*REVIEW*

# Gut-liver axis in liver cirrhosis: How to manage leaky gut and endotoxemia

Hiroshi Fukui

Hiroshi Fukui, Department of Gastroenterology, Endocrinology and Metabolism, Nara Medical University, Kashihara, Nara 634-8522, Japan

Author contributions: Fukui H wrote this review.

Conflict-of-interest: The author has no conflict of interest to declare.

Correspondence to: Hiroshi Fukui, MD, PhD, Department of Gastroenterology, Endocrinology and Metabolism, Nara Medical University, 840 Shijo-cho Kashihara, Nara 634-8522, Japan. hfukui@naramed-u.ac.jp
Telephone: +81-744-223051
Fax: +81-744-247122

## Abstract

A "leaky gut" may be the cutting edge for the passage of toxins, antigens or bacteria into the body, and may play a pathogenic role in advanced liver cirrhosis and its complications. Plasma endotoxin levels have been admitted as a surrogate marker of bacterial translocation and close relations of endotoxemia to hyperdynamic circulation, portal hypertension, renal, cardiac, pulmonary and coagulation disturbances have been reported. Bacterial overgrowth, increased intestinal permeability, failure to inactivate endotoxin, activated innate immunity are all likely to play a role in the pathological states of bacterial translocation. Therapeutic approach by management of the gut-liver axis by antibiotics, probiotics, synbiotics, prebiotics and their combinations may improve the clinical course of cirrhotic patients. Special concern should be paid on anti-endotoxin treatment. Adequate management of the gut-liver axis may be effective for prevention of liver cirrhosis itself by inhibiting the progression of fibrosis.

Key words: Gut-liver axis; Liver cirrhosis; Pathogenesis; Complications; Endotoxemia; Bacterial translocation; Leaky gut; Toll-like receptors; Selective intestinal decontamination; Probiotics

Core tip: A "leaky gut" may be the cutting edge for the passage of toxins, antigens or bacteria into the body, and may play a pathogenic role in advanced liver cirrhosis and its complications. More attention should be paid to the role of intestinal bacteria and bacterial products in the field of Hepatology. Here, I would like to overview the history of endotoxin assay in the blood, clinical significance of endotoxemia in liver cirrhosis and then shift to the topic of gut and liver in general. Understanding of the gut-liver axis, leaky gut and endotoxemia in cirrhosis may give us new ideas.

## INTRODUCTION

Bacterial infections account for significant morbidity

and mortality in patients with liver cirrhosis[1]. Infections increase mortality 4-fold in cirrhotic patients[2]. Although urinary, respiratory, ascitic fluid infections and bacteremia are common infectious complications, spontaneous bacterial peritonitis (SBP) occurs most frequently. A vast majority of such infections are due to enteric gram-negative bacteria, mainly *Enterobacteriaceae*[3]. Passage of viable bacteria from the intestinal lumen through the intestinal wall and to mesenteric lymph nodes (MLNs) and other sites, defined as bacterial translocation (BT), explains the development of SBP[4]. The concept of BT was later broadened to microbial products or their fragments, such as endotoxin [lipopolysaccharide (LPS)], peptidoglycan, lipopeptides and bacterial DNA. The liver receives portal blood containing these microbial products and acts as the initial site of their filtration and detoxication. These defense mechanisms are impaired in the cirrhotic liver, which finally results in spillover of these products and secretion of various inflammatory mediators.

LPS is a major component of the gram-negative bacterial wall. Its detection in the blood has a long history and is still not complete with several methodological difficulties. However, the endotoxemia detected by the Limulus-lysate test or its modifications has been well correlated to the severity, complications and mortality of liver cirrhosis. Control of endotoxemia is still considered to be the mainstay of therapy for advanced liver cirrhosis.

Here, I would like to overview the history of endotoxin assay in the blood, clinical significance of endotoxemia in liver cirrhosis and then shift to the topic of gut and liver in general. Understanding of the gut-liver axis, leaky gut and endotoxemia in cirrhosis may give us new ideas for hepatology tomorrow.

## DETECTION OF ENDOTOXEMIA IN PATIENTS WITH LIVER CIRRHOSIS

Levels of bacterial LPS are increased in the portal and/ or systemic circulation in liver cirrhosis. Endotoxemia was first demonstrated by the Limulus amebocyte lysate (LAL) test and later by several quantitative assay, such as the chromogenic Limulus assay and the turbidimetric endotoxin assay. Although endotoxemia is now considered to be a common feature of liver cirrhosis, there has been much debate and still disagreement about plasma endotoxin levels.

Early study using LAL test revealed that systemic endotoxemia in liver cirrhosis occurred with a frequency of 15 of 31 compared with 2 of 21 venous samples and 9 of 21 portal venous samples from patients without liver disease[5]. Tarao et al[6] reported that death occurred within 6 mo in 47.8% of the patients with a positive endotoxin test, whereas only 16.7% of those with a negative test died in the same period. Clemente et al[7] showed that a positive

LAL test was almost exclusively associated with a progressive functional renal failure (8 of 10 patients) and all but one of them died. Endotoxemia was also associated with hemorrhage due to acute erosions of the gastric mucosa (6 of 7 patients)[7]. On the contrary, Gaeta et al[8] found no significant difference in the frequency of endotoxemia between patients with impaired and unimpaired renal blood flow. Moreover, no relation was found between endotoxin plasma levels and renal blood flow in their study. Bode et al[9] reported that the prevalence of endotoxemia was not significantly higher in cirrhotics with ascites or esophageal varices when compared to the subgroup without ascites or esophageal varices. They additionally found endotoxemia more frequently in patients with alcoholic cirrhosis (67.3%) than in patients with non-alcoholic cirrhosis (45.5%)[9].

In general, nonspecific gelation has been reported by this LAL test, and interpretation of the results has often caused confusion[10]. Finally, Fulenwider et al[11] reported that they could not detect any endotoxin by the LAL test in peripheral plasma, portal plasma and ascites. They concluded that the ubiquity of endotoxin, with the attendant opportunities for specimen contamination, is the most likely explanation for the high prevalence of endotoxin in the plasma and ascites of cirrhotic patients.

In 1978, Iwanaga et al[12] developed a quantitative endotoxin assay using a synthetic chromogenic peptide as a substrate for the endotoxin-sensitive Limulus enzyme. This assay has been shown to give reliable results for minute amount of endotoxin in water. However, measurements of endotoxin in blood present some difficulties. Major problems in plasma endotoxin assay are (1) disagreement about the best way of preparing standard curves[13-15]; (2) lack of an optimal method for eliminating plasma inhibitors for endotoxin assay[15-17]; and (3) necessity of endotoxin-specific chromogenic substrate[18]. We have insisted that a standard curve should be prepared for each individual plasma sample in the endotoxin determination, because ideal 100% recovery of endotoxin could not be validated in any trial of plasma pretreatment[15]. The internal standard is especially necessary in the perchloric acid treatment, where strict adjustment of pH in samples is difficult before the chromogenic assay[19]. Our study demonstrated hidden extra portion of endotoxin in plasma of patients with chronic liver diseases, using new way of plasma pretreatment either by Tween 80 in the dilution and heating method or by triethylamine in the perchloric acid method[20]. The chromogenic substrate widely used in the world is considered to react not only endotoxin but also other substances such as (1,3)-β-D-glucan, component of cell wall of fungus[21]. Endotoxin-specific chromogenic substrate was produced by removing G-factor from the lysate[21].

Although most studies on plasma endotoxin

levels ignored these points, using simply the dilution and heating method and non-specific chromogenic substrate, results obtained were generally useful for evaluation of the clinical significance in liver cirrhosis. Lumsden et al[22] found that endotoxin levels in the portal venous blood was significantly higher than that in the peripheral venous blood, although there was a wide variability. However, neither hepatic nor peripheral venous endotoxin levels correlated significantly with a variety of clinical, biochemical or radiological parameters[22]. Tachiyama et al[23] also confirmed that endotoxin levels in the portal blood was higher than that in the peripheral venous blood by their Limulus gelation turbidimetric LAL assay. Moreover, they found that portal endotoxin levels in patients with cirrhosis was higher than those without cirrhosis[23]. This finding suggests an enhanced intestinal production and/or absorption of endotoxin in liver cirrhosis, which later developed the discussion on bacterial overgrowth and leaky gut in liver cirrhosis. Bigatello et al[24] detected endotoxemia in 36 of 39 cirrhotic patients and in none of healthy volunteers by their chromogenic LAL test. They found that systemic endotoxemia was higher in patients with hepatic encephalopathy after esophagogastric hemorrhage than in well-compensated cirrhotics. It was higher in patients with deep coma than in those with light coma and also higher in those who died than in those who survived. They concluded endotoxemia without sepsis is a constant finding in cirrhosis and increasing levels of endotoxemia are associated with hepatic failure, encephalopathy, and death[24].

We found higher plasma endotoxin level in patients with alcoholic cirrhosis than in patients with non-alcoholic cirrhosis with an improved chromogenic substrate assay, using individual standard curves for each plasma sample[25]. On admission endotoxin concentrations in alcoholics with fatty liver were similarly elevated as observed in alcoholic cirrhosis. In 6 out of 12 patients with fatty liver or alcoholic hepatitis, in whom a second sample of plasma was investigated after 6 to 8 d, endotoxemia was no longer detectable. The results indicate that, irrespective of the stage of liver disease, alcohol abuse favors the development of endotoxemia[25]. In the measurement of plasma endotoxin by the dilution and heating method, we frequently experienced that plasma endotoxin level in patients with advanced liver cirrhosis was unexpectedly low and speculated that some part of plasma endotoxin might be lost in the procedure of dilution and heating. To overcome this situation, we added a detergent Tween 80 after heating plasma and discovered much hidden endotoxin in the sample[20]. Significantly higher plasma endotoxin levels in cirrhotics with upper gastrointestinal (GI) bleeding compared with those without upper GI bleeding was detected by this Tween 80 method[26].

To attain endotoxin-specific chromogenic assay,

we further improved our method using triethylamine in the perchloric acid method and endotoxin-specific substrate Endospecy (Seikagaku Kogyo Co., Tokyo, Japan) with kinetic analysis[19,20]. A final pH of the assay sample was always adjusted to 7 by careful titration of triethylamine[19]. The results of our measurement of plasma endotoxin in 90 patients with liver cirrhosis and 11 patients with chronic hepatitis with this method were summarized as follows: (1) there was an increase of plasma endotoxin with the progression of chronic liver disease; (2) in patients with bleeding from esophageal varices, plasma endotoxin increased for 3 d after the bleeding and thereafter decreased; and (3) endotoxin level increased as the progression of Child-Pugh grades and was negatively related to prothrombin time[18].

## RELATIONSHIP OF ENDOTOXEMIA TO PATHOGENESIS OF CIRRHOSIS AND ITS COMPLICATIONS

### Hyperdynamic circulation

Hyperdynamic circulation characterized by hypotension, low systemic vascular resistance, high cardiac output and a reduced sensitivity to vasoconstrictors are features of cirrhosis. These cardiovascular changes might be the result of increased synthesis of a vasodilator[27]. Nitric oxide derived from vascular endothelium is a potent vasodilator that plays a key role in the homeostasis of blood pressure[28]. Cirrhotic patients showed significant increases in serum nitrite/nitrate which was significantly correlated with endotoxemia[28]. Oral administration of colistin to 15 cirrhotic patients reduced significantly plasma endotoxin levels and serum nitrite/nitrate levels[28]. A lower systemic vascular resistance and a higher cardiac output were found in cirrhotics with endotoxemia than in those without endotoxemia[29]. These studies support that endotoxemia may be responsible, at least in part, for the hyperdynamic circulation found in patients with liver cirrhosis. On the contrary, Campillo et al[30] showed that serum nitrate levels did not correlate with endotoxemia and that cardiac index did not correlate with serum nitrate levels, urine nitrate excretion and endotoxemia. Plasma interleukin (IL)-6 levels were correlated negatively with systemic vascular resistance in patients with cirrhosis, but no correlation was observed between plasma endotoxin levels and plasma IL-6 levels[31]. Finaly, Bhimani et al[32] concluded from their experiments that an endotoxin-induced increase in mesenteric iNOS activity and a decrease in hepatic cNOS activity may account for, respectively, the hyperdynamic visceral circulation and the increased intrahepatic resistance of cirrhosis. Although the role of endotoxin on the hyperdynamic circulation still remains controversial, endotoxemia, possibly from gut-derived bacterial translocation, causes induction of

nitric oxide (NO) synthase (NOS) leading to increased vascular NO production, which is the primary stimulus for the development of vasodilatation in cirrhosis and its accompanying clinical manifestations[33].

### Portal hypertension

In cirrhosis, portal hypertension can promote bacterial translocation and increase serum endotoxin levels. Vice versa, endotoxin aggravates portal hypertension by induction of systemic and splanchnic vasodilation, and by triggering hepatic inflammatory response *via* tumor necrosis factor $\alpha$ (TNF-$\alpha$)[34]. Endotoxin levels correlated with hemodynamic derangement in cirrhotic severe portal hypertension, and with levels of soluble TNF-$\alpha$ receptors in patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt[34]. Thromboxane (TX) A2 has been suggested to play a significant role in the development of portal hypertension in fibrosis, and Kupffer cell (KC) derived TXA2 has been shown to mediate the hyperresponsiveness of the portal circulation to the vasoconstrictive actions of endothelin-1 during endotoxemia[35]. The double stresses of early fibrosis additively activate KC and release increased amount of TXA2 in response to ET-1, which leads to the increased portal resistance and ultimately hepatic microcirculatory dysfunction[35]. Steib *et al*[36] concluded from their experiment in bile-duct ligated rats that upregulation of Toll-like receptors (TLR)4 and MyD88 expression in fibrotic livers confers hypersensitivity to LPS. This may lead to escalation of portal hypertension by production of TX and Cys-leucotriene after LPS-induced KC activation.

### Hepatic encephalopathy

Except for the early human study by Bigatello *et al*[24], the role of endotoxin on hepatic encephalopathy had not been investigated until recently. Wright *et al*[37] showed that the injection of endotoxin into cirrhotic rats induced pre-coma and exacerbates cytotoxic edema because of the synergistic effect of hyperammonemia and the induced inflammatory response. Bajaj *et al*[38] further extended the problem to microbiome in the intestine and concluded that cirrhosis with hepatic encephalopathy is associated with significant alterations in the stool microbiome compared with healthy individuals. Specific bacterial families (*Alcaligeneceae, Porphyromonadaceae, Enterobacteriaceae*) are strongly associated with cognition and inflammation in hepatic encephalopathy. The central role of ammonia in the pathogenesis of hepatic encephalopathy is incontrovertible. However, there is a robust evidence indicating the importance of inflammation in exacerbating the neurological effects of hepatic encephalopathy[39]. Sterile inflammation by circulating endotoxin from the gut (bacterial translocation) inducing immune dysfunction may have some effect *via* the release of pro-inflammatory mediators which directly signal to the brain[39].

### Renal disturbance

Endotoxin is a well-known renal vasoconstrictor. Deleterious effect of endotoxin on kidney has been confirmed in various animals, *i.e.*, dogs[40], mouse[41] and bile-duct ligated rats[42]. Uchihara *et al*[43] found that plasma endothelin levels were significantly higher in patients with endotoxemia than in those without and were negatively correlated to creatinine clearance in cirrhotics. They concluded that plasma endothelin closely related to endotoxemia, may play a contributory role in kidney dysfunction in patients with cirrhosis[43]. As stated above, there has been a disagreement about the relation of endotoxemia to renal disturbance in cirrhosis. Close correlation reported in the early period using LAL test[7,44,45] was not always validated in the later period using quantitative LAL test[30]. However, experimental evidences together with beneficial effect of non-absorbable antibiotics support the pathogenetic roles of endotoxin in the renal disturbance.

Shah *et al*[46] demonstrated in their bile-duct ligated rats that kidneys in cirrhosis show an increased expression of TLR4, NF$\kappa$B, and the pro-inflammatory cytokine TNF-$\alpha$, which makes them susceptible to a further inflammatory insult. This increased susceptibility to LPS can be prevented with selective decontamination by norfloxacin[46]. The effect of selective decontamination for renal disturbance to patients with liver cirrhosis was first reported with paromomycin sulfate[47] and recently by rifaximin[48].

SBP is the most dangerous infectious complication arising in patients with cirrhosis and ascites. It is associated with high serum and ascitic fluid levels of proinflammatory cytokines. These patients are predisposed to the development of renal impairment, type 1 hepatorenal syndrome[49]. Albumin infusion improves renal function in acutely decompensated cirrhotic patients with acute kidney injury by impacting on renal blood flow autoregulation. This is possibly achieved through endothelial stabilization and a reduction in the sympathetic tone, endotoxemia and oxidative stress[50].

### Cirrhotic cardiomyopathy

Liver cirrhosis is associated with several cardiovascular abnormalities. Despite an increased baseline cardiac output, cirrhotic patients have a suboptimal ventricular response to stress. This phenomenon is called cirrhotic cardiomyopathy. The pathogenesis of this syndrome is multifactorial and includes diminished $\beta$-adrenergic receptor signal transduction, cardiomyocyte cellular plasma membrane dysfunction, and increased activity or levels of cardiodepressant substances such as cytokines, endogenous cannabinoids, and nitric oxide[51]. Patients with severe cirrhotic cardiomyopathy have higher lipopolysaccharide binding protein (LBP) levels, which are significantly correlated with the degree of diastolic dysfunction. This findings support a potential role of bacterial endotoxemia on the aggravation of cardiomyopathy in cirrhotic patients[52].

The development of severe renal failure type 1 HRS seems to be related to a cardiac systolic dysfunction[53]. In addition to the above myocardial dysfunction, the release of endotoxins and biologically active substances such as inflammatory cytokines, nitric oxide, carbon monoxide related to bacterial infection may further impair cardiac function in patients with advanced cirrhosis[54].

### Hepatopulmonary syndrome

Hepatopulmonary syndrome (HPS) is an important cause of dyspnea and hypoxia in 10%-30% of patients with cirrhosis[55]. It is due to vasodilation and angiogenesis in the pulmonary vascular bed, which leads to ventilation-perfusion mismatching, diffusion limitation to oxygen exchange, and arteriovenous shunting[55].

In experimental studies, Zhang et al[56] demonstrated that progression and severity of HPS as indicated by both increased pulmonary capillaries and histological changes are closely associated with endotoxin levels in the cirrhotic rat model. They thought that overproduction of TNF-$\alpha$ due to endotoxin stimulation of KCs via mitogen-activated protein kinase signal transduction pathway may be a major mechanism mediating the pathologic alterations of HPS[57]. There was a case report[58] that showed a beneficial effect of oral norfloxacin for hypoxia in a patient with this syndrome. The authors speculated that norfloxacin reduced endotoxemia and concomitant nitric oxide production in patients with cirrhosis. However, a following pilot study of intestinal decontamination with norfloxacin in patients with HPS, in an attempt to reduce endotoxemia, failed to produce any improvement in gas exchange[55].

### Coagulation and platelet abnormalities

Patients with advanced liver cirrhosis paradoxically have both risks of bleeding and thrombosis. They should face fragile balance between hypercoagulability and hypocoagulability related to reduced synthesis of clotting factors, accelerated fibrinolysis, platelet dysfunction and low-grade intravascular clotting. Hyperfibrinolysis is not a primary phenomenon but occurs as a consequence of clotting activation and that endotoxemia might play a pathophysiological role[59]. Cirrhotic patients are at increased risk for thrombotic events, particularly in the portal venous system[60]. In cirrhotics, plasma levels of von Willebrand factor (vWF) antigen and endotoxemia progressively increased from Child Pugh's classification A to class C[61]. vWF is a marker of endothelial perturbation and endotoxin releases vWF from endothelial cells in vitro[61]. Endothelial procoagulant activation induced by low-grade endotoxemia may represent a trigger for systemic clotting activation in liver cirrhosis patients[62]. Violi et al[63] reported that endotoxemia was directly correlated with F1 + 2[59] and D-dimer. These studies show that an ongoing prothrombotic state is present in the portal circulation of cirrhotic patients and may play a pivotal role in the thrombotic episodes[63]. They further confirmed that monocyte expression of tissue factor (TF) was significantly correlated with plasma levels of F1 + 2 and with endotoxemia[64]. TF mRNA expression was detected only in three patients with endotoxemia[64].

Decreased plasma ADAMTS13 activity results in the accumulation of unusually large vWF multimer (UL-VWFM) and the formation of platelet thrombi[65]. Uemura et al[65] showed that ADAMTS13 activity decreased with increasing severity of liver disease (controls means 100%, chronic hepatitis 87%, Child A cirrhosis 79%, Child B cirrhosis 63%, and Child C cirrhosis 31%), and showed severe deficiency (< 3% of controls) in five end-stage cirrhotics. This ADAMTS13 activity may be a useful prognostic marker that is equal or superior to the Child-Turcotte-Pugh score and the Model for End-Stage Liver Disease score to predict not only the short-term prognosis but also the long-term survival of the cirrhotic patients[66]. As we found that endotoxemia was inversely correlated with ADAMTS13 activity and was higher in patients with UL-VWFM than those without in patients with alcoholic hepatitis[67], this relation should be estimated in liver cirrhosis.

## GUT-LIVER AXIS IN HEALTH AND LIVER CIRRHOSIS

The gut and the liver are the key organs in nutrient absorption and metabolism. Bile acids, drugs, and toxins undergo extensive enterohepatic circulation. Bile acids play a major role in several hepatic and intestinal diseases. Endotoxins deriving from intestinal Gram-negative bacteria are important in the pathogenesis of liver and systemic diseases[68]. Gut flora and bacterial translocation play important roles in the pathogenesis of chronic liver disease, including cirrhosis and its complications[69]. Intestinal bacterial overgrowth and increased bacterial translocation of gut flora from the intestinal lumen predispose patients to bacterial infections and major complications[69].

### Bacterial translocation

BT or microbial translocation is defined as the migration of viable microorganisms or bacterial products (i.e., bacterial LPS, peptidoglycan, and lipopeptides) from the intestinal lumen to the mesenteric lymph nodes and other extraintestinal sites[70]. Passage of viable bacteria from the intestinal lumen through the intestinal wall and its translocation to mesenteric lymph nodes and other sites is the accepted pathogenic mechanism for the development of spontaneous infections, such as SBP or bacteremia[4]. Bacterial products, such as endotoxin, or bacterial DNA can translocate to extra-intestinal sites

and promote an immunological response similar to that produced by viable bacteria. Pathological BT is a contributing factor in the development of complications in cirrhosis, not only in infections, but by exerting a profound inflammatory state and exacerbating the hemodynamic derangement[4,71].

### Small intestinal bacterial overgrowth

Small intestinal bacterial overgrowth (SIBO), defined as ≥ 10^5 total colony-forming units per milliliter of proximal jejunal aspirations, was present in 59% of cirrhotic patients and is associated with systemic endotoxemia[72]. SIBO related with a slowed intestinal transit, low acid gastric secretion, intestinal immunological factors and pancreatic and biliary secretions, is important factor promoting BT[4]. Cirrhotic rats with intestinal bacterial overgrowth had a significantly higher rate of translocation and slower intestinal transit than those without it[73].

SIBO, determined by the breath hydrogen test, is common in patients with cirrhosis, especially in those with advanced liver dysfunction and in those with a history of SBP[4,74,75]. In a study that estimated SIBO by more reliable quantitative cultures of jejunal aspirates, the occurrence of SBP did not correlate with the presence of SIBO[4]. Sánchez et al[76] reported that the increase of intestinal aerobic bacteria in experimental cirrhosis is associated with translocation and is supposed to play an important role in the development of BT. Impaired motility may be implicated in the pathogenesis of intestinal bacterial overgrowth[76]. Gut flora imbalances, higher levels of *Enterobacteriaceae* result in significant changes in BT and liver function in cirrhotic rats[77].

Dietary habits, by increasing the percentage of intestinal Gram-negative endotoxin producers, may accelerate liver fibrogenesis, introducing dysbiosis as a cofactor contributing to chronic liver injury in nonalcoholic fatty liver disease[78]. Liver cirrhosis disturbs intestinal microbiota and innate immunity-related genes, which contributes to endotoxemia and bacterial translocation. These had not completely recovered in cirrhotic rats until 1 mo after orthotopic liver transplantation[79].

### Increased intestinal permeability

The gut epithelium plays an important role in the immune homeostasis in the gut as the first barrier against the bacterial translocation[80,81]. Because gut barrier system by intestinal epithelial cells prevent translocation of large amounts of bacteria and bacterial products, very small amount of them can reach the liver in a healthy state[82]. The intestinal barrier is formed mainly by intestinal epithelial cells and their mucinous components[4]. In addition, intercellular junctions such as tight junctions and gap junctions allow selective passage of substances[4]. Structural and functional changes in the intestinal mucosa

that increase intestinal permeability of bacteria and its products have been found in patients with liver cirrhosis[4]. This intestinal barrier dysfunction may be an important pathogenetic factor for several complications of liver cirrhosis[83]. Characteristics of cirrhosis itself, including portal hypertension, alterations in the intestinal microbiota, inflammation and oxidative stress can affect barrier function of both small and large intestine and may contribute to the development of complications[71]. Although gut barrier function did not show a significant relationship with endotoxemia, increased intestinal permeability may be a significant finding that at least in part is associated with the pathophysiology of viral liver cirrhosis[84].

There is a long-standing debate about the presence and role of increased intestinal permeability in patients with cirrhosis[85]. Some authors have shown an association between increased intestinal permeability and severity of liver cirrhosis assessed by the Child-Pugh classification[85-87], but others have failed to reproduce these results[88-90]. Methodological problems should be taken into account when interpreting these conflicting results[91]. Some authors used sugars[86,87,92], and others used isotope probes[85,88-90], the latter considered to be the gold standard as the probes are not synthesized or digested in the human body[85]. However, the assessment of the mucosal intestinal permeability by urinary excretion of orally administered non-metabolizable sugars gave us some information on the discrimination between transcellular and para-cellular fluxes[93].

Monosaccharides, such as mannitol, are absorbed through the transcellular pathway and reflect the extent of absorption of small molecules. Disaccharides, such as lactulose, are absorbed through the paracellular junction complex (the tight junctions) and extrusion zones of the intervillous spaces, which corresponds to the permeability of larger molecules[92,94]. Mannitol absorption as assessed by urinary excretion can be considered as an indicator of the mucosal absorptive area, and lactulose absorption as a measure of the integrity of intestinal mucosal tight junctions[94,95].

The lactulose/mannitol (L/M) ratio (LMR) thus comprises an index to appraise intestinal permeability and its increase has been traditionally used as a marker of hyperpermeability[96]; this ratio has been reported to be elevated in patients with liver cirrhosis[92] and to be markedly elevated in advanced stage[86,87]. This increase in intestinal permeability determined by LMR has been reported in several previous studies[96,97]. Alcoholics with liver disease also had marked and statistically significant increases in lactulose excretion in addition to increased LMR[96]. The abnormally elevated LMR in alcoholics with liver disease is not simply due to decreased mannitol excretion but represents increased gut permeability[96]. Pascual et al[87] found a significantly higher lactulose excretion (%L) with a comparable mannitol excretion

(%M) in patients with liver cirrhosis as compared to controls.

Parlesak et al[98] reported that permeability of polyethylene glycol (PEG) with high molecular mass (PEG 1500 and PEG 4000) was increased in patients with alcoholic liver diseases. They discussed PEG is an appropriate probe for the assessment of endotoxin translocation on the basis of its homogeneous chemical properties, appropriately adaptable molecular mass and linear, chain-like shape mimicking the structure of endotoxin[98]. These demands cannot be met by other commonly used permeability marker compounds described above[99]. Lee et al[99] reported that intestinal permeability determined by PEG 400 and 3500 was significantly high in cirrhotics with ascites. Kim et al[100] reported that the intestinal permeability index, the percentage of permeability of PEG 3350 to that of PEG 400, was increased on admission for active GI bleeding in patients with liver cirrhosis and infections.

Recently, Assimakopoulos et al[101] showed that human liver cirrhosis induces significant alterations in tight junctions of enterocytes. They found a significantly reduced expression of the tight junction proteins occludin and claudin-1 in duodenal biopsies of the total patient group compared with healthy controls and this correlated inversely with endotoxemia. In addition, the cirrhotic patients with ascites showed a significantly reduced expression of occluding and claudin-1 compared with those without ascites. These changes might represent an important cellular mechanism for intestinal barrier dysfunction and hyperpermeability in patients with liver cirrhosis[101]. They further showed that human liver cirrhosis is associated with decreased intestinal mucosal proliferation and proliferation/apoptosis ratio even at early stages of cirrhosis and increased intestinal oxidative stress in advanced liver disease[102].

### Disturbance of the liver-bile acid-microbiome axis[103]
Bile acids also play a role in the prevention of BT by inhibiting bacterial overgrowth, exerting a trophic effect on intestinal mucosa and neutralizing endotoxin[4,104]. Therefore, bile acids prevent BT and avoid the passage of bacterial products from the lumen of intestine[4,105,106]. During progression of cirrhosis, BT leads to inflammation, which suppresses synthesis of total bile acids in the liver via inhibition of CYP7A1 and induces a shift toward chenodeoxycholic acid production through the alternate pathway[103]. Decrease in bile acids entering the intestines appears to favor overgrowth of pathogenic and pro-inflammatory members of the microbiome including Porphyromonadaceae and Enterobacteriaceae[103]. Decreasing bile acid concentration in the colon in cirrhosis is also associated with decreases in Clostridium cluster XIVa, which includes bile acid 7α-dehydroxylating bacteria which produce deoxycholic acid[103]. Lorenzo-Zúñiga et al[106] found that conjugated bile acid administration

reduced bacterial content to normal levels and improves bacterial translocation and endotoxemia in cirrhotic rats. Further studies should evaluate the potential benefits of bile acids in humans[70].

### Toll-like receptors and liver disease
Translocated microbial products activate KCs in the liver through pattern recognition receptors, such as toll-like receptors (TLRs) and NOD-like receptors[82]. TLRs, recognize pathogen-derived molecules, i.e., structural components unique to bacteria, fungi, and virus- and activate innate immune responses including cytokine production in the liver[82,107,108]. It should be noted that hepatic non-immune cells, such as hepatic stellate cells (HSCs) and endothelial cells, also respond to bacterial products through TLRs[82].

Currently, more than 10 members of the TLR family have been identified. TLR4 was the first identified isoform that responds primarily to LPS[82]. LPS binds to TLR4 with co-receptor CD14 and MD-2. TLR2 heterodimerizes with TLR1 or TLR6 to recognize lipoprotein and peptidoglycan derived from Gram-positive bacteria. Bacterial flagellin is recognized by TLR5. Intracellular TLR3 and TLR9 are activated by microbe-derived nucleic acids including double stranded RNA and CpG motif containing unmethylated DNA, respectively[108].

After the binding of corresponding ligands, TLRs activate MyD88-dependent and MyD88-independent signaling pathways, which are related to the production of inflammatory mediators, anti-microbial peptides and induction of acquired immunity to eradicate invading microorganisms. The downstream signaling has now been extensively studied, which should be referred to excellent reviews[82, 108].

It is postulated that TLR4 and gut microflora-derived LPS contribute to the progression of liver fibrosis[108]. Alcohol induces LBP and TLR4, and increases responsiveness to gut-derived endotoxin. Binding of LPS to CD14/TLR4 on KCs activates production of cytokines and oxidants, which leads to T cell recruitment, HSC activation and collagen production in the liver of patients with alcoholic steatohepatitis[107]. The study of TLR4 to fibrosis progression was further extended to viral hepatitis C. A large patient cohort demonstrating that the TLR4 single nucleotide polymorphism (SNP) is one of seven SNPs that may predict the risk of liver cirrhosis in patients with chronic hepatitis C infection[109].

Recent studies suggested that TLR4 in hepatic HSCs also responds to LPS to activate Jun N-terminal kinases and NFκB[110]. Seki et al[111] demonstrated that TLR4 signaling in HSCs, but not in KCs, is crucial for the development of liver fibrosis, from the experiment by two different types of TLR4 BM chimeric mice; one group contains TLR4 mutant KCs and TLR4 intact HSCs and hepatocytes, while the other type contains TLR4 intact KCs and TLR4 mutant HSCs and hepatocytes.

Notably, aberrant activation of innate immune signaling due to enhanced BT may trigger "harmful inflammation" that contributes to sepsis, chronic inflammation, autoimmune diseases, tissue and organ injuries, fibrosis and carcinogenesis[112].

### Failure to inactivate endotoxin in the blood

It has been proposed that LPS from the portal blood initially is taken up by KCs and then by hepatocytes in the liver[113]. LPS is removed *via* several mechanisms, including molecules that bind LPS and prevent it from activating TLR4, enzymes that degrade the lipid A moiety to decrease its activity, and inactivation of LPS following uptake into the liver and spleen[114]. Another mechanism for LPS neutralization is by serum lipoproteins, high-density lipoprotein (HDL), LDL, VLDL, and chylomicrons, apolipoproteins apoE and apoA-I[115-118]. All of these mechanisms can chaperone endotoxin to hepatocytes, KCs, or sinusoidal endothelial cells, resulting in clearance of LPS without significant inflammatory cell activation[118].

Due to its lipophilic structure, LPS also adheres to plasma lipoproteins, particularly HDL[119]. HDL particles are multifunctional lipoprotein complexes that transport lipids and have several anti-inflammatory properties. Patients with alcoholic cirrhosis had a significantly decreased whole blood endotoxin-binding capacity together with decreased HDL plasma concentrations, which might result in an increase of the portion of unbound and, possibly more toxic, endotoxin[120]. LBP is an acute phase protein induced by LPS, IL-6, and IL-1$\beta$. Interestingly enough, this protein has a bi-directional action on inflammation (pro-inflammatory and anti-inflammatory) induced by LPS. It usually catalyzes the transfer of LPS to CD14, and thus enhances the LPS-induced activation of monocytes, macrophages, and other immune cells. However, in the blood rich in HDL, LBP transfers LPS to HDL with an aid of apolipoprotein (Apo) A1[121,122]. Two experimental studies have shown that HDL administration reduced the effects of LPS on tumor necrosis factor-$\alpha$ production[123,124] and systemic hemodynamics, restoring liver endothelial nitric oxide synthase activity and decreasing portal pressure[123]. Incubation of whole blood with reconstituted HDL prevents LPS-induced tumor necrosis factor-$\alpha$ and interleukin-6 overproduction by monocytes of patients with cirrhosis[125].

Until now endotoxin binding and inactivating capacity of albumin have been relatively ignored[18]. In a trial of stabilizing standard endotoxin by an addition of albumin, we happened to notice that albumin inhibits endotoxin activity in the chromogenic assay system[25]. This observations have led us to hypothesize that albumin may act as a protective protein against endotoxemia. In our preliminary study, we first noted that 250 pg/mL endotoxin lost most of their activity in the presence of albumin at physiological concentrations. Another interesting result was that albumin inhibits LPS-stimulated IL-1 secretion in the macrophage culture system[25]. We further noted that the capacity of albumin to bind exogenous $^3$H-labelled endotoxin decreased in plasma of cirrhotics[18]. In patients with Child A and Child B cirrhosis, the plasma endotoxin inactivating rate was positively correlated to the endotoxin binding capacity of plasma albumin[126]. Albumin has a protective effect against encephalopathy in advanced cirrhosis[127]. Substances which require albumin binding-such as bilirubin, free fatty acids and organic anions[128] markedly increase in the blood in this situation. The increase of these substances may limit endotoxin binding capacity of albumin. In Child C cirrhotics in whom albumin shows very low endotoxin binding capacity, additional microbial loads by acute infection may result in overwhelming endotoxemia and serious clinical results[18] Mechanisms of LPS clearance in the blood and LPS-induced inflammation are summarized in Figure 1.

### How to evaluate BT clinically

As described above, plasma endotoxin assay has been most widely used. Despite the development of various new assay technique based on LAL test, there is no standard accepted method for clinical use. We have established chromogenic LAL test with kinetic analysis and compared various methods of plasma pretreatment, including the dilution and heating method and the perchloric acid method under the strict control by internal endotoxin standard. We have compared endotoxin-specific chromogenic substrate with the conventional chromogenic substrate and could not find out any superiority of the endotoxin-specific test for evaluation of BT. Both tests were well correlated to clinical course and considered to be useful markers. It should be noted that (1-3)-$\beta$-D-glucan powerfully co-stimulate cytokine production (IL-6/IL-8) induced by ligands for TLR1/2, TLR2/6, TLR4, and TLR5[129]. Plasma glucan in patients with bacterial infections, and the low levels of glucan found in normal individuals, may be attributable to movement of glucan from the GI tract into the blood and not necessarily to the presence of a pathogen[130]. Although most quantitative LAL test reacts (1-3)-$\beta$-D-glucan and is not endotoxin-specific, both endotoxin from Gram-negative bacteria and (1-3)-$\beta$-D-glucan from fungus are microbial products which translocate from the intestine. They strongly co-stimulate innate immune system and induce the production of inflammatory mediators. The drawbacks of the tests are complexity of the measurement and difficulty in standardization. I could not recommend other endotoxin assay method with very low sensitivity. It was designed to detect endotoxemia with bacteremia but not suitable for detection of spillover endotoxemia in liver diseases. Endotoxin activity assay[131] using a novel chemiluminescent assay will be evaluated for this purpose. I list up current detection methods of
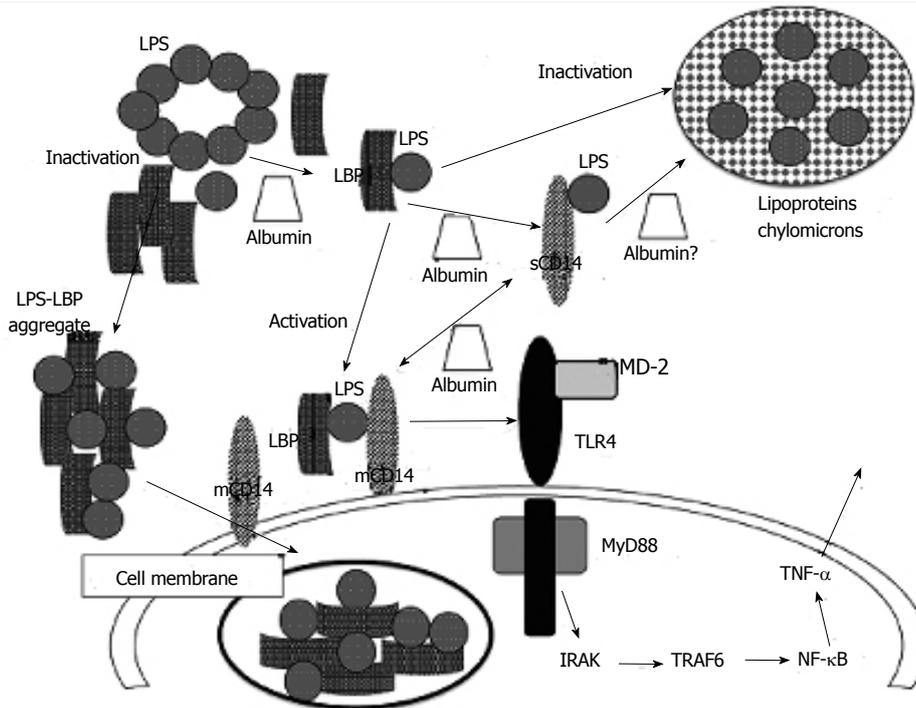
**Figure 1 Mechanism of lipopolysaccharide clearance in the blood and LPS-toll like receptors-MyD88 signal transduction.** LBP enhances cell responses to LPS by accelerating the binding of LPS to CD14. LBP can also inhibit cell responses to LPS; It transfers LPS to plasma lipoproteins and it combines with LPS aggregates to form large LPS-LBP complexes that are internalized[176]. sCD14 can remove, or divert, LPS from mCD14 and transfer it to plasma lipoproteins, where LPS is inactivated[176]. Albumin is essential during the interaction of LBP with LPS aggregate to produce a LBP: LPS aggregate and the efficient transfer of LPS from the aggregate to a molecule of sCD14[177]. Albumin stabilizes LPS: CD14 complexes for cell activation. Mechanism of inhibitory effect of albumin on LPS is still unknown. It may directly inactivate minute amount of LPS and may also enhance LPS transport to lipoproteins. LPS: Lipopolysaccharide; LBP: Lipopolysaccharide binding protein; TNF-α: Tumor necrosis factor α; TLR4: Toll-like receptors 4; NF-κB: Nuclear factor kappa B; TRAF6: TNF receptor-associated factor.

endotoxemia with their pros and cons in Table 1.

LBP can be evaluated as another useful surrogate marker of BT, although both endotoxin and LBP reflect only translocation of *Gram-negative bacilli*[4]. Elevated LBP levels in cirrhosis were related to pro-inflammatory state and haemodynamic derangement, which were shown to be ameliorated by intestinal decontamination with norfloxacin[132]. A prospective study in non-infected cirrhotics with ascites showed that increased serum LBP was the only factor independently associated with first severe bacterial infection in a multivariate analysis[133]. Detection of serum peptidoglycan, a polymer consisting of sugars and amino acids that forms cell wall of gram-positive bacteria, has been considered as a marker of BT in an experimental model of hemorrhagic shock[4,134].

Recently, detection of bacterial DNA (bactDNA) by polymerase chain reaction has been proposed as a surrogate marker for BT. It has been simultaneously detected in blood and ascites in 9 of 28 cirrhotics with culture-negative ascites[135]. Detection of bactDNA in biological fluids in experimental cirrhosis and ascites is associated with its simultaneous presence in MLNs[136]. Bellot *et al*[137] reported that bactDNA (+) patients had significantly lower mean arterial pressure and systemic vascular resistance. The increase in hepatic venous pressure gradient after the test meal significantly correlated with serum bactDNA concentration.

Uniformity of analytical methods is needed to ascertain its real value in clinical setting[4] .

## THERAPEUTIC APPROACH TO LIVER CIRRHOSIS BY MANAGEMENT OF THE GUT-LIVER AXIS

Our hypothesis about the mechanism of endotoxemia and its consequences related to complications in advanced liver cirrhosis are shown in Figure 2. Gram-negative bacteria and endotoxins are more likely than other types of bacteria to stimulate tumor necrosis factor and cytokines that would lead to the production of nitric oxide (NO)[138]. Endotoxemia in relation to bacterial translocation, causes induction of NO synthase leading to increased vascular NO production, which is the primary stimulus for the development of vasodilatation and its accompanying clinical manifestations in cirrhosis[99]. Nitric oxide is also a potent inducer of increased membrane permeability in the vascular endothelium and intestinal mucosa, possibly contributing to bacterial translocation[28,99]. In patients with advanced cirrhosis, there may be a vicious cycle among endotoxemia, induction of NO and increased intestinal permeability, which may further induce derangement of the hyperdynamic circulatory status and renal failure. New clues to improving

| | **Name of the test (manufacturer)** | **Pro** | **Con** |
|---|---|---|---|
| **Table 1 Detection methods of endotoxemia** | | | |
| LAL test | ToxinSensorTM Gel clot Endotoxin Assay Kit (GenScript) PYROGENTTM-5000 LAL Reagent (Lonza) | Good marker of BT | Not specific, react βd-Glucan, plasma preparation difficult |
| Chromogenic substrate assay | Toxicolr test (Seikagaku Corporation) | Good marker of BT available for both end-point assay and kinetic assay | Not specific, react βd-Glucan PCA method: poor endotoxin recovery plasama pretreatment reagent currently unavailable |
| | QCL1000 (BioWhittaker/Cambrex Lonza end-point assay Kinetic-QCL™ Kinetic Chromogenic LAL Assays (Lonza) | Good marker of BT available for both end-point assay and kinetic assay | Not specific, react βd-Glucan |
| | LAL Coatest, S-2423 (Kabi Vitrum Diagnostica, Chromogenix) | Good marker of BT available for both end-point assay and kinetic assay | Not specific, react βd-Glucan, not available now |
| | EndosafePTS (Charles River Lab) | Good marker of BT handy and quick | Not specific, react βd-Glucan (Concomitasnt use of Endosafe PTS glucan assay may be necessary for specificity) |
| | LAL chromogenic endpoint assay Hycult Biotech Mini-LAL assay (Hycult Biotechnology) ToxinSensor™ Chromogenic LAL Endotoxin Assay (GenScript) end-point method Pyrochrome (Associates of Cape Cod Inc) | Good marker of BT available for both end-point assay and kinetic assay | Not specific, react βd-Glucan |
| | | Easy plasma pretreatment (dilution and heatig) available for both end-point assay and kinetic assay | Not specific, react βd-Glucan |
| | Endospecy (Seikagaku Corporation) | Endotoxin-specific available for both end-point assay and kinetic assay | PCA method: poor endotoxin recovery plasama pretreatment reagent currently unavailable New PCA method: plasma pretreatment reagent currently unavailable |
| Turbidometric assay | Limulus ES II test (Wako) PYROSTAR™ ES-F LAL Reagent (Charles River Lab) | Endotoxin-specific | Unnable to detect spillover endotoxemia in cirrhosaas |
| Recombinant factor C (rFC) system assay | Recombinant factor C (rFC) system PyroGene (Lonza) fluorescent EndoLISA Hyglos ELISA EndoZyme® recombinant Factor C (rFC) Assaye HyGlos fluorescent | Endotoxin-specific | Very few reports, usefullness for liver disease unclear |
| Endotoxin activity assay | EAA™ (Spectral Dianostics Inc) | Received FDA clearance rapid diagnostic for endotoxin activity in human whole blood. Higher EAA™ levels are correlated with a higher risk of mortality, as well as an increasing risk for developing sepsis | Addition of methylprednisolone decreased the EAA levels. Indiredt endotoxin assay to reflect the primed state of polymorphonuclear leukocytes |

LAL: Limulus amebocyte lysate; FDA: Food and Drug Administration.

prognosis of advanced liver cirrhosis may be found in better management of gut-liver axis.

### Selective intestinal decontamination
Selective intestinal decontamination (SID) for management of complications of liver cirrhosis has a long and evolving history. At first, the word SID was not used but the trial to eliminate the endogenous source of gram-negative aerobic bacteria was reported early in 1982. Adachi et al[139] reported that oral administration of polymyxin B is useful in the treatment of hyperammonemia and endotoxemia in liver cirrhosis, as a poorly absorbed antibiotic and as an antiendotoxin agent. Similarly, Tarao et al[47] stated that paromomycin sulfate (2 g/d for 4 wk) is effective in the prevention of endotoxemia and the associated renal impairment in cirrhosis. Although the effect of the latter was not reproduced later in patients with alcoholic liver disease[140].

Long-term use of norfloxacin (400 mg/d, mean follow-up period 6.4 ± 0.6 mo) has been reported to eliminate aerobic *gram-negative bacilli* from the fecal flora without significant changes in other microorganisms throughout the study[141]. A double blind, placebo-controlled trial evaluating its efficacy in cirrhotics who recovered from an episode of SBP, revealed a significant reduction of SBP recurrence by the treatement (20% *vs* 68%) at one year of follow-up[141]. In cirrhotic patients with low ascitic fluid protein concentrations (≤ 1 g/dL) or hyperbilirubinemia (> 2.5mg/dL), long-term prophylactic treatment with norfloxacin was also effective in the prevention of the first episode of SBP (1.8% *vs* 16.9%)[142]. However, these promising results were followed by the problem of quinolone-resistant SBP[142,143]. Prior antibiotic therapy and norfloxacin prophylaxis is confirmed to increase the risk of carriage of methicillin-resistant Staphylococcus aureus[144]. After that, primary prophylaxis of SBP has been targeted to high-risk patients. When cirrhotics with low protein ascitic levels (< 1.5 g/dL), advanced
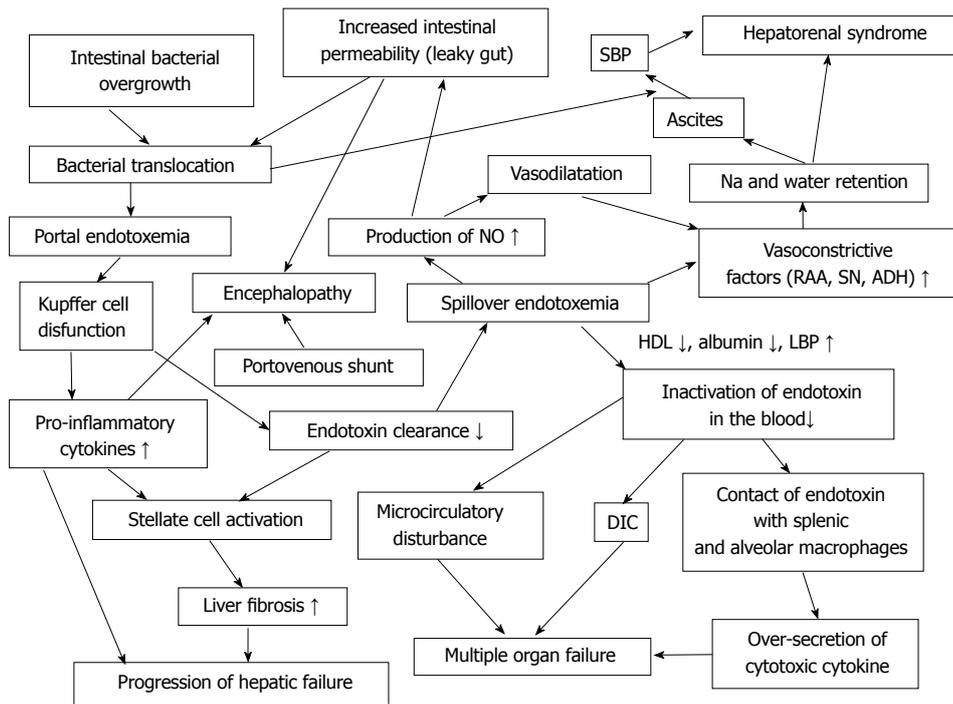
**Figure 2 Mechanism of endotoxemia and its consequences in advanced liver cirrhosis (hypothesis).** Depressed elimination of endotoxin by Kupffer cells (KCs) is considered to induce spillover endotoxemia and processing of endoxin by extrahepatic macrophages which secrete larger amount of TNF than KCs. The excessive cytokine response to endotoxin by splenic and alveolar macrophages may be important in the pathogenesis of ARDS and multiple organ failure. Endotoxemia enhances vascular NO production, which is the primary stimulus for the development of vasodilatation. Enhanced vasoconstrictive factors in response to vasodilatation and endotoxemia are responsible for ascites and hepatorenal syndrome. Hepatic encephalopathy is also closely related to inflammatory reaction attributable to leaky gut amd endotoxemia. RAA: Renin-angiotensin-aldosterone system; SN: Sympathetic nerves; ADH: Antiduretic hormone (vasopressin); SBP: Spontaneous bacterial peritonitis; NO: Nitric oxide; LBP: Lipopolysaccharide binding protein; HDL: High-density lipoprotein.

liver failure (Child-Pugh score $\geq$ 9 points with serum bilirubin level $\geq$ 3 mg/dL) or impaired renal function were selected, primary prophylaxis with norfloxacin reduced the incidence of SBP, delayed the development of hepatorenal syndrome, and improved survival[145].

Rifaximin is a minimally absorbed oral antimicrobial agent that is concentrated in the gastrointestinal tract[146]. It has broad-spectrum *in vitro* activity against gram-positive and gram-negative aerobic and anaerobic enteric bacteria, and has a low risk of inducing bacterial resistance[147-149]. Given its pharmacologic characteristics this drug has been used in the treatment of hepatic encephalopathy. Rifaximin has been compared with neomycin[150], paromomycin[151], and lactulose[152], showing similar results in both clinical improvement and reducing blood ammonia[153]. Recently its effect on advanced liver cirrhosis as SID agent has been intensively studied. Vlachogiannakos *et al*[154] reported that a 4-wk rifaximin regimen significantly ameliorated endotoxemia and lowered hepatic venous pressure gradient in patients with decompensated alcohol-related cirrhosis. Kalambokis *et al*[48] noted that rifaximin treatment reduced cardiac output and increased systemic vascular resistance, glomerular filtration rate and natriuresis, in association with decreases in plasma renin activity, endotoxin, IL-6, and TNF-$\alpha$ levels. These data supported that intestinal decontamination with rifaximin improved systemic hemodynamics and renal function in patients with advanced cirrhosis. Decrease of

ascitic neutrophil count in cirrhotic patients with sterile ascites[155] and improvement of thrombocytopenia[156] ware also reported by the same group. Dănulescu *et al*[157] further reported that rifaximin causes a significant decrease in ascitic neutrophil count, producing a decrease in SBP frequency and improvement of life in cirrhotic patients with refractory ascites.

Several recent studies evaluated its effect on brain function based on the concept of gut-liver-brain axis. Rifaximin is associated with improved cognitive function and endotoxemia in minimal hepatic encephalopathy (MHE), which is accompanied by alteration of gut bacterial linkages with metabolites without significant change in microbial abundance[158]. A significant improvement in cognition including working memory and inhibitory control, and fractional anisotropy without effect on MD or MR spectroscopy, through modulation of fronto-parietal and subcortical activation and connectivity was seen after open-label rifaximin therapy in MHE[159].

### Probiotics
Probiotics, lactose-fermenting *Lactobacilli* and *Bifidobacteria*, have been reported to stabilize mucosal barrier function and modulate the gut microflora, suppressing pathogenic microbial growth[70]. They acidify the gut lumen, compete with pathogenic bacteria for nutrients, and produce antimicrobial substance[70,160,161]. Administration of VSL#3, a probiotic combination

of eight strains of *Lactobacilli*, *Bifidobacteria* and *Streptococcus*, has been reported to reduce oxidative/nitrosative stress parameters in patients with alcoholic liver cirrhosis[162]. Cirrhotic subjects receiving *Escherichia coli Nissle* for 42 d showed an effectiveness in the restoration of normal colonic colonization and a trend of significant lowering of the endotoxemia and improvement of liver functions evaluated by Child-Pugh score[163]. Stadlbauer *et al*[164] proved that probiotics restore neutrophil phagocytic capacity in cirrhosis, possibly by changing IL-10 secretion and TLR4 expression, warranting larger randomized controlled and mechanistic studies. Probiotics are able to decrease the permeability of the intestinal wall, and decrease bacterial translocation and endotoxemia in animal models as well as in clinical studies, which is extremely important in the prevention of complications of liver cirrhosis and infection after liver transplantation[165]. Probiotics could limit oxidative and inflammatory liver damage and, in some situations, improve the histological state[165]. Recent meta-analysis could not confirm that probiotics are effective to hepatic encephalopathy[166]. Further clinical trials are needed to know an ideal probiotic therapy for this purpose[166].

### Synbiotics
Synbiotic treatment was also associated with a significant reduction in endotoxemia[164]. The Child-Pugh functional class improved in nearly 50% of cases[164]. Synbiotic preparation consisting of 4 freeze-dried, non-urease-producing lactic acid bacteria and four fermentable fiber, Synbiotic 2000®, was effective to patients with liver cirrhosis and minimal hepatic encephalopathy[167]. Synbiotic treatment for 30 d significantly increased the fecal content of non-urease-producing *Lactobacillus* species, which was associated with a significant reduction in blood ammonia and endotoxin[167]. An improvement in Child-Pugh class occurred in 47% of patients receiving synbiotic preparation, compared with 29% or 8% of patients receiving fermentable fiber alone or placebo, respectively[167]. For the prevention of infections, this synbiotic regimen (Synbiotic 2000®) was more effective than fiber alone in reducing the incidence of bacterial infections in liver transplant recipients[168]. Additionally, Wan *et al*[169] reported that taurine and oat fiber achieved an additive inhibitory effect on intestinal endotoxin release in a rat liver ischemia/reperfusion model, which might be an effective approach for the treatment of intestinal endotoxemia.

### Prebiotics
Lactitol and lactulose are synthetic non-absorbable disaccharides. They remain undigested until they reach the large bowel, where they are metabolized by colonic bacteria, generating acetic and lactic acids. The resulting lower pH may inhibit urease-producing intestinal bacteria and promote the growth of non-urease-producing *Lactobacilli*[70,170]. Chen *et al*[171]

reported that lactitol increased beneficial bacteria, such as *Bifidobacteria* and *Lactobacilli* with a significant decrease in plasma endotoxin levels in patients with chronic viral hepatic diseases.

### New cocktails and others
Current approaches for hepatic encephalopathy include the use of non-absorbable antibiotics (*i.e.*, neomycin, paromomycin, metronidazole, or rifaximin) and non-absorbable disaccharides[70]. Probiotics may be a promising therapeutic option in the management of hepatic encephalopathy[167,172-174], although we need more clinical studies to get a final conclusion[166]. Some authors have shown that probiotics may positively modulate the gut microflora, reducing the amount of bacterial ammonia reaching the portal vein[70]. The long-term oral administration of *Enterococcus faecium SF 68* was equally effective as lactulose and its effect on mental status persisted longer than lactulose[172]. Oral intake of *Bifidobacterium longum* plus fructo-oligosaccharides for 90 d was also effective in biochemical and neuropsychological tests of cirrhotics with minimal hepatic encephalopathy[175].

## CONCLUSION
It has been proposed that a "leaky gut" may be the cutting edge for the passage of toxins, antigens or bacteria into the body, and may play a pathogenic role in advanced liver cirrhosis and its complications. More attention should be paid to the role of intestinal bacteria and bacterial products in the field of hepatology. The usefulness and the limitations of selective intestinal decontamination should be more clearly defined. Rifaximin may be promising. However, more judicious combinations of probiotics and prebiotics should be explored. Infusion of albumin or HDL in addition to endotoxin adsorption may be theoretically effective for intractable endotoxemia. Moreover, adequate management of the gut-liver axis is even effective for prevention of fibrosis in alcoholic and nonalcoholic steatohepatitis. This seems to be a radical therapy to decrease liver cirrhosis itself. Readers interested in the topics are recommended to read recent excellent reviews[4,70,71,82,103,108]. The research in these fields may open a new possibility in the next decade.

## REFERENCES
1   **Tandon P**, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008; **28**: 26-42 [PMID: 18293275 DOI: 10.1055/s-2008-1040319]

2   **Arvaniti V**, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010; **139**: 1246-1256, 1256e1-e5 [PMID: 20558165]

3   **Campillo B**, Richardet JP, Kheo T, Dupeyron C. Nosocomial spontaneous bacterial peritonitis and bacteremia in cirrhotic

patients: impact of isolate type on prognosis and characteristics of infection. *Clin Infect Dis* 2002; **35**: 1-10 [PMID: 12060868 DOI: 10.1086/340617]

4     **Bellot P**, Francés R, Such J. Pathological bacterial translocation in cirrhosis: pathophysiology, diagnosis and clinical implications. *Liver Int* 2013; **33**: 31-39 [PMID: 23121656 DOI: 10.1111/liv.12021]

5     **Prytz H**, Holst-Christensen J, Korner B, Liehr H. Portal venous and systemic endotoxaemia in patients without liver disease and systemic endotoxaemia in patients with cirrhosis. *Scand J Gastroenterol* 1976; **11**: 857-863 [PMID: 794999]

6     **Tarao K**, So K, Moroi T, Ikeuchi T, Suyama T. Detection of endotoxin in plasma and ascitic fluid of patients with cirrhosis: its clinical significance. *Gastroenterology* 1977; **73**: 539-542 [PMID: 892353]

7     **Clemente C**, Bosch J, Rodés J, Arroyo V, Mas A, Maragall S. Functional renal failure and haemorrhagic gastritis associated with endotoxaemia in cirrhosis. *Gut* 1977; **18**: 556-560 [PMID: 301485 DOI: 10.1136/gut.18.7.556]

8     **Gaeta GB**, Perna P, Adinolfi LE, Utili R, Ruggiero G. Endotoxemia in a series of 104 patients with chronic liver diseases: prevalence and significance. *Digestion* 1982; **23**: 239-244 [PMID: 6183161 DOI: 10.1159/000198756]

9     **Bode C**, Kugler V, Bode JC. Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. *J Hepatol* 1987; **4**: 8-14 [PMID: 3571935 DOI: 10.1016/S0168-8278(87)80003-X]

10    **Scully MF**. Measurement of endotoxaemia by the Limulus test. *Intensive Care Med* 1984; **10**: 1-2 [PMID: 6699263 DOI: 10.1007/BF00258060]

11    **Fulenwider JT**, Sibley C, Stein SF, Evatt B, Nordlinger BM, Ivey GL. Endotoxemia of cirrhosis: an observation not substantiated. *Gastroenterology* 1980; **78**: 1001-1004 [PMID: 6991352]

12    **Iwanaga S**, Morita T, Harada T, Nakamura S, Niwa M, Takada K, Kimura T, Sakakibara S. Chromogenic substrates for horseshoe crab clotting enzyme. Its application for the assay of bacterial endotoxins. *Haemostasis* 1978; **7**: 183-188 [PMID: 658779]

13    **Sturk A**, Joop K, ten Cate JW, Thomas LL. Optimalization of a chromogenic assay for endotoxin in blood. *Prog Clin Biol Res* 1985; **189**: 117-137 [PMID: 4048199]

14    **Friberger P**. The design of a reliable endotoxin test. *Prog Clin Biol Res* 1985; **189**: 139-149 [PMID: 4048200]

15    **Fukui H**, Brauner B, Bode JC, Bode C. Chromogenic endotoxin assay in plasma. Selection of plasma pretreatment and production of standard curves. *J Clin Chem Clin Biochem* 1989; **27**: 941-946 [PMID: 2693575]

16    **Obayashi T**. Addition of perchloric acid to blood samples for colorimetric limulus test using chromogenic substrate: comparison with conventional procedures and clinical applications. *J Lab Clin Med* 1984; **104**: 321-330 [PMID: 6088654]

17    **Tsuji K**, Martin PA, Gaunnac GL. Recovery of endotoxin from human plasma by acid oxidative treatments as monitored by an automated microtiter plate-chromogenic substrate Limulus amebocyte lysate (LAL) assay method. *Prog Clin Biol Res* 1987; **231**: 443-457 [PMID: 3035582]

18    **Fukui H**, Tsujