

bacteria and multi-resistant gram-positive bacteria such as *Enterococci* or MRSA) has been increasingly reported in the healthcare associated and especially in nosocomial settings[37,57]. In patients with typical presentation and clinical improvement after ATB, a repeat of paracentesis is not necessary to assess for resolution of SBP[1,37,40,41]. However, in cases with questionable diagnosis or in those who did not satisfactorily improve with ATB, repeated paracentesis should be performed to document the response of treatment[37,40]. If the PMN count does not reduce by at least 25% after 2 d of ATB, changing treatment and/or reevaluation for other possible cause(s) of symptoms should be considered[37,59].

Renal impairment develops in 30%-40% of SBP cases and is a strong predictor of death during hospitalization[39,40,60]. The use of intravenous albumin (1.5 g/kg within 6 h of SBP diagnosis followed by 1 g/kg on day 3) in conjunction with intravenous (*IV*) antibiotic was found to reduce the incidence of renal impairment from 33% to 10% and mortality from 29% to 10%[61]. Notably, albumin infusion was particularly effective in patients with baseline serum creatinine ⩾ 1 mg/dL, blood urea nitrogen ⩾ 30 mg/dL or bilirubin ⩾ 4 mg/dL[39,61]. Unfortunately, albumin infusion in high-risk SBP has been underutilized, even in the United States, with > 50% of cases did not follow the guidelines[62]. It is unclear whether crystalloids or artificial colloids could replace albumin in this setting[39-41,63].

Prophylaxis of SBP

After recovering from SBP, the rate of recurrence is around 43% at 6 mo and 69% at 1 year[46]. Therefore, secondary prophylaxis of SBP should be given indefinitely or until LT[37,40,61,64]. Intermittent dosing of prophylactic ATB may select resistant flora, thus daily dosing is preferred[37,40] (Table 4).

Primary prophylaxis of SBP is justified for patients with high risk for developing SBP. A meta-analysis of ATB prophylaxis in cirrhotic patients with GI hemorrhage (5 RCT; n = 534) revealed 32% reduction of infections including SBP and/or bacteremia (P < 0.001) and 9% increase in survival (P = 0.004)[28]. Further, a subsequent meta-analysis of 8 oral antibiotic trials (n = 647) demonstrated 72% reduction in mortality at 3 mo; only 6 patients were additionally treated in order to prevent another death[65]. Oral norfloxacin is often utilized for primary prophylaxis in most settings, however *IV* ceftriaxone has been shown to be more effective than oral norfloxacin in patients with particularly advanced cirrhosis[66] (Table 4).

In cirrhotic patients with low ascitic fluid protein < 1.5 g/dL, the risk of developing a first episode of SBP is 13%-45% at 1 year[32,39]. However, several studies evaluating primary prophylaxis of SBP with norfloxacin in this setting yielded heterogeneous results[39]. Notably, a well-designed, randomized, controlled trial conducted in patients with severe liver disease and ascites protein < 1.5 g/dL without prior SBP demonstrated that norfloxacin (400 mg/d) reduced the development of SBP (from 61% to 7%) and improved survival at 1 year (from 48% to 60%)[67]. Notably, primary prophylactic ATB for SBP should be considered only for selected patients with

Table 4 Vaccinations and other preventive measures for bacterial infections in patients with cirrhosis

Avoidance	
Raw/uncooked foods, especially seafood	
Close contact to at-risk animals or sick people	
Wound exposure to flood or seawater	
Vaccination[87]	
Influenza	Recommended yearly for all patients with chronic liver disease
Pneumococcal (polysaccharide)	Recommended for all cirrhotic patient Booster dose after 3-5 yr
Hepatitis A	Recommended for all non-immune, cirrhotic patient, 2 injections 6-12 mo apart Anti-HAV should be checked 1-2 mo after the second dose
Hepatitis B	Recommended for all cirrhotic patient without serological markers of HBV (*e.g.*, negative HBsAg, anti-HBs, and anti-HBc antibodies) 3 injections (at month 0, 1 and 6) Anti-HBs should be checked 1-2 mo after the last dose Patients with advanced cirrhosis should receive 1 dose of 40 μg/mL (Recombivax HB) administered on a 3-dose schedule or 2 doses of 20 μg/mL (Engerix-B) administered simultaneously on a 4-dose schedule at 0, 1, 2 and 6 mo
Other vaccines, *e.g.*, Td, Tdap, MMR, varicella	Recommendations are as same as general adult population
Prophylactic antibiotics	
Secondary prophylaxis for SBP[32,41]	Recommended for all cirrhotic patients who recovered from SBP Norfloxacin 400 mg PO daily Alternatives: TMP/SMX 1 double-strength tablet or ciprofloxacin 500 mg PO daily
Primary prophylaxis in GI bleeding[32,41]	Recommended for all cirrhotic patients with GI hemorrhage Norfloxacin 400 mg PO twice daily or ceftriaxone 1 g *IV* daily for 7 d *IV* ceftriaxone is preferred, in patients with advanced cirrhosis as defined by the presence of at least two of the following: Ascites, severe malnutrition, encephalopathy or bilirubin > 3 mg/dL
Primary prophylaxis in patients with low ascitic fluid protein[32,41]	Recommended for cirrhotic patients with ascitic fluid protein < 1.5 g/dL and at least one of the following is present: Serum creatinine > 1.2 mg/dL, blood urea nitrogen > 25 mg/dL, serum sodium < 130 mEq/L or Child-Pugh > 9 points with bilirubin > 3 mg/dL
Prophylaxis before undergoing endoscopic and surgical procedures	Prophylactic antiobiotics are recommended for the moderate-high risk invasive endoscopic or surgical procedures (choice of antibiotics should be individualized) Prophylactic antibiotics are not routinely recommended for diagnostic endoscopy, elective variceal band ligation or sclerotherapy, and abdominal paracentesis

HBV: Hepatitis B virus; SBP: Spontaneous bacterial peritonitis; Td: Tetanus-Diphtheria; Tdap: Tetanus-Diphtheria-Pertussis; MMR: Measles/Mumps/Rubella; GI: Gastrointestinal; TMP/SMX: Trimethoprim/sulfamethoxazole; PO: Per oral; *IV*: Intravenous.

advanced cirrhosis and ascitic fluid protein < 1.5 g/dL since more liberal use of these ATB in long-term would lead to subsequent infection by resistant bacteria as well as *Clostridium difficile*-associated diarrhea (Table 4)[39-41].

Consequences of bacterial infections in cirrhosis

Bacterial infections in cirrhosis are associated with poor outcomes (increased mortality about 4 folds)[47]. Both short- and long-term mortality rates of sepsis in cirrhotic patients are very high; 26%-44% of patients die within 1 mo after infection and another one-third die in 1 year[4,47]. The clinical predictors of death during or following infection are advanced liver disease, nosocomial origin, gastrointestinal hemorrhage, encephalopathy, liver cancer, presence of shock and organ failure (especially renal failure)[4,47].

The suggested strategies for the management of cirrhotic patients with severe sepsis are discussed in depth in other articles[23,32,68,69]. Broad spectrum empirical ATB[70] and fluid resuscitation, with either cystalloids or colloids (albumin, gelatins or hydroxyethyl starches), should be promptly initiated and followed an early goal-directed therapy approach (stepwise emergent resuscitation with predefined goals to keep mean arterial pressure ⩾ 65 mmHg, central venous pressure between 8-12 mmHg, central venous oxygen saturation ⩾ 70% and urine output ⩾ 0.5 mL/kg per hour)[23,32,68]. Resuscitation with crystalloids requires more fluid to attain the same targets and results in more edema, particularly in cirrhotic patients with hypoalbuminemia[32]. The benefit of resuscitation with albumin in non-cirrhotic patients with sepsis has been reported[71]. However, the role of albumin infusion for sepsis other than from SBP in cirrhosis is still unclear. The RCT from Spain found beneficial effects on renal and circulatory functions with a potential benefit on survival[72]. Conversely, more recent RCT from France reported that albumin delayed the onset of renal failure, but did not significantly improve 3-mo renal failure and survival rates. Thus, pulmonary edema developed in 8% of patients in the albumin group[73]. Norepinephrine and dopamine have been considered as the first-choice vasopressor agents in patients with septic shock[23,32,68,69]. Cirrhotic patients with septic shock are often associated with vascular hyporeactivity to these vasopressor agents. Thus, inotropic drugs are not generally effective since they already present high cardiac outputs[23,32,68]. Relative adrenal insufficiency is common (51%-77%) in cirrhotic patients with septic shock, however the effects of corticosteroids on such patients' outcomes

are unclear[23,32,68]. Therefore, stress dose corticosteroid is currently recommended only for patients with vasopressor-unresponsive septic shock[23,32,68]. Blood sugar should be maintained in the range of 140-180 mg/dL[69].

Acute kidney injury following infections develop in 27%-34% of patients with advanced cirrhosis[2,61,74,75], and is a strong predictor of death (40%-50% mortality)[47,74,75]. Risk factors for infection-induced renal failure in cirrhosis include advanced liver disease[74-76], pre-existing kidney disease[76], hypovolemia or low cardiac output[2,75], unresolved infection[74] and not receiving prompt albumin infusion[61]. It should be noted that most studies that reported poor survival in patients with infection-induced renal failure have defined renal failure as a serum creatinine level of > 1.5 mg/dL. Recently, the International Ascites Club and the Acute Dialysis Quality Initiative group proposed that acute kidney injury (AKI) in cirrhosis should be redefined as an increase in serum creatinine level of 0.3 mg/dL in less than 48 h or a 50% increase in serum creatinine level from a stable baseline reading within the previous 6 mo, irrespective of the final serum creatinine level[77,78]. This new definition was then evaluated and found to accurately predict 30-d mortality in patients with cirrhosis and infection (10-fold higher among those with irreversible AKI than those without AKI)[79]. Renal failure during infection (without septic shock) that does not respond to albumin infusion is considered hepatorenal syndrome[80].

Bacterial infection can trigger a rapid deterioration of liver functions in patients with cirrhosis and it is one of the most common precipitating cause of ACLF, which represents > 30% of the cases[3,23,81,82]. The most common sites of bacterial infection are ascites and lungs[81]. Moreover, infections were the second most common cause of death at 28 d among patients with ACLF (28%), behind multiple organ failure without septic or hypovolemic shock (44%). However, there was no difference in 28 d mortality among ACLF patients with or without the bacterial infection at admission (37% and 33%, respectively)[81]. Independent predictors of poor survival in patients with bacterial infections and ACLF were presence of organ(s) failure, second infections, admission values of high MELD, low blood pressure, leukocutosis, and low albumin[83].

Pulmonary complications are commonly observed in cirrhotic patients with infections. Aspiration is common in encephalopathic patients. Acute respiratory distress syndrome is increasingly seen in cirrhosis that may develop is association with exaggerated SIRS in severe sepsis[84]. Prognosis of cirrhotic patients with respiratory failure is poor, with a mortality rate up to 33%-60%[69,85]. Additionally, sepsis-induced cytokines can further worsen pre-existing coagulation and platelet abnormalities in patients with cirrhosis[2,24].

Prevention measures

Preventive measures must be emphasized to all patients with cirrhosis and prophylactic ATB is suggested for those who are at high risk of developing infections (Table 4)[2]. Notably, antibiotic prophylaxis has been associated with the development of multi-drug resistant bacteria and C. difficile infection. Therefore it should be judiciously used in those patients with proper indications.

Active immunization against hepatitis A and B viruses, influenza and pneumococcus are recommended since these preventable infections carry accompanied by higher morbidity and mortality in patients with cirrhosis (Table 4)[86-88]. Both cellular and humoral immune responses are suboptimal in cirrhosis, particularly in the advanced stage, which can be associated with inadequate post-vaccination antibody response, as well as loss of immunogenicity in the long-term[86-88]. Therefore, it is important to address immunization needs in patients with chronic liver disease or compensated cirrhosis early on, when immunizations are most effective.

Although there is no clear recommendation whether we can safely utilize live and attenuated vaccines in patients with cirrhosis, inactivated or killed-type vaccinations are generally preferable[86-88]. The incidence and severity of *Streptococcus pneumoniae* infections are increased in patients with cirrhosis[89-92]. Pneumococcal vaccination is less effective in patients with cirrhosis, with a further decline in protective antibodies after LT[93]. It is therefore recommended with booster doses every 5 years[86-88]. Incidence of seasonal flu is not obviously increased in cirrhosis; however, influenza may precipitate liver decompensation[86,87,94]. Influenza vaccine is well-tolerated and effective in cirrhotic patients, despite a mildly decreased immunogenicity[95,96]. All other vaccinations recommended for general adult population are also indicated in patients with cirrhosis as the Centers for Disease Control and Prevention recommendation for adults[97].

Proton pump inhibitors and the risk of infections in cirrhosis

Proton pump inhibitors (PPIs) have been widely used in patients with cirrhosis (sometimes over-utilized)[98]. Patients with cirrhosis have high prevalence of gastroduodenal mucosal lesions[99,100] and are associated with increased mortality rate from peptic ulcer bleeding (adjusted OR = 3.3; 95%CI: 2.2-4.9)[101]. However, clear evidence for a protective role of PPIs in cirrhosis is limited.

A state of gastric acid suppression induced by PPIs, particularly in long-term users, is known to be associated with small bowel bacterial overgrowth, alteration of gut flora and reduction of gastrointestinal motility[102-104]. By these effects, PPIs may enhance BT and possibly increase the risk of various infections in patients with cirrhosis. In addition, impairment of neutrophil function caused by PPIs has also been reported[105-107]. There have been several studies, including case-control, retrospective and prospective cohorts, and meta-analyses, suggesting that PPIs are associated with increased risk of bacterial infections, such as SBP, bacteremia, *Clostridium difficile*-associated diarrhea, and enteric

Table 5 Studies demonstrated risk of bacterial infections in cirrhotic patients receiving proton pump inhibitors

Ref.	Design	*n*	Results
Campbell *et al*[116]	Case-control	116	NS for SBP (OR = 1.05; 95%CI: 0.43-2.57)
Bajaj *et al*[108]	Case-control	83230	PPI use were significantly higher in those with CDAD (74% *vs* 31%, *P* = 0.0001)
Bajaj *et al*[112]	Retrospective, propensity-matched	1268	↑ Serious infections (HR = 1.66; 95%CI: 1.31-2.12)
de Vos *et al*[119]	Case-control	102	PPI were more frequently used in SBP patients than in controls, but did not influence prognosis of SBP
Min *et al*[113]	Retrospective cohort	1554	↑ SBP (HR = 1.39; 95%CI: 1.057-1.843)
Mandorfer *et al*[117]	Retrospective	607	PPI neither predisposes to SBP (HR = 1.38; 95%CI: 0.63-3.01) or other infections (HR = 1.71; 95%CI: 0.85-3.44)
Terg *et al*[118]	Prospective	770	PPI therapy was not associated with a higher risk of SBP and other infections
Merli *et al*[114]	Cross-sectional	400	↑ Bacterial infections (OR = 2; 95%CI: 1.2-3.2)
O'Leary *et al*[115]	Prospective	188	↑ Infections: CDAD and SBP (OR = 2.94; 95%CI: 1.39-6.20)

NS: Not significance; SBP: Spontaneous bacterial peritonitis; PPI: Proton pump inhibitor; CDAD: Clostridium difficile associate disease.

infections, in patients with cirrhosis[108-115]. However, the association between PPIs and infections in cirrhosis remains somewhat controversial since many studies have reported conflicting results[116-119] (Table 5). Though randomized controlled studies are required to draw firm conclusions whether or not PPIs increase infections in cirrhosis, PPI should be used only if clinically indicated.

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ORIGINAL ARTICLE

Basic Study

Burn injury induces histopathological changes and cell proliferation in liver of rats

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Abstract

AIM: To investigate effects of severe burn injury (BI) in rat liver through the histopathological and inflammatory markers analysis.

METHODS: Forty-two male Wistar rats were distributed into two groups, control (C) and subjected to scald BI (SBI). The animals were euthanized one, four and 14 d post sham or 45% of the total body surface BI. Liver fragments were submitted to histopathological, morphoquantitative (hepatocyte area and cell density), ciclooxigenase-2 (COX-2) immunoexpression, and gene expression [real-time polymerase chain reaction for tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS) and caspase-3] methods.

RESULTS: Histopathological findings showed inflammatory process in all periods investigated and hepatocyte degeneration added to increased amount of connective tissue 14 d post injury. Hepatocyte area, the density of binucleated hepatocytes and density of sinusoidal cells of SBI groups were increased when compared with control. COX-2 immunoexpression was stronger in SBI groups. No differences were found in TNF-α, iNOS and caspase-3 gene expression.

CONCLUSION: BI induces histopathological changes, upregulation of COX-2 immunoexpression, and cell proliferation in liver of rats.

Key words: Burn injury; Morphology; Histophatology; Liver; Ciclooxigenase-2

Core tip: Severe burn injuries result in serious complications that involve host response related to inflammation and multiple organ dysfunction. The goal of this study was to investigate the temporal effects of extensive experimental burn injury (BI) in rat liver through the histopathological and morphoquantitative aspects, immunoexpression of ciclooxigenase-2 (COX-2) and liver gene expression of tumor necrosis factor-α, inducible nitric oxide synthase and caspase-3. Our results revealed that BI induces histopathological changes, upregulation of COX-2 immunoexpression, and cell proliferation in liver of rats.

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INTRODUCTION

Burn injuries (BIs) represent one of the greatest public health problems, which induce to significant patient morbidity and mortality[1]. Scalds are most common cause of BI and preferentially occurs in children under the five years[2]. In pediatric patient population persistent protein catabolism may lead to delay in growth for up to 2 years after burn[3].

Severe BI greater than 40% is a process that involves several host responses, including organ damage by inflammation and immune response[4]. Hypermetabolism is characterized by the inflammatory response, negative nitrogen balance, increase in resting energy consumption, and alterations in glucose and lipid metabolism[5]. Excessive systemic inflammatory response syndrome (SIRS) following burns to damage distant organ and provoke multiple organ dysfunction syndrome[6].

According to Jeschke *et al*[7] liver has been shown to play a crucial role after a BI. In a study of 102 children, the authors found that liver size and weight increased during the first week post BI, peaked at 2 wk post burn, and remained increased at 6, 9 and 12 mo after trauma. In autopsy of severely burned pediatric patients hepatomegaly with fatty infiltration was related to elevated occurrence of sepsis and mortality[8]. Jeschke *et al*[9] showed liver weight was increased by 140% to 150% compared with estimated liver weight at 6, 9 and 12 mo post BI, indicating prolonged alterations in hepato-structure.

Apoptosis from liver cells was evaluated by terminal deoxyuridine nick end labeling (TUNEL) assay in rats that have severe BI greater than 40% total body surface area[10]. Moreover, compensatory hepatocyte proliferation is detected due to increased apoptosis[7].

Severe BI is associated with host responses related to inflammation and apoptotic process and liver clearly plays an important role in metabolic processes post burn. The present study proposes to investigate effects of severe BI in liver of rats through the histopathological and morphoquantitative aspects, immunoexpression of ciclooxigenase-2 (COX-2) and liver gene expression of tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS) and caspase-3.

MATERIALS AND METHODS

Experimental design

Male Wistar rats (*n* = 42), Rattus Norvegicus, with 21-d-old was chosen in the present study to mimic a developing organism. The rats were individually housed cages for five days, distributed into two groups: Control (C) and subjected to scald BI (SBI). The temperature room was controlled (22 ℃) with regular light-dark cycle with 12 h, water and food were offered ad libitum. On the sixth day, the animals were anesthetized with an intraperitoneal (IP) injection of Ketamine (50 mg/mL) and Xilazyne (10 mg/mL) and dorsal and ventral hair were removed. The group SBI (*n* = 21) was submitted to nonlethal scald BI by immersing 45% of each rat's body, in 87 ℃ water as described by Walker *et al*[11]. The C group (*n* = 21) were submitted to sham of the SBI. Each animal had 30% of its dorsal and 15% of ventral area exposed to SBI for 10 and 3 s, respectively[12]. The rats in both groups were subcutaneously injected with the analgesic Buprenorphine (0.2 mg/kg) immediately after sham or SBI and again 24 h later. One, 4 and 14 d following the SBI, all animals from each group were euthanized with a lethal IP injection of Ketamine (150 mg/kg) and Xilazyne (30 mg/kg).

Compliance with ethical requirements

All institutional and national guidelines for the care and use of laboratory animals were followed. The procedures were approved by the Committee of Ethics and Research from Federal University of São Paulo (protocol No. 329/12).

Histopathological and morphoquantitative analysis

Liver of euthanized rats from SBI and C groups were examined. The specimens was immediately fixed in 10% formalin phosphate buffer for 24 h for histological analyzes and routinely embedded in paraffin blocks and cut in transversal sections (4 μm). The slides were stained with hematoxylin and eosin (H and E) and Sirius Red[13], whose photomicrographs were made under normal and polarized light to differentiate type Ⅰ (red and yellow) and Ⅲ (green) collagen.

The hepatocyte area (μm^2) was determined from

Table 1 Primers sequences

Gene	Forward	Reverse
TNF-α	5′-CCCAGAAAAGCAAGCAACCA-3′	5′-GCCTCGGGCCAGTGTATG-3′
Caspase-3	5′-TCTACCGCACCCGGTTACTA-3′	5′-TGTCGTCATGTCCACCACTG-3′
GAPDH	5′-GCTCTCTGCTCCTCCCTGTTC-3′	5′-GACGCTGGCACTGCACAA-3′

TNF-α: Tumor necrosis factor-α; *GAPDH*: Glyceraldehyde-3-Phosphate Dehydrogenase.

the measurement of 50 cells stained with H and E per animal. These fibers were randomly chosen from each animal comprising each experimental group. The cell density (number of cells/mm^2) was determined as described by Mandarim-de-Lacerda *et al*[14]. For this purpose, it was used five sections chosen randomly and stained with H and E and two fields of each section was analyzed, totaling ten photomicrographs per animal. It was determinate the density of mononucleated hepatocytes, binucleated hepatocytes and sinusoidal cells. For to investigate the hepatocyte area and density, it was used a computerized imaging equipment (Axio Visio 4.5 - Zeiss®) attached to a binocular microscope (Axio Observer D1, Zeiss®) with a 63 × objective.

COX-2 immunohistochemical analysis

The paraffin of liver sections (4 μm) was removed with xylene and cuts were rehydrated in graded ethanol, after pre-treated with 0.01 mol/L citric acid buffer (pH 6) in a microwave for 15 min at 850 W for antigen retrieval. The sections were pre-incubated for 5 min in 0.3% hydrogen peroxide in phosphate buffered saline (PBS) solution to inactive the endogenous peroxidase. Then the material blocked was with 5% normal goat serum in PBS solution for 10 min and then incubated with anti-COX-2 polyclonal primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA), at concentration of 1:200. Sections were incubated overnight at 4 ℃ in a refrigerator. After this was washes in PBS and incubated with biotin conjugated secondary antibody anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at a concentration of 1:200 in PBS for 30 min, washed with PBS: Followed by the application of avidin-biotin complex conjugated to peroxidase (Vector Laboratories) for 30 min. Then continued with the application of a 0.05% solution of 3-3-diaminobenzidine solution and counterstained with Harris hematoxylin (Merck).

Real-time polymerase chain reaction for TNF-α and caspase-3

Liver of animals was homogenized with 1 mL Trizol® (Invitrogen®, CA, United States), was added chloroform, isopropanol and ethanol 75% and centrifuged. In 40 μL of DEPC-treated water the pellet formed was re-suspended. The RNA purity and integrity were guaranteed by optical density (260/280 nm ratio > 1.9; Nanodrop® 2000 c, Thermo Scientific, Canada). Successively, the samples were kept in -80 ℃. And were treated with DNAase (deoxyribonuclease I Amp Grade®, Invitrogen®, CA, United States) as fixed by the producer. The total RNA extraction was according with the protocol adapted by Chomczynski *et al*[15].

The total RNA was treated with DNAase and was built the cDNA by reverse transcriptase [real-time polymerase chain reaction (RT-PCR)], with the High-Capacity cDNA kit Reverser Transcription® (Applied Biosystems®, United States). The primers previously designed for genes of interest and endogenous control (Glyceraldehyde-3-Phosphate Dehydrogenase) were used for gene expression analysis and the detection of amplification was through intercalating DNA (Sybr Green®, Applied Biosystems®, United States). The primers sequences are show in the Table 1.

The samples in duplicate are pipetted on the equipment RT-PCR 7500 Fast (Applied Biosystems®, United States) and subsequent the program of cycling was selected: Holding stage -95 ℃ for 10 min, 30 cycles of 15 s at 95 ℃ and 60 ℃ for 1 min, finish with the melting curve: 95 ℃ for 15 s, 60 ℃ for 1 min and 95 ℃ for 15 s. The results were acquired by relative quantification (method2$^{-\Delta\Delta Ct}$) at which the Cycle-threshold (Ct) values obtained for C group was compared to the SBI group.

Statistical analysis

Statistical analysis for hepatocyte area, cell density and RT-PCR were evaluated by analysis of variance with two factors (group and time), and followed with a Tukey's test for multiple comparisons, when necessary. $P < 0.05$ was considered to statistical significance.

RESULTS

Histopathological and morphoquantitative analysis

Liver cuts from Control group revealed hepatocytes arranged equidistantly with sinusoidal cells distributed in sinusoidal space (Figure 1A-C). Histopathological evaluation of SBI group investigated one day post injury revealed the presence of erythrocytes in sinusoidal space associated with inflammatory infiltrate (Figure 1D). Four days after injury, an increased sinusoidal space persisted (Figure 1E) and fourteen days post injury, liver sections showed inflammatory cells rounding hepatocytes in degeneration (Figure 1F).

SBI group following 14 d after lesion exhibited increase in connective tissue in the hepatic parenchyma (Figure 2C and D) when compared with controls (Figure 2A and B). The data concerning SBI group 1 and 4 d post BI not have been present for the reason that similar to control groups. Under polarized light connective tissue analysis showed type Ⅲ collagen (green) preponderance.

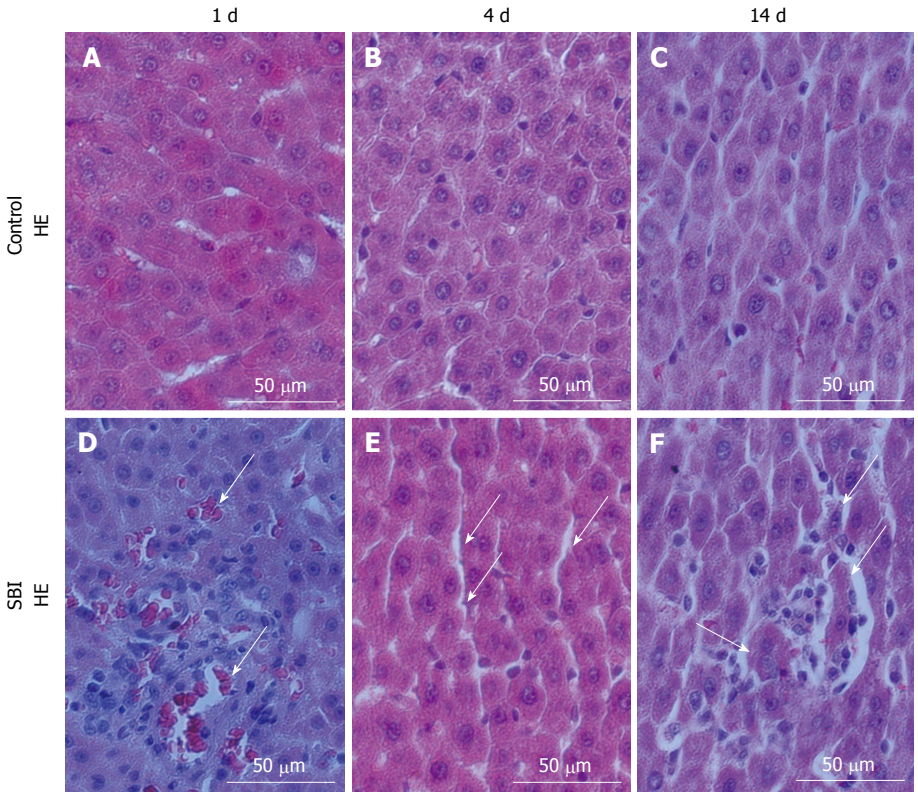

Figure 1 Sections of rat liver stained with hematoxylin and eosin; panels show groups control (A-C) and submitted to scald burn injury (D-F) evaluated in different periods. A-C: Hepatocytes and sinusoidal cells with normal aspect; D: Sinusoidal space filled by erythrocytes (arrows) and inflammatory infiltrate; E: Sinusoidal space increased (arrows); F: Sinusoidal space increased and inflammatory cells rounding hepatocytes in degeneration process (arrows). SBI: Scald burn injury.

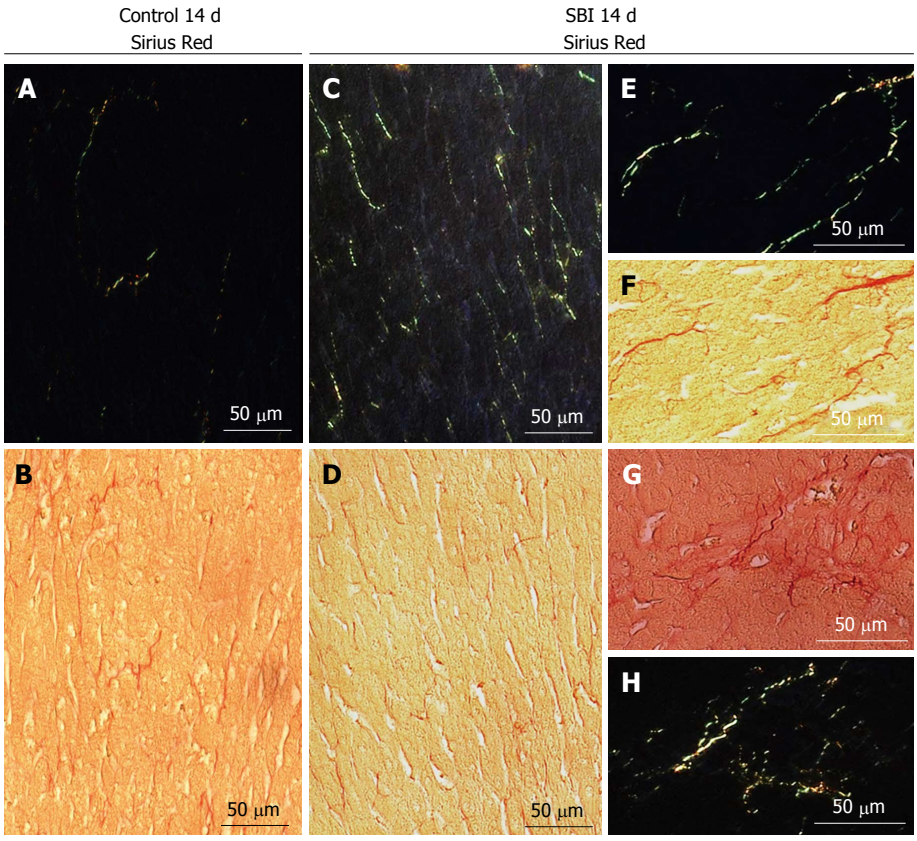

Figure 2 Liver sections stained with Sirius Red with normal (B, D, F and G) and polarized light (A, C, E and H). Panels show control group 14 d after sham (A and B) or burn injury (C-H). Notes connective tissue was increased in SBI group when compared with Control. SBI: Scald burn injury.

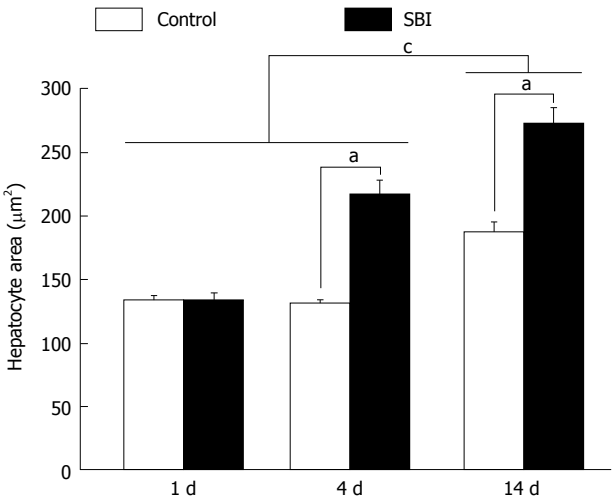

Figure 3 Mean + SD of hepatocyte area. [a]*P* < 0.05 - hepatocyte area of SBI group increased than control; [c]*P* < 0.05 - animals with 14 d sham or post burn injury showed hepatocyte larger than other periods investigated. SBI: Scald burn injury.

Further details in increased magnification of SBI group are demonstrated in Figure 2E-H.

Hepatocytes area was significantly higher in the SBI group investigated 4 and 14 d after BI (Figure 3). Moreover, the mean of cells area in animals with 14 d was statistically larger than 1 and 4 d post BI.

In relation to cell density (cells number/mm^2) represented in Figure 4, mononucleated hepatocyte density decrease in SBI groups 1 and 14 d post BI ($P < 0.05$). Binucleated hepatocyte density in SBI groups showed significantly decreased cell density one day post injury and increased cell density in 4 and 14 d after BI when compared with respectively controls. Sinusoidal cells presented significantly increased cell density for SBI group in all periods investigated.

COX-2 immunohistochemistry

COX-2 immunoexpression was encountered in the cytoplasm of hepatocytes. Control groups presented weak immunoexpression for all periods investigated in this setting. However a strong and focal immunoexpression was detected in SBI groups after 1 and 4 d and persisted weakly 14 d post BI (Figure 5).

RT-PCR for TNF-α, iNOS and caspase-3

TNF-α and iNOS, related to inflammation, and caspase-3 related to apoptosis, were evaluated in liver (Figure 6). The results showed no statistically differences between control and SBI groups for all periods investigated.

DISCUSSION

Extensive burn injuries outcomes in critical complications that involve host response related to SIRS and multiple organ dysfunction being liver as a putative target-organ. The dynamic of organism adaptation as a result of liver response of hypermetabolism has been recognized in numerous studies but molecular and morphological occurrences still need to be better clarified. The aim of this paper was to investigate effects of severe BI in rat liver through the histopathological and morphoquantitative aspects, immunoexpression of COX-2 and liver gene expression of TNF-α, iNOS and caspase-3.

The results showed morphological alterations in liver such as sinusoidal space filled by erythrocytes and inflammatory infiltrate associated with hepatocytes in degeneration following 14 d post injury. Damage resulting from severe BI initiates a SIRS as far as serious metabolic disturbances. Systemic signs manifested in the first hours post severe burns is related to enlarged systemic capillary permeability with protein escapement into the interstitial space[16]. Burns greater than 40% of body surface area commonly are followed by stress, inflammation, hypermetabolism, in addition the circulatory response associated to altered glycolysis, proteolysis, glycogenolysis, gluconeogenesis and lipolysis[9]. Our current results are consistent with this stress response of liver after BI.

BI causes liver injury which persists over a prolonged time. In children, at 6, 9 and 12 mo post burn, liver weight was incremented by 140% to 150% compared with estimated liver weight, showing longstanding alterations in liver morphology up to 12 mo after BI[7,9]. Morphoquantitative aspects on hepatocytes investigated in this setting detected increased hepatocyte area 4 and 14 d after BI in SBI group. Additionally, hepatocyte proliferation was present as result of increased binucleated hepatocyte density (number of cells/mm^2) 4 and 14 d post BI. These data show that despite liver increase weight gain is caused by edema formation[7], hepatocyte area gain and proliferation should be an important factor for hepatomegaly in burns. The compensatory hepatic cell proliferation are related to liver necrosis and liver apoptosis[10] but the underlying mechanisms, in which extensive burn provoke apoptosis in hepatocytes are not established so far[7]. This requires further study.

Regarding binucleated hepatocyte density, it is important to emphasize that because mitotic figures do not occur in adult liver, binucleated cells are usually assumed to be result of amitosis which implies splitting of the nucleus and these amitosis has been associated both with replacement of aged cells in abnormal tissue and with regenerative growth after injury[17]. Although binucleated hepatocytes are present in both groups (C and SBI), the presence of binucleated hepatocytes in SBI groups are related to regenerative growth as a response of skin BI. Interestingly mononucleated hepatocyte density decrease in SBI groups 1 and 14 d after trauma probably related to degeneration process showed in histopathological findings.

To further elucidate the molecular mechanisms induced by cutaneous BI, caspase-3 gene expression was evaluated in liver and no remarkable differences between groups were detected. Studies using TUNEL assay in liver of post BI experimental models showed apoptosis process[9,10]. Jayaraman *et al*[18] related that

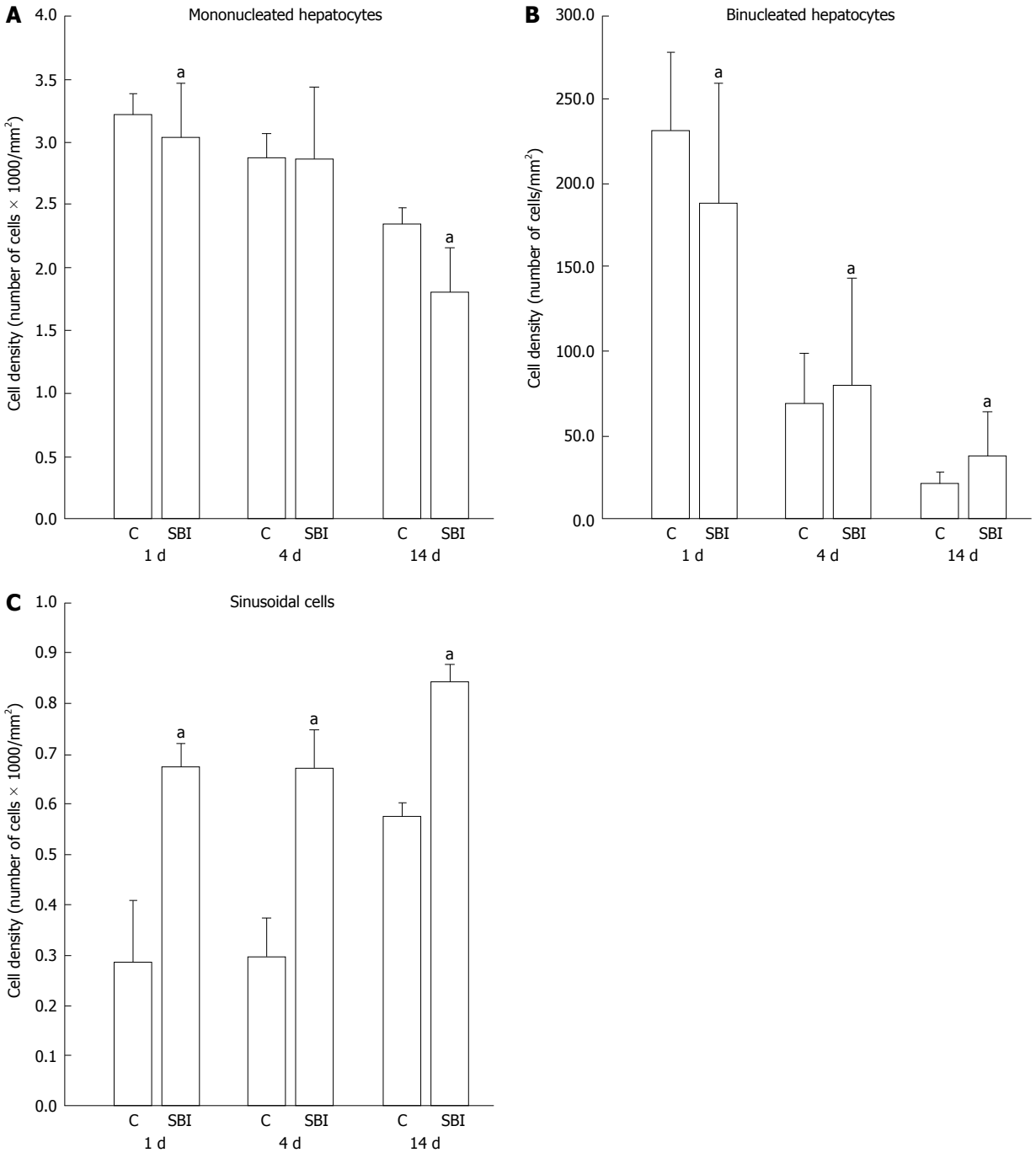

Figure 4 Mean + SD of cell density (number of cells/mm^2). Cell density of mononucleated hepatocytes (A), binucleated hepatocytes (B) and sinusoidal cells (C). $^aP < 0.05$, SBI different from control. SBI: Scald burn injury; C: Control.

the up-regulation of some acute-phase genes as STAT3, leptin receptor and HNF4α, are related to infection 7 d post injury in 20% of total body surface area in rats, suggesting bacterial infection of the wound post BI in animals. These authors showed up-regulation of Birc4, a protein that block caspase-3 and caspase-7 that are associated to apoptosis post burn. In this way, the present study suggests that the proliferation of hepatocytes cells was due to inflammatory process following necrosis. Following hepatocyte death, growth factors are secreted, and hepatocyte proliferation is trigged in liver.

Post BI, liver modulates the immune responses and the inflammatory processes. Protein catabolism owing to extensive BI is all guided by systemic inflammatory response, with enhanced activation of pro-inflammatory cytokines[19]. In a local investigation of liver, inflammatory infiltrate was viewed in histopathological findings and confirmed with immunohistochemical investigation a result of strong and focal immunoexpression COX-2 in SBI group. COX-2 is responsible for the conversion of arachidonic acid to prostaglandins[20]. Although severe burn in children is related to increased blood cytokine levels[21], experimental studies with murine model

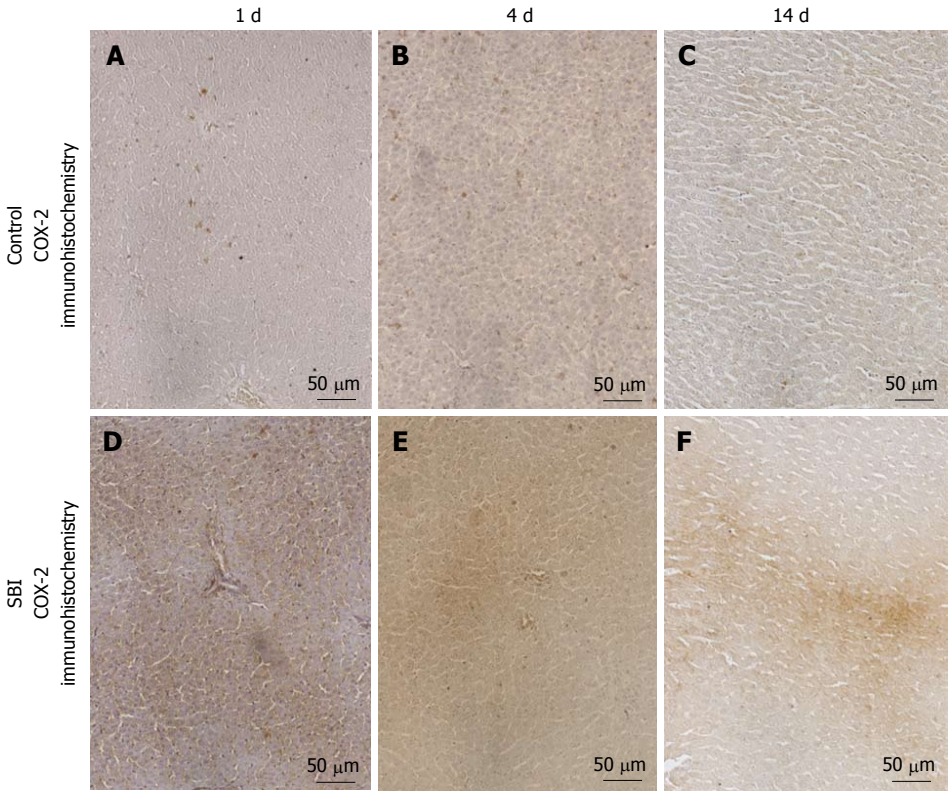

Figure 5 Liver ciclooxigenase-2 immunohistochemistry. Control (A-C) and SBI (D-F) evaluated 1, 4 or 14 d after burn injury. Notes stronger cytoplasmic immunoexpression in SBI groups when compared with control. SBI: Scald burn injury; COX-2: Ciclooxigenase-2.

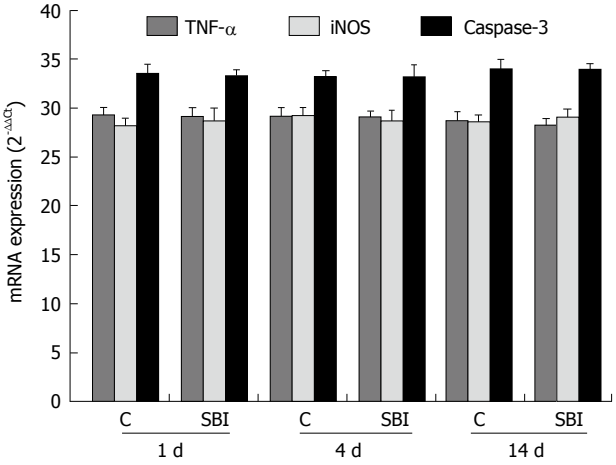

Figure 6 Mean + SD of liver tumor necrosis factor-α, inducible nitric oxide synthase and caspase-3 mRNA expression. TNF-α: Tumor necrosis factor-α; iNOS: Inducible nitric oxide synthase; C: Control; SBI: Scald burn injury.

submitted to BI for water vapor of 18% of the body surface showed enhanced expression of TNF-α and iNOS in initial stages of BI evaluated by means of peritoneal fluid of RT-PCR analysis[22]. Conversely, the present investigation of these inflammatory mediators (directly on the liver and not in a corporal fluid evaluation) showed no differences between groups.

Liver sinusoidal endothelial cells (LSECs) provide liver regeneration post injury of this organ[23]. In the sinusoidal space, there are four cell types: LSECs, Kupffer cells, stellate cells and pit cells. Kupffer cells generate cytokines and pro-inflammatory factors that stimulate neutrophils activation and change sinusoids porosity and may lead to cirrhosis[24]. Herein, LSECs and Kupffer cells constitute the hepatic reticuloendothelial system[25]. In the present study the density of sinusoidal cells was significantly increased in SBI group for all periods when compared with controls. This should be associated to phagocytic activity of Kupffer cells since local inflammation, erythrocytes invasion activation of neutrophils and disturbance of porosity in the sinusoids walls leading to erythrocytes invasion of sinusoidal space. Severe haemolysis was observed immediately after BI[26]. LSECs are separated from liver parenchyma by space of Disse, which is represented for a perisinusoidal extravascular space. Space of Disse contains collagen type Ⅰ, Ⅲ, Ⅴ and Ⅵ and the changes related with perisinusoidal basal lamina in livers, should increase collagen deposition in the space of Disse[25]. Furthermore, agglomeration of connective tissue inside the space of Disse may obstruct the normal traffic between blood and hepatocytes, reducing the release of macromolecules, difficulting the interation between cells and leading to a liver dysfunction[27].

Hepatocyte growth factor (HGF) develops a key function in cell regeneration, motility, growth and morphogenesis. Hepatic stellate cells provide as the main source of HGF in liver, however, after lesion, HGF expression is increased in LSECs[23,28]. In addition, hepatic stellate cells activated may initiate liver fibrosis process. Healthy LSECs inhibit the activation of hepatic stellate

cells[23]. In this study, increased density of sinusoidal cells should be related with enhanced of accumulation of the collagen, specially type Ⅲ, in hepatic parenchyma in SBI animals when compared with controls 14 d after injury.

In conclusion, severe burn in greater than 40% of the body surface induces, in liver, histopathological changes, inflammation related to COX-2 immunoexpression, and cell proliferation not related to caspase-3 expression. Because modulating function of liver after burn injuries, the treatment of severe burns can be focused in liver disarrangements.

COMMENTS

Background

Scalds are most common cause of burn injury (BI) and preferentially occur in children under the five years. The persistent protein catabolism may lead to delay in growth for up to 2 years after injury. In addition, severe burn injuries result in serious complications that involve host response related to inflammation and multiple organ dysfunctions, including liver damage.

Research frontiers

Studies involving autopsy of severely burned pediatric patients showed data about liver weight increased with fatty infiltration, but molecular and morphological investigation *in vivo* is necessary to elucidate better the liver damage process during great BI. For this, the present study investigated effects of severe BI in liver of young rats through the histopathological and morphoquantitative aspects, immunoexpression of COX-2 and liver gene expression of tumor necrosis factor-α, inducible nitric oxide synthase and caspase-3.

Innovations and breakthroughs

The liver damage process during severe BI are related with histopathological and morphoquantitative changes such as presence of erythrocytes in sinusoidal space associated with inflammatory infiltrate and inflammatory cells rounding hepatocytes in degeneration. Moreover, increased connective tissue, hepatocyte area larger than control, altered binucleated hepatocyte and sinusoidal cells density, were described in the present study.

Applications

Emphasize the importance of global treatment in burn great than 40% of total body surface area mainly in children. Highlight the damage caused in liver morphology clarifying morphological changes to possible treatments to prevent major consequences of BI.

Terminology

Severe BI greater than 40% in children causes chronic morphological liver consequences ignored in numerous treatment centers. To emphasize the liver dysfunction as a result of extensive skin BI because hypermetabolic consequences was the purpose of the present paper.

Peer-review

The aim of the authors was to investigate the temporal effects of extensive experimental burn injury in rat liver. The are some studies focused on this issue. This is well designed study. This article will provide new information about liver problems developing after BI.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Boceprevir plus peginterferon/ribavirin for treatment of chronic hepatitis C in Russia

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Author contributions: Isakov V, Long J, Wahl J and Helmand FA designed the study; Isakov V, Nikitin I, Chulano V and Ogurtsov P enrolled patients; Isakov V, Lukyanova E, Long J and Helmand FA analyzed the data; Isakov V and Helmand FA wrote the first draft; all authors critically reviewed and approved the final draft of the paper.

Supported by Merck & Co., Inc., Kenilworth, NJ, United States.

Institutional review board statement: The protocol P08160 of the study registered at ClinicalTrials.gov with identifier NCT01425203 presented in the manuscript of Isakov V *et al* "Boceprevir plus peginterferon/ribavirin for treatment of chronic hepatitis C in Russia" was reviewed and approved by the Institute of Nutrition (Moscow, Russia) Institutional Review Board.

Clinical trial registration statement: Protocol P08160 (The Effect of Boceprevir in Russian Participants Diagnosed with Chronic Hepatitis C Genotype 1) is registered at ClinicalTrials.gov (Identifier: NCT01425203).

Informed consent statement: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients included in the study.

Conflict-of-interest statement: Isakov V has received research/grant support from MSD, has served as a board member and or consultant for Abbvie, Vertex, Roche, Novartis, Janssen, and Bristol-Myers Squibb; has served on speakers' bureaus for Bristol-Myers Squibb, Janssen, Roche, and Novartis; and has received travel funding from Bristol-Myers Squibb and MSD Russia. Chulanov V has served on advisory boards for Roche, Bristol-Myers Squibb, Janssen, Novartis, and MSD Russia; has received research grants from Bristol-Myers Squibb; and has served on speakers' bureaus for Bristol-Myers Squibb, Roche, Janssen, Novartis, Gilead, AbbVie, and MSD. Ogurtsov P has served on advisory boards for Schering-Plough and delivered lectures on behalf of Abbott, Solvay, PRO.MED.CS Praha a.s., and Veropharm. Nikitin I has nothing to disclose. Lukyanova E, Long J, Wahl J and Helmond FA are current employees of Merck & Co., Inc., Kenilworth, NJ, United States.

Data sharing statement: These data were presented in part at the 64th Annual Meeting of the American Association for the Study of Liver Diseases; November 1-5, 2013; Washington, DC, United States.

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Core tip: Compared to the standard-of care treatment with peginterferon and ribavirin (PR), addition of boceprevir to PR results in a significant increase in rates of sustained virologic response achieved with substantially shorter treatment durations across a broad cross-section of patients with chronic hepatitis C virus infection in Russia.

Isakov V, Nikitin I, Chulanov V, Ogurtsov P, Lukyanova E, Long J, Wahl J, Helmond FA; The P08160 Trial Investigators. Boceprevir plus peginterferon/ribavirin for treatment of chronic hepatitis C in Russia. *World J Hepatol* 2016; 8(6): 331-339 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i6/331.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i6.331

Abstract

AIM: To evaluate addition of boceprevir to peginterferon/ribavirin (PR) in Russian patients with chronic hepatitis C virus (HCV).

METHODS: Treatment-naive (TN) and treatment-experienced (TE) patients (who had failed prior treatment with PR for ⩾ 12 wk) with chronic HCV genotype 1 infection were enrolled in this placebo-controlled, double-blind study. All patients initially received PR for 4 wk. Patients randomized to control treatment then received PR for an additional 44 wk. TN patients randomized to triple therapy received boceprevir (800 mg three times daily) plus PR for 24 wk and then further therapy according to treatment week 8 (TW8) HCV RNA levels. TE patients received boceprevir plus PR for 32 wk and then further therapy according to TW8 HCV RNA levels. Treatment was discontinued for TN patients with detectable HCV RNA at TW24 and TE patients with detectable HCV RNA at TW12 because of futility. The primary efficacy end point was sustained virologic response (SVR) defined as undetectable HCV RNA 24 wk after completing all study therapy.

RESULTS: SVR was 74.8% in the boceprevir plus PR arm compared with 46.2% in the control arm, with a stratification-adjusted treatment difference of 29.2% (95%CI: 16.4-41.5; $P < 0.0001$). Rates of SVR were higher in the boceprevir arm in both TN and TE patient groups (TN 78.4% *vs* 56.3%; TE 69.4% *vs* 30.0%). Within TE patients, the rates of SVR were higher with boceprevir plus PR compared with PR, regardless of treatment failure type (null responder, partial responder, and relapser). Most patients receiving boceprevir plus PR in both TN (86%) and TE (71%) populations were eligible for reduced treatment duration. Anemia was increased in patients receiving boceprevir plus PR *vs* PR alone (47.2% *vs* 24.4%); there was a corresponding increase in ribavirin dose reduction and erythropoietin use. Among patients receiving boceprevir plus PR, SVR rates were similar in patients with anemia (< 10 g/dL) and those without anemia (71.2% *vs* 77.4%).

CONCLUSION: Regulatory approval has been obtained for boceprevir plus PR in Russian patients with HCV genotype 1 infection based on the results of this study.

Key words: Hepatitis C virus; Boceprevir; Peginterferon; Ribavirin; Randomized; Clinical trial; Sustained virologic response

INTRODUCTION

Boceprevir is an orally administered, serine protease inhibitor of the hepatitis C virus (HCV) nonstructural protein 3 protease[1]. The addition of boceprevir to peginterferon and ribavirin (PR) improves rates of sustained virologic response (SVR) in adult patients with HCV genotype 1 (GT1) infection[2,3]. In the phase 3 SPRINT-2 study in previously untreated patients and the RESPOND-2 study in patients who had failed previous treatment, the addition of boceprevir to PR increased SVR rates compared with PR alone. In both studies, the implementation of response-guided therapy (RGT) permitted a shortened treatment duration for patients with an early response to therapy. In SPRINT-2, 44% of patients receiving boceprevir RGT required only 28 wk of treatment with triple therapy, and the SVR rate in this group was 96%[3]. Similarly, in RESPOND-2, 46% of patients had undetectable HCV RNA at treatment week 8 (TW8) and were eligible for a shortened 36-wk treatment regimen: SVR in this population was 86%[2]. In these studies, the safety profile of boceprevir plus PR largely resembled the safety profile of PR alone, with the notable exceptions of increased rates of dysgeusia and anemia in patients receiving boceprevir.

According to the World Health Organization (WHO), there were an estimated 5.8 million patients with HCV infection in Russia in 2010, accounting for 4.1% of the total Russian population[4]. In Western countries, treatment of HCV infection has advanced dramatically over the last 5 years with the introduction of new targeted therapies that substantially shorten treatment duration and improve SVR rates[5,6]. However, in resource-constrained countries, standard treatment protocols are lacking, and PR dual therapy frequently remains the cornerstone of treatment[7,8]. Recent guidelines from the WHO note the low rates of treatment uptake for patients in low- and middle-income countries. The aim of this study was to evaluate the safety and

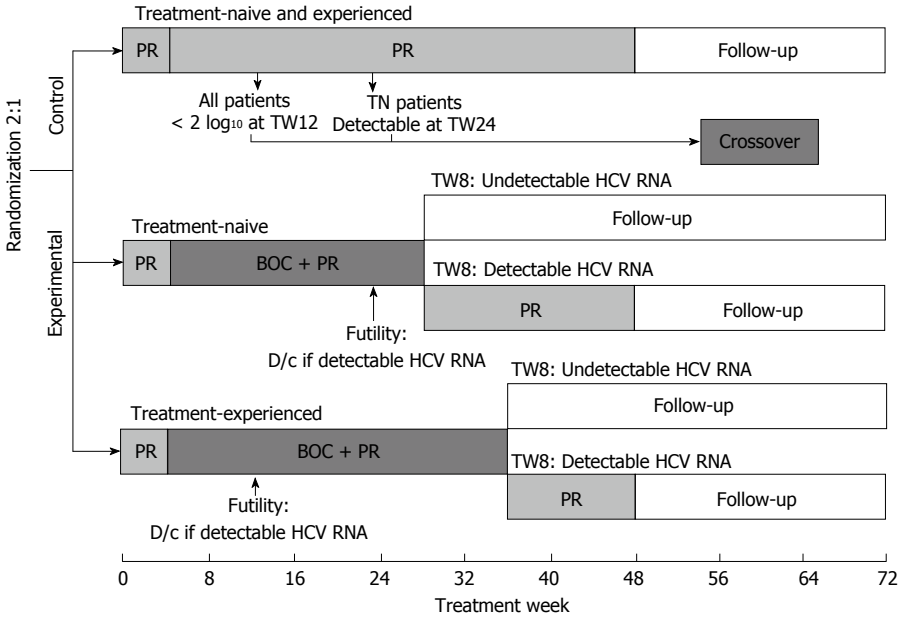

Figure 1 Study design. BOC: Boceprevir; D/c: Discontinued; HCV: Hepatitis C virus; PR: Peginterferon/ribavirin; TW: Treatment week.

efficacy of boceprevir plus PR therapy in treatment-naive (TN) and treatment-experienced (TE) Russian patients with chronic HCV GT1 infection.

MATERIALS AND METHODS

This was a randomized, placebo-controlled, double-blind clinical trial (ClinicalTrials.gov identifier, NCT01425203; protocol P08160), carried out in accordance with the Declaration of Helsinki, current guidelines on Good Clinical Practice, and local ethical and legal requirements. All patients provided voluntary written informed consent before trial entry.

Study design

Patients were randomized in a 2:1 ratio to receive experimental or control therapy, stratified by previous treatment (naive *vs* experienced) and interleukin-28B (*IL28B*) status (CC allele *vs* non-CC allele) (Figure 1). All patients initially received PR [peginterferon alfa-2b (1.5 μg/kg per week) plus ribavirin (800-1400 mg/d)] for 4 wk. Patients in the control arm then received PR for an additional 44 wk. In the experimental arm, TN patients received boceprevir [800 mg three times daily (TID)] plus PR for 24 wk and then further therapy according to TW8 HCV RNA levels. Patients with undetectable HCV RNA at TW8 concluded treatment at week 28 while those with detectable HCV RNA at TW8 continued therapy with PR from weeks 28-48. TE patients received boceprevir (800 mg TID) plus PR for 32 wk and then further therapy according to TW8 HCV RNA levels. Patients with undetectable HCV RNA at TW8 concluded treatment at week 36, while those with detectable HCV RNA at TW8 continued PR therapy from weeks 36-48. Treatment was discontinued for TN patients with detectable HCV RNA at TW24 and TE patients with detectable HCV RNA at TW12 because of futility. Patients in the control arm (PR only) who failed treatment because of the futility rule could cross over to receive triple therapy. TN patients with < 2 $\log_{10}$ decline in HCV RNA at TW12, or with detectable HCV RNA at TW24 could cross over to receive boceprevir plus PR for 32 wk. TE patients with detectable HCV RNA at TW12 could also cross over to receive boceprevir plus PR for 32 wk. Duration of further therapy depended on HCV RNA detectability at crossover week 4 (COW4). Crossover treatment duration was 32 (COW4 HCV RNA undetectable) or 44 wk (COW4 HCV RNA detectable).

Patients

The study population included TN and TE adult patients with chronic HCV infection (enrollment ratio 60:40). TN patients had received no previous therapy for HCV infection, whereas TE patients were required to have received prior treatment with PR for ⩾ 12 wk without interruption or dose reduction. Inclusion criteria for the study included a baseline viral load of ⩾ 10000 IU/mL, and a liver biopsy consistent with chronic HCV infection. Cirrhotic patients were required to have an ultrasound within 6 mo of screening with no evidence of hepatocellular carcinoma. Exclusion criteria included a platelet count of < 100000/mm^3; hemoglobin levels < 12 g/dL for females or < 13 g/dL for males; human immuno-deficiency virus or hepatitis B virus infection; previous discontinuation of PR due to a treatment-related adverse event (AE); or decompensated liver disease, including a history or presence of ascites, bleeding varices, or hepatic encephalopathy.

Assessments

The primary efficacy end point was SVR, defined as undetectable HCV RNA 24 wk after completing treatment in randomized patients who received at least 1 dose

Table 1 Patient demographics *n* (%)

	Boceprevir plus PR (*n* = 159)	PR (*n* = 78)
Sex		
Male	94 (59.1)	45 (57.7)
Female	65 (40.9)	33 (42.3)
Age (yr), mean (SD)	38.6 (9.8)	38.1 (10.0)
Race		
White	158 (99.4)	77 (98.7)
Asian	1 (0.6)	1 (1.3)
Ethnicity		
Not Hispanic or Latino	159 (100)	78 (100)
Weight (kg), mean (SD)	78.1 (16.6)	78.5 (16.8)
BMI (kg/m^2), mean (SD)	25.9 (4.2)	26.0 (4.4)
Previous treatment		
Naive	97 (61.0)	48 (61.5)
Experienced	62 (39.0)	30 (38.5)
IL28B genotype		
CC allele	22 (13.8)	11 (14.1)
Non-CC allele	137 (86.2)	67 (85.9)
HCV genotype		
GT1a	4 (2.5)	0 (0)
GT1b	155 (97.5)	78 (100)
Baseline HCV RNA		
⩽ 800000 IU/mL	89 (56.0)	53 (67.9)
> 800000 IU/mL	70 (44.0)	25 (32.1)
Hemoglobin (g/dL), mean (SD)	15.0 (1.5)	14.9 (1.5)
Liver histology		
Cirrhosis	7 (4.4)	2 (2.6)
No cirrhosis	152 (95.6)	76 (97.4)

GT: Genotype; HCV: Hepatitis C virus; PR: Peginterferon/ribavirin; SD: Standard deviation; BMI: Body mass index; *IL28B*: Interleukin-28B.

of any trial medication. HCV RNA was detected using COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, version 1.0 (Roche Diagnostics, Basel Switzerland); lower limit of quantification = 43 IU/mL; limit of detectability = 18.0 IU/mL. The key secondary end point was the achievement of SVR in randomized patients who received at least 1 dose of boceprevir or boceprevir placebo therapy. Other end points included the relationship between early virologic response and SVR (summarized using the proportion of patients who achieved SVR among those with undetectable HCV RNA at TW4, TW8 or TW12), the proportion of patients with virologic breakthrough (undetectable HCV RNA and subsequent HCV RNA above the limit of quantification while on study therapy), the proportion with incomplete virologic response (> 1 $\log_{10}$ increase in HCV RNA from nadir value while on study therapy), and safety.

Statistical analysis

The statistical methods of this study were reviewed by Jianmin Long from Merck and Co., Inc. Analyses were based on the full analysis set population, which included all randomized and treated patients. Target enrollment was 70 patients in the PR control group and 140 in the boceprevir plus PR arm, providing 98% power to demonstrate the superiority of boceprevir plus PR *vs* PR at an overall 1-sided, 2.5% alpha level, if the underlying difference in SVR was 30%. The power and sample size calculations were based on the assumption of an underlying response rate of 30% for the PR control arm. The minimum criterion for success was that the *P* value for the comparison of SVR between the boceprevir plus PR arm and the control PR arm was < 0.05. An interim analysis was performed when all patients had completed at least 8 wk of treatment or had discontinued therapy. The results of this interim analysis were used as the basis for regulatory submission in Russia.

Achievement of SVR was summarized using descriptive statistics. The primary statistical comparison was conducted on the full analysis set using the stratified Miettinen and Nurminen method at alpha level of 0.05 adjusted for stratification factors (*IL28B* genotype CC *vs* non-CC and TN *vs* TE) as specified at the time of randomization. Multiplicity adjustment for controlling the type 1 error for the primary and key secondary comparisons was based on the step-down approach. The key secondary comparison was tested only if the statistical significance of the primary comparison reached an alpha level of 0.05. Any patient with missing data at, or after follow-up week 24, and undetectable HCV RNA at follow-up week 12, was considered a sustained virologic responder. For efficacy analyses, patients in the PR control arm who rolled over to the crossover arm were considered as failures at and after the time of the crossover.

RESULTS

Patients

A total of 238 patients were randomly assigned: 159 were assigned to receive boceprevir plus PR and 79 were assigned to PR (Figure 2). One patient assigned to PR did not receive any study medication and was therefore excluded from the full analysis set population. Four patients discontinued during lead-in (boceprevir plus PR, *n* = 3; PR, *n* = 1), yielding 233 patients in the modified intent-to-treat data set. Fifty-nine patients (boceprevir plus PR, *n* = 24; PR, *n* = 35) discontinued after adding boceprevir/placebo, with the most common reason for discontinuation being treatment failure (5% of patients receiving boceprevir plus PR and 34% of those receiving PR alone were discontinued based on futility criteria, Figure 2). Twenty-seven patients in the PR control arm entered crossover because of treatment failure at the futility time points. In total, 229 patients entered the follow-up phase (Figure 2). The majority of patients were white, with GT1b infection, and the *IL28B* non-CC genotype (Table 1). Few patients were cirrhotic. Compliance rates with boceprevir therapy were high (97.5% of patients had ⩾ 80% compliance).

SVR

SVR at follow-up week 24 was higher in the boceprevir plus PR arm compared with the control arm [74.8% (119/159) *vs* 46.2% (36/78)], with a stratification-adjusted treatment difference of 29.2% (95%CI: 16.4-41.5; *P* < 0.0001) (Figure 3). The end of treatment response rate was 87.4% (139/159) for the boceprevir

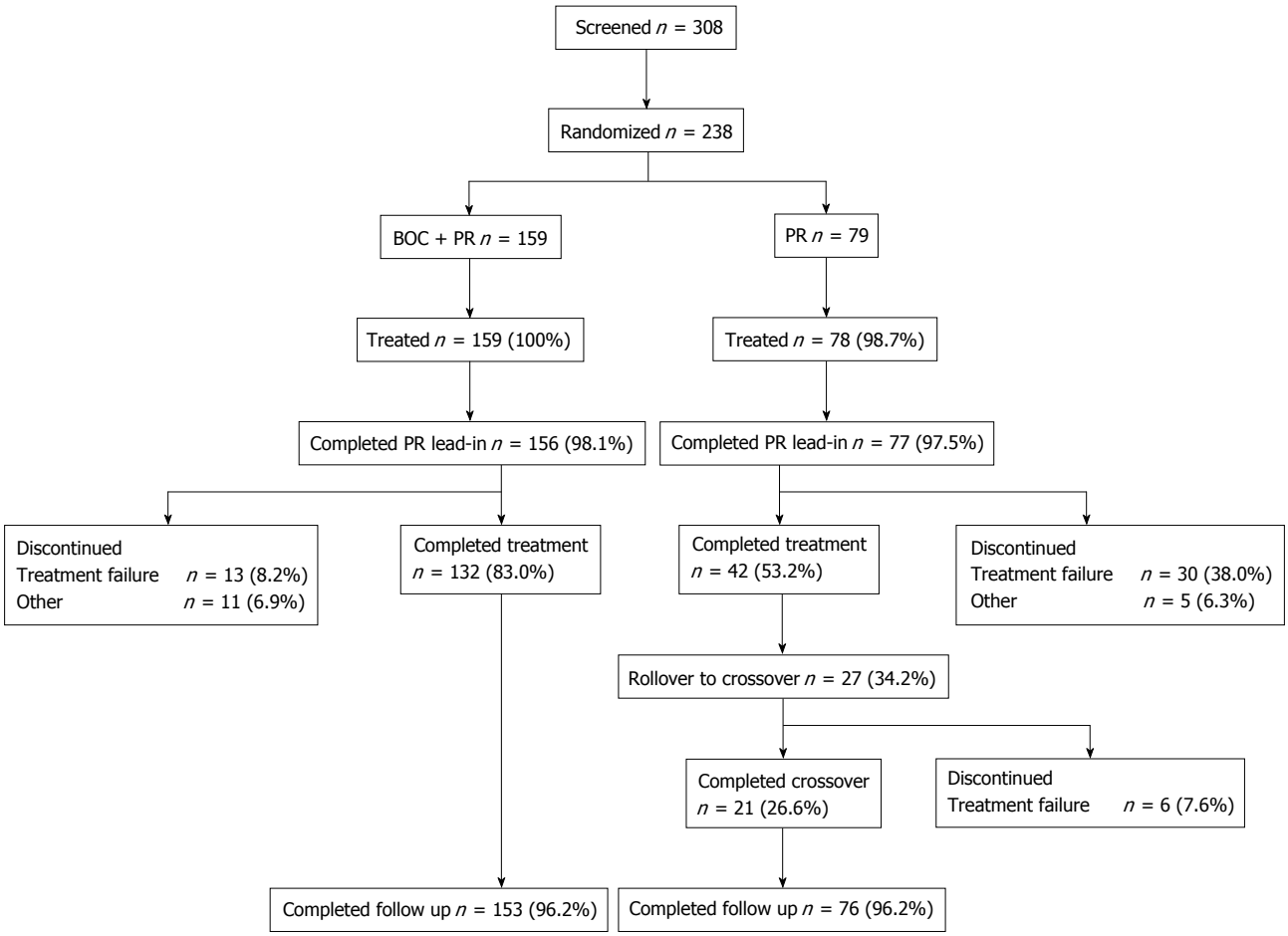

Figure 2 Patient disposition. BOC: Boceprevir; PR: Peginterferon/ribavirin.

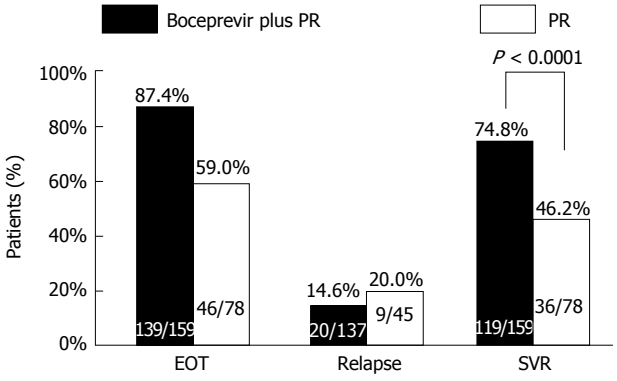

Figure 3 Analysis of sustained virologic response, end of treatment response, and relapse rate. If a patient had missing data at and after the FW24 window and had undetectable HCV RNA at FW12, the patient was considered a sustained virologic responder. The *P* value was adjusted for stratification factors of *IL28B* genotype (CC *vs* non-CC) and previous treatment (TN *vs* TE), based on the Miettinen and Nurminen method. EOT was defined as the last dose date in treatment phase ± 14 d inclusive. The closest value to the last dose date was considered to be the EOT value. If there was no value within this window, the closest available value after this window was used. Relapse was defined as any patient who had detectable HCV RNA following end of all study therapy, after becoming undetectable and remaining so until end of treatment. EOT: End of treatment; HCV: Hepatitis C virus; PR: Peginterferon/ribavirin; SVR: Sustained virologic response; TE: Treatment experienced; TN: Treatment naive; *IL28B*: Interleukin-28B.

plus PR arm, and 59.0% (46/78) for the PR control arm. The relapse rate was 14.6% (20/137) for the boceprevir plus PR arm, and 20.0% (9/45) for the PR control arm.

Virologic failure

Rates of virologic breakthrough were 3.8% (6/159) in the boceprevir plus PR arm, and 5.1% (4/78) in the PR control arm. No patients in the PR control arm exhibited virologic rebound. Incomplete virologic response/rebound rate in the boceprevir plus PR arm was 3.1% (5/159). Five patients with incomplete virologic response had samples sequenced, of which 3 samples had variants detected (V36M, *n* = 1; T54A, *n* = 2; T54S, *n* = 1; T54T, *n* = 2). Similarly, 5 patients with virologic breakthrough had samples sequenced, of which 3 had detectable HCV variants (T54A, *n* = 1; T54S, *n* = 1; T54T, *n* = 2; V55A, *n* = 1).

SVR according to on-treatment virologic response

All patients with undetectable HCV RNA at TW4 in both treatment arms attained SVR (Table 2). In both treatment arms, all patients received PR alone for the first 4 wk of therapy. The proportions of patients with < 1 log drop [boceprevir 43/159 (27%) and PR 22/78

Table 2 Sustained virologic response by previous treatment, interleukin-28B genotype, and on-treatment virologic response *n* (%)

	Boceprevir plus PR (*n* = 159)	PR (*n* = 78)
Treatment naive	76/97 (78.4)	27/48 (56.3)
Treatment experienced	43/62 (69.4)	9/30 (30.0)
Null responder	8/17 (47.1)	1/6 (16.7)
Partial responder	5/8 (62.5)	1/4 (25.0)
Relapser	30/37 (81.1)	7/20 (35.0)
Treatment naive		
IL28B CC genotype	19/20 (95.0)	11/11 (100.0)
IL28B non-CC genotype	57/77 (74.0)	16/37 (43.2)
Treatment experienced		
IL28B CC genotype	2/2 (100.0)	0/0
IL28B non-CC genotype	41/60 (68.3)	9/30 (30.0)
SVR according to baseline HCV RNA		
All patients		
⩽ 800000 IU/mL	71/89 (79.8)	25/53 (47.2)
> 800000 IU/mL	48/70 (68.8)	11/25 (44.0)
Treatment naive		
⩽ 800000 IU/mL	45/52 (86.5)	16/27 (59.3)
> 800000 IU/mL	31/45 (68.9)	11/21 (52.4)
Treatment experienced		
⩽ 800000 IU/mL	26/37 (70.3)	9/26 (34.6)
> 800000 IU/mL	17/25 (68.0)	0/4 (0)
SVR according to TW4 response		
TW4 < 1 log drop	20/43 (46.5)	0/22 (0)
TW4 ⩾ 1 log drop	75/90 (83.3)	26/45 (57.8)
TW4 undetectable	23/23 (100)	10/10 (100)
Missing	1/3	0/1
SVR according to TW8 response		
TW8 undetectable	115/139 (82.7)	29/33 (87.9)
TW8 detectable	4/16 (25)	7/44 (15.9)
Missing	0/4	0/1
SVR according to presence of anemia		
Yes	47/66 (71.2)	6/11 (54.5)
No	72/93 (77.4)	30/67 (44.8)
SVR according to EPO use		
Yes	10/15 (66.7)	3/3 (100)
No	109/144 (75.7)	33/75 (44)
SVR according to ribavirin dose reduction		
Yes	46/67 (68.7)	12/17 (70.6)
No	73/92 (79.4)	24/61 (39.3)

SVR is defined as the virologic response at follow-up week 24. If a patient had missing data at and after the follow-up week 24 window and had undetectable HCV RNA at follow-up week 12, the patient was considered a sustained virologic responder. EPO: Erythropoietin; HCV: Hepatitis C virus; SVR: Sustained virologic response; TW: Treatment week; PR: Peginterferon/ribavirin; *IL28B*: Interleukin-28B.

(28%)] and ⩾ 1 log drop [boceprevir 90/159 (57%) and PR 45/78 (58%)] in HCV RNA at TW4 were similar in both treatment arms. However, SVR was higher in patients receiving boceprevir + PR compared with PR within the subgroups of patients with < 1 log drop in HCV RNA at TW4 (46.5% *vs* 0%) and those with ⩾ 1 log drop in HCV RNA at TW4 (83.3% *vs* 57.8%).

A TW8 interim analysis was submitted for regulatory approval in Russia. In this analysis, rates of undetectable HCV RNA at TW8 in the boceprevir RGT and PR arms were 91% (88/97) *vs* 48% (23/48) in TN patients and 82% (51/62) *vs* 33% (22/67) in TE patients. Overall, the rates of undetectable HCV RNA at TW8 in all patients were higher in patients receiving boceprevir plus PR compared with control therapy (87.4% *vs* 42.3%, *P* < 0.0001). SVR rates in patients with undetectable HCV RNA at TW8 were similar between treatment arms [boceprevir + PR 82.7% (115/139) *vs* PR 87.9% (29/33)].

SVR according to baseline variables

SVR rates are presented by previous treatment and response, and *IL28B* genotype (Table 2). SVR rates were higher in patients receiving boceprevir plus PR compared with PR in both TN (78.4% *vs* 56.3%) and TE (69.4% *vs* 30.0%) subgroups. Within TE patients, the rates of SVR were higher with boceprevir plus PR compared with PR, regardless of treatment failure type (null responder, partial responder, and relapser). SVR rates were high among all patients with *IL28B* CC genotype, regardless of treatment arm or previous treatment history. Conversely, the rates of SVR in patients with *IL28B* CT or TT genotypes were higher with boceprevir plus PR compared with PR alone (Table 2). SVR rates were also higher with boceprevir compared with PR, regardless of baseline viral load. SVR was 87% in TN patients with baseline viral load ⩽ 800000 IU/mL. Among patients receiving boceprevir, rates of SVR were generally higher in TN patients with low viral load compared with those with high baseline viral load (86.5% *vs* 68.9%); however, SVR was similar in TE patients with high *vs* low baseline viral load receiving boceprevir (70.3% *vs* 68.0%) (Table 2).

SVR in patients requiring anemia management

Among patients receiving boceprevir plus PR, SVR rates were similar in patients with anemia (< 10 g/dL) and those without anemia (71.2% *vs* 77.4%). SVR rates were also relatively similar in boceprevir recipients requiring erythropoietin (EPO) for anemia management and those not using EPO (66.7% *vs* 75.7%, Table 2), and in those who received ribavirin dose reduction and those who did not (68.7% *vs* 79.4%).

Crossover therapy

The SVR rates for the crossover group are presented in Table 3. Overall, 70.4% of patients who crossed over from PR alone to boceprevir plus PR had SVR at follow-up week 24.

Safety

The reported AEs were consistent with the known safety profile of boceprevir (Table 4), with treatment-emergent AEs noted frequently in both treatment arms (97.5% in the boceprevir plus PR arm and 91.0% in the PR control arm). The number of patients discontinuing treatment because of AEs was 4.4% in the boceprevir plus PR arm (*n* = 7, of which 5 were considered treatment related) and 2.6% in the PR control arm (*n* = 2, of which 1 was considered treatment related). Serious AEs were reported in 10.7% (*n* = 17, of which

Table 3 Sustained virologic response at follow-up week 24 in the crossover group *n* (%)

	SVR
Total	19/27 (70.4)
TN TW12 failure (< 2 log decline HCV RNA)	8/11 (72.7)
TE TW12 failure (detectable HCV RNA)	11/16 (68.8)
TN TW24 failure (detectable HCV RNA)	0/0

HCV: Hepatitis C virus; SVR: Sustained virologic response; TE: Treatment-experienced; TN: Treatment-naive; TW: Treatment week.

12 were considered drug related) and 11.5% (n = 9, of which 5 were considered drug related) of patients in the boceprevir plus PR and PR arms, respectively. Dose modifications due to an AE were reported in 56% (89/159) in the boceprevir plus PR arm, and 33.3% (26/78) for PR alone. There were no deaths reported during the study.

Anemia was reported at a higher rate in patients receiving boceprevir plus PR compared with those receiving PR alone (47.2% *vs* 24.4%). However, few patients in either treatment group had on-treatment hemoglobin levels < 8.5 g/dL (boceprevir + PR 6.3% *vs* PR 2.6%). EPO use was reported for 9.4% of patients receiving boceprevir plus PR and 3.8% of those receiving PR alone. Ribavirin dose reduction was required for 65 patients (40.9%) receiving boceprevir plus PR and 14 patients (17.9%) receiving PR alone.

DISCUSSION

Data from the present study indicate that, similar to activity seen in Western populations, boceprevir added to PR results in a marked improvement in SVR rates compared with PR alone in TN and TE Russian patients with HCV GT1 infection. The high rate of undetectable HCV RNA at TW8 in TN and TE patients receiving boceprevir plus PR resulted in a high proportion of patients being deemed eligible for RGT with consequent reductions in their treatment durations. The treatment effect (*i.e.*, difference in response between boceprevir plus PR and PR alone) was comparable between this study in Russian patients, and the phase 3 trials (Table 5). However, whereas 42%-46% of patients receiving boceprevir RGT in the phase 3 studies had undetectable HCV RNA at TW8, in the present study 87.4% of boceprevir recipients had undetectable HCV RNA at TW8. This suggests that the proportion of Russian patients eligible for shortened treatment duration may be higher than reported in the phase 3 studies, and is suggestive of a favorable cost/efficacy ratio in Russian patients. Response rates were particularly high among patients with favorable disease characteristics such as the *IL28B* CC genotype. In patients with this genotype, SVR rates were high regardless of treatment regimen; however, patients with the *IL28B* non-CC genotype derived a substantial benefit from boceprevir therapy.

The tolerability profile seen with boceprevir in Russian patients was consistent with the established tolerability profile documented in Western patients. The majority of AEs were associated with PR therapy. As seen in Western patients, anemia was increased with boceprevir, and there was also a corresponding increase in the use of anemia management strategies (ribavirin dose reduction and EPO use) among patients receiving boceprevir. In SPRINT-2 and RESPOND-2, approximately 3%-8% of patients receiving boceprevir plus PR had hemoglobin levels < 8.0 g/dL: EPO use was required in 41%-46% of patients, and 21% required dose reduction due to anemia[2,3]. In the present study, 6.3% of patients receiving boceprevir plus PR had nadir hemoglobin < 8.5 g/dL. There were also differences in the rates of anemia management strategies with lower rates of EPO use (9.4%) but higher rates of dose reduction (41%) in the present study compared with the phase 3 studies in Western patients[2,3]. These differences between studies are a reflection of the different anemia management strategies. In the phase 3 protocols, investigators were free to choose between ribavirin dose reduction and EPO use as a first-line strategy while in the present study ribavirin dose reduction was the first-line strategy and EPO use was the second-line strategy.

Table 4 Adverse events (≥ 20% in any treatment arm) *n* (%)

	Boceprevir plus PR (n = 159)	PR (n = 78)
Any AE	155 (97.5)	71 (91.0)
Neutropenia	84 (52.8)	31 (41.0)
Pyrexia	77 (48.4)	36 (46.2)
Anemia	75 (47.2)	19 (24.4)
Leukopenia	62 (39.0)	25 (32.1)
Dysgeusia	59 (37.1)	3 (3.8)
Asthenia	44 (27.7)	23 (29.5)
Headache	43 (27.0)	25 (32.1)
Influenza-like illness	39 (24.5)	14 (17.9)
Nausea	39 (24.5)	9 (11.5)
Anemia		
8.5-10 g/dL	56 (35.2)	9 (11.5)
< 8.5 g/dL	10 (6.3)	2 (2.6)
Ribavirin dose reduction	65 (40.9)	14 (17.9)
EPO use	15 (9.4)	3 (3.8)
Serious AE	17 (10.7)	9 (11.5)
Discontinued because of an AE	7 (4.4)	2 (2.6)
Dose modification due to an AE	89 (56.0)	26 (33.3)

AE: Adverse event; EPO: Erythropoietin; PR: Peginterferon/ribavirin.

Response rates in this study are higher for both boceprevir plus PR and PR alone, compared with rates seen in previous phase 3 studies (Table 5). This increase in response may be explained by differences in the patient populations enrolled in the current study and the phase 3 studies[2,3]. Compared with patients enrolled in the boceprevir phase 3 studies, more Russian patients were aged ≤ 40 years (62% *vs* 13%), had baseline viral load ≤ 800000 IU/mL (60% *vs* 14%), and had HCV GT1b infection (98% *vs* 35%).

Data from the present study support the use of boceprevir in Russian patients with HCV GT1 infection. However, boceprevir-based triple therapy may not be

Table 5 Comparison of virologic response rates between Russian patients and western patients receiving boceprevir-based triple therapy in the serine protease inhibitor therapy 2 and retreatment with hepatitis C virus serine protease inhibitor boceprevir and pegIntron/rebetol 2 studies *n* (%)

	Russian patients		SPRINT-2		RESPOND-2	
	RGT of BOC	PR	RGT of BOC	PR	RGT of BOC	PR
TN						
EOT	89/97 (91.8)	33/48 (68.8)	277/366 (76)	191/363 (53)	-	-
SVR	76/97 (78.4)	27/48 (56.3)	242/366 (66)	137/363 (38)	-	-
Relapse	13/89 (14.6)	6/33 (18.2)	24/265 (9)	39/176 (22)	-	-
TE						
EOT	50/62 (80.6)	13/30 (43.3)	-	-	114/162 (70.4)	25/80 (31)
SVR	43/62 (69.4)	9/30 (30)	-	-	107/161 (66)	17/80 (21)
Relapse	7/48 (14.6)	3/12 (25.0)	-	-	14/121 (12)	8/25 (32)

BOC: Boceprevir; EOT: End of treatment response; PR: Peginterferon/ribavirin; RESPOND-2: Retreatment with hepatitis C virus serine protease inhibitor boceprevir and pegIntron/rebetol 2 study; RGT: Response-guided therapy; SPRINT-2: Serine protease inhibitor therapy 2 study; SVR: Sustained virologic response; TE: Treatment experienced; TN: Treatment naive.

appropriate for all patients with GT1 infection. Patients with low viral load at baseline who achieve undetectable HCV RNA at TW4 may achieve high SVR rates with 24-wk of therapy with PR alone and would not require the addition of boceprevir[9]. Despite the world-wide acceptance of interferon-free regimens as a standard of care due to the near 100% efficacy and low adverse events rate, some patients will continue to receive interferon-based treatment. This is due largely to the fact that the approval of interferon-free regimens is not immediately followed by total reimbursement in many countries, or that access to these regimens is dependent on the stage of the liver disease, prioritizing treatment of cirrhotic patients[10-12]. Easy-to-treat patients can be successfully treated with interferon-based regimens which may be easier to access through reimbursement.

In conclusion, data from the present study support the use of boceprevir plus PR for the treatment of Russian patients with HCV GT1 infection. The safety and efficacy profile of boceprevir in Russian patients was generally similar to that previously reported in phase 3 studies in Western patients; however, this treatment may be more cost-effective in Russia as approximately 88% of patients had undetectable HCV RNA at TW8, suggesting that a higher proportion of Russian patients receiving boceprevir plus PR would be eligible for reduced treatment duration with RGT compared with Western patients. Regulatory approval has been obtained for boceprevir in Russia based on the results of this study.

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COMMENTS

Background

In the treatment of hepatitis C virus (HCV), genotype 1 infection, peginterferon plus ribavirin is associated with low efficacy and poor tolerability. Phase 3 studies have shown that addition of a direct-acting antiviral agent such as boceprevir can improve efficacy and shorten treatment durations.

Research frontiers

The safety and efficacy of boceprevir plus peginterferon and ribavirin in Russian patients with HCV infection is currently unknown.

Innovations and breakthroughs

In the present study, patients receiving boceprevir plus peginterferon and ribavirin achieved significantly higher rates of sustained virologic response compared with patients treated with peginterferon and ribavirin alone. Patients receiving boceprevir-based therapy frequently required substantially shorter treatment durations compared to patients receiving PR alone alone. Rates of anemia were higher among patients receiving boceprevir.

Applications

Regulatory approval has been obtained for boceprevir in Russia based on the results of this study.

Peer-review

This manuscript evaluates the efficacy and safety of boceprevir plus peginterferon and ribavirin in treatment-naïve and treatment-experienced Russian patients with HCV genotype 1 infection.

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CASE REPORT

Primary hepatic amyloidosis: A case report and review of literature

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Informed consent statement: The patient involved in the study gave her written informed consent authorizing use and disclosure of her protected health information.

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Abstract

We describe a case of 42-year-old female presenting with abdominal pain associated with loss of weight and fever for 8 mo. On evaluation she had gross hepatomegaly with raised alkaline phosphatase and raised GGT levels with normal transaminases and bilirubin. On imaging she had diffuse enlargement of liver with heterogeneous contrast uptake in liver. Her viral marker and autoimmune markers were negative. Liver biopsy depicted massive deposition of amyloid in peri-sinusoidal spaces which revealed apple green birefringence on polarizing microscopy after Congo red staining. Cardiac and renal evaluation was unremarkable. Abdominal fat pad and rectum biopsy was negative for amyloid deposit. There was no evidence of primary amyloidosis as bone marrow examination was normal. Serum and urine immunofixation electrophoresis were normal. Immunoperoxidase staining for serum amyloid associated protein for secondary amyloidosis was negative from liver biopsy. We present this rare case of primary hepatic amyloidosis and review the literature regarding varied presentations of hepatic involvement in amyloidosis.

Key words: Amyloidosis; Congo red staining; Isolated hepatic amyloidosis; Amyloid associated protein; Immunofixation electrophoresis

Core tip: Amyloidosis is a pathological process that encompasses a spectrum of disease resulting from the extracellular deposition of fibrillar amyloid protein. It can involve any organ isolated or in conjunction with other organs and can do so in the form of a focal, tumour-like lesion, or an infiltrative process. Amyloidosis localized to the liver has been rarely described. This case represents a rare instance of primary hepatic amyloidosis without

evidence of primary or secondary cause of amyloid deposit posing considerable diagnostic and therapeutic challenge for the clinicians.

Sonthalia N, Jain S, Pawar S, Zanwar V, Surude R, Rathi PM. Primary hepatic amyloidosis: A case report and review of literature. *World J Hepatol* 2016; 8(6): 340-344 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i6/340.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i6.340

INTRODUCTION

Amyloidosis is a pathological process that encompasses a spectrum of disease resulting from the extracellular deposition of fibrillar amyloid protein, which can involve any organ in isolation or in conjunction with other organs and can do so in the form of a focal, tumour-like lesion, or an infiltrative process. Amyloidosis localized to the liver has been rarely described, although it is possible that these patients have yet to exhibit evidence of systemic disease. Hepatic involvement in both primary (AL) and secondary (AA) forms of systemic amyloidosis is common; however, clinically dominant hepatic amyloidosis is unusual[1]. Accumulation of amyloids in the liver produces hepatomegaly in 33%-92% of patients, as well as moderate jaundice and moderate to severe cholestasis[2,3]. Our patient presented with constitutional symptoms of fever, weight loss, and hepatomegaly without jaundice with evidence of amyloid deposit in perisinusoidal spaces without any systemic evidence of primary or secondary amyloidosis. Isolated hepatic amyloidosis has rarely been described in literature which poses great diagnostic and therapeutic challenge.

CASE REPORT

A 42-year-old female, presented with complaints of right hypochondriac and epigastric pain, which was dull aching, occurring intermittently, associated with weight loss of 15 kg over 8 mo. There was no history of jaundice, hematemesis, melena, and abdominal distension, alteration in bowel habit or bleeding from any site. There was no history of joint pain, rash, oral ulceration, cough, skin tightness, peripheral tingling, and weakness in limbs or breathlessness. She had an episode of acute febrile illness due to uncomplicated plasmodium vivax malaria in the recent past. There was history of acute viral hepatitis two times in remote past. She was hypothyroid on supplementation since 2 years. There was no history of tuberculosis in past.

On General examination patient was afebrile with pulse rate 80/min, blood pressure 16.2/10 kPa and had pallor. Systemic examination revealed liver enlarged for 2.4 inch below right costal margin which was firm, with sharp margin, smooth surface, non-tender, without hepatic rub with liver span of 8.8 inch. Spleen was also enlarged.

On evaluation investigations revealed normocytic normochromic anemia, aspartate aminotransferase level of 0.52 μkat/L (upper normal limit 0.6 μkat/L) and alanine aminotransferase level of 0.47 μkat/L (upper normal limit 0.51 μkat/L), alkaline phosphatase value of 7.46 μkat/L (upper normal limit 2 μkat/L) raised to 3.5 times the upper limit of normal, gamma glutamyl transferase value of 9.29 μkat/L (upper normal limit 0.51 μkat/L). Prothrombin time was 14 s with INR of 1, serum urea was 3.57 mmol/L (normal up to 8.2 mmol/L) and creatinine was 70.72 μmol/L (normal upto 106 μmol/L). Her thyroid stimulating hormone was 3.1 μIU/mL (normal upto 5 μIU/mL), FT4 was 14.16 pmol/L (normal 12 to 30 pmol/L), and FT3 was 4.62 pmol/L (normal 2 to 7 pmol/L). Erythrocyte sedimentation rate was 55 mm at end of 1st h by Westergreen method, C-reactive proteins was 47.62 nmol/L (normal up to 28.5 nmol/L). Her serum calcium was 2.23 mmol/L, fasting blood sugar was 5 mmol/L, triglycerides were 1.02 mmol/L and cholesterol was 3.37 mmol/L. Vitamin D3 was 239.2 nmol/L (normal > 150 nmol/L); intact-parathyroid hormone was 13 pg/mL (normal 10-65 ng/mL). Human immunodeficiency virus antibodies, HBsAg, anti-hepatitis C virus antibody were negative. Autoimmune markers including anti-nuclear antibodies, anti-Liver Kidney Microsome type 1 antibody (anti-LKM 1), anti-smooth muscle antibodies, anti-mitochondrial antibodies were negative.

Ultrasonography abdomen revealed hepatomegaly (7 inch) with coarse echo texture with compressed intrahepatic inferior vene cava and splenomegaly. Oesophagus-DuodenoScopy revealed mild antral gastritis. Pre-contrast computed tomography (CT) abdomen revealed gross hepatomegaly with tiny foci of calcification (Figure 1A). There was heterogeneous post contrast enhancement with diffuse low density areas in liver on venous phase (Figure 1). On further evaluation for infiltrative liver disorders, liver biopsy was done which revealed diffuse eosinophilic homogenous material throughout sinusoids with compressed hepatocytes (Figure 2). These areas were Congo red stain positive with apple green birefringence on polarizing microscopy suggestive of Amyloid deposits (Figure 3). Ultrasonography and fine needle aspiration cytology from the thyroid gland was done which was suggestive of colloid goiter without any evidence of any evidence of amyloid deposit.

The patient had normal serum and urine protein immunofixation electrophoresis, with normal serum free light chain assay. There was no albuminuria or no bence-jones proteinuria. Her electrocardiogram and echocardiogram was normal. Bone marrow biopsy did not reveal any plasma cell dyscrasia or amyloid deposit. Contrast enhanced CT of thorax and nuclear medicine whole body bone scan was normal with no evidence of extra osseous uptake. Skeletal survey showed no gross abnormalities. Abdominal fat pad biopsy and rectum biopsy was negative for amyloid deposit. Her serum Rheumatoid factor, pAnti-neutrophilic cytoplasmic antibodies (ANCA), cANCA, anti-rho, anti-

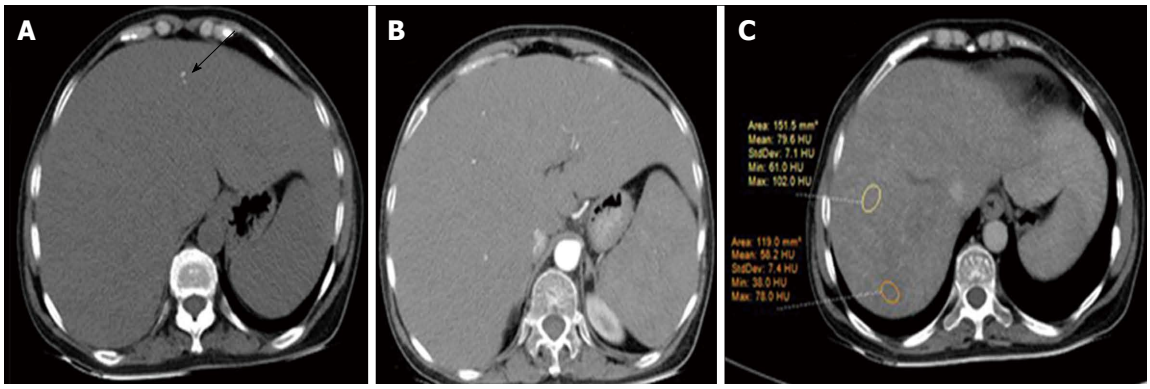

Figure 1 Pre-contrast computed tomography image of abdomen. A: Gross hepatomegaly with tiny foci of calcification in segment Ⅳ of liver (black arrow); arterial phase; B: Diffuse low contrast attenuation; C: Venous show heterogeneous contrast enhancement with diffuse low density areas scattered throughout liver parenchyma (yellow and orange spherical).

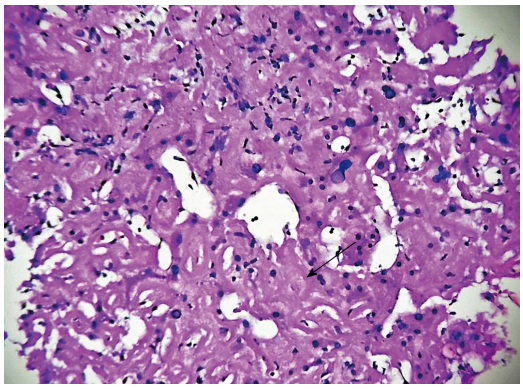

Figure 2 Haemotoxylin and eosin stain of the liver biopsy specimen shows diffuse extracellular amyloid deposit in peri-sinusoidal spaces with compression of hepatocytes (black arrow).

la antibody, tuberculin test, and tumour markers were negative. Immunohistochemistry using anti serum amyloid associated (SAA) protein immunoperoxidase staining for secondary amyloidosis was done from the liver biopsy specimen which was also negative. Immunohistochemistry of liver biopsy using anti-kappa and anti-lambda antibody was not done as the serum free light chain assay and serum protein immunofixation electrophoresis was normal. She was managed symptomatically with colchicine and other supportive therapies including intravenous fluids. The patient was followed up for 6 mo. Subsequently she was lost to follow up. Her constitutional symptoms improved marginally on colchicine. But hepatomegaly and altered biochemical parameters did not show any improvement.

DISCUSSION

Though amyloidosis is considered as a systemic disease, 10%-20% cases can be localised[4]. To our knowledge primarily isolated hepatic involvement of liver in amyloidosis has rarely been described in the literature. Though it is possible that the patient has yet exhibited the evidence of systemic disease, hepatic involvement can occur in both primary and secondary types of amyloidosis (AL/AA). In primary type the characteristic fibrillar protein is a fragment of the variable immunoglobulin light (and/or rarely heavy) chain and in secondary type the protein is the amino acid terminus of the acute phase protein SAA. Secondary amyloidosis with hepatic involvement can be seen in chronic inflammatory disorders and infections including multiple myeloma, tuberculosis, rheumatoid arthritis, familial Mediterranean fever, Crohn's disease, Reiter's syndrome, ankylosing spondylitis, Sjögren's syndrome, dermatomyositis, vasculitis, chronic osteomyelitis, bronchiectasis, cystic fibrosis, systemic lupus erythematosus (SLE)[4], *etc.* Currently the AA/AL ratio has been 1:17 to 1:38 due to fewer chronic infections and an increasing recognition of AL amyloidosis. Other types of amyloidosis that are rarely seen include dialysis-related amyloidosis with the deposition of β2-microglobulins, and autosomal dominant systemic amyloidosis, such as familial amyloidotic polyneuropathy with the deposition of genetically variant transthyretin[5].

Hepatic amyloidosis is usually characterised by amyloid deposits in the liver parenchyma along the sinusoids within the spaces of disse or within the blood vessel walls. As a result of extensive compression of hepatocytes by the amyloid deposits there may be atrophy of hepatocyte. More massive infiltration results in enlarged liver with rubber elastic consistency. This results in "lardaceous liver" appearance on cut-surface[6].

The clinical spectrum of hepatic amyloidosis can range from hepatomegaly and borderline abnormal liver function test to more severe form resulting in portal hypertension, hepatic failure and rarely spontaneous rupture[7]. Around 70%-80% of the cases have associated nephrotic syndrome, congestive cardiac failure, orthostatic hypotension or peripheral neuropathy. In another series, other frequent findings in cases of hepatic amyloidosis included proteinuria (88%), elevated serum alkaline phospatase (86%), abnormal serum protein electrophoresis (monoclonal protein or hypogammaglobulinemia, 64%), hyposplenism on the peripheral blood smear (62%), defined by the presence

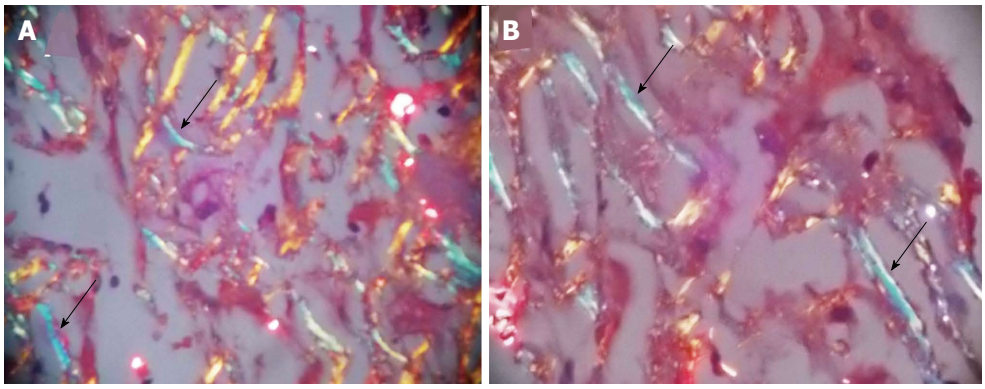

Figure 3 Apple green birefringence demonstrated by amyloid fibrils on polarizing light microscopy (black arrows in A and B) is in liver biopsy specimen.

of Howell-Jolly bodies; and hepatomegaly (81%) disproportional to the liver enzyme abnormalities[8]. The median survival rate in these patients is 9 mo[7,8]. Our patient had gross hepatomegaly with constitutional symptoms together with raised alkaline phosphatase but no other associated feature.

Radiological findings of hepatic involvement are non-specific. Pre-contrast and contrast enhanced CT reveals enlarged liver with heterogeneous decrease attenuation with delayed enhancement. There may be focal area of hypo attenuation owing to impaired blood flow due to extensive infiltration by amyloid deposit. Our patient had gross hepatomegaly with tiny foci of parenchymal calcification on pre-contrast CT which is rarely seen[9]. Her arterial phase CT shows lack of parenchymal enhancement in liver. Venous phase shows heterogeneous enhancement with diffuse low density areas in liver. Rarely typical hepatic contour characterised by asymmetric and triangular appearance of liver with apex towards falciform ligament may occur owing to selective atrophy of the lateral margins of both the lobes[10].

Immunohistochemistry using anti-kappa and anti-lambda antibodies are useful in immunohistochemical classification and diagnosis of AL type amyloidosis. However it has its own limitations owing to cross reactivity between anti-kappa and anti-lambda antibodies. A study using antibodies against three different regions of immunoglobulin lambda light chain for the immunohistochemical analysis of liver biopsy samples from the cases of immunoglobulin lambda light chain amyloidosis showed that the amyloid deposits may not be homogeneous in the liver and that molecular heterogeneity of amyloid fibril protein or a difference in the mode of deposition results in the histopathological heterogeneity of AL amyloid deposits[11].

Our case represents a diagnostic challenge where specific type of amyloid deposit in liver was difficult to determine. Normal bone marrow examination with normal serum and urine immunofixation electrophoresis ruled out primary amyloidosis. Anti-SAA immunoperoxide staining from liver biopsy was also negative. Hereditary forms of amyloidosis including lysosome form were considered. There was no facility for mass spectroscopic analysis for varied type of amyloid protein at our centre. There was no evidence of hepatocellular failure or spontaneous rupture. There was no cardiac, renal or nervous system involvement. There was no evidence of tuberculosis, rheumatoid arthritis, SLE, crohns disease, or evidence of other common inflammatory diseases. This case represents need for high level of suspicion to diagnose a case of isolated hepatomegaly due to amyloidosis. There have been case reports of hepatic involvement in amyloidosis including one where the presentation was a liver SOL in the setting of plasma cell dyscrasias, but isolated hepatic involvement is a rare entity[12]. In absence of systemic evidence of amyloidosis liver transplant was considered for the patient but she did not have requisite Model for End Stage Liver Disease points to make her eligible candidate. She was managed symptomatically with colchicine and other supportive therapies.

This case represents diagnostic and therapeutic difficulty in managing a case of primary hepatic amyloidosis of undetermined aetiology.

COMMENTS

Case characteristics

A middle aged female presented with long-standing abdominal pain with loss of weight without jaundice.

Clinical diagnosis

On examination her vitals were stable and she had gross hepatomegaly.

Differential diagnosis

Infiltrative liver disorders (amyloidosis, lymphoma, sarcoidosis), metabolic liver disease (hemochromatosis), autoimmune liver disease.

Laboratory diagnosis

She had normal alanine and aspartate aminotransferase levels, markedly raised alkaline phosphatase level of 447 U/L (upper normal limit 310 U/L) raised to 1.5 times the upper limit of normal, gamma Glutamyl transferase value of 566 U/L (upper normal limit 45 U/L) raised to 12 times the upper limit of normal and other parameters within normal limit.

Imaging diagnosis

Computed tomography whole abdomen showed gross hepatomegaly with heterogeneous post contrast enhancement with diffuse low-density areas on

venous phase.

Pathological diagnosis

Liver biopsy was suggestive of diffuse eosinophilic homogenous material throughout sinusoids with compressed hepatocytes which were Congo red stain positive showing apple green birefringence on polarizing microscopy suggestive of amyloid deposits.

Treatment

She was managed symptomatically with colchicine and other supportive therapies as there was no definite cause of hepatic amyloidosis that could be found out.

Related reports

There have been case reports of hepatic involvement in amyloidosis including being presented as a mass in setting of plasma cell dyscrasias, but isolated hepatic involvement is a rare entity published series have described varied presentation of liver involment in amyloidosis which can range from asymptomatic hepatomegaly to fulminant hepatic failure.

Term explanations

Amyloidosis is a pathological process that encompasses a spectrum of disease resulting from the extracellular deposition of fibrillar amyloid protein which can involve any organ isolated or in conjunction with other organs and can do so in the form of a focal, tumour-like lesion, or an infiltrative process.

Experiences and lessons

This case represents diagnostic and therapeutic difficulty in managing a case of isolated hepatic amyloidosis of undetermined aetiology.

Peer-review

The paper is well written.

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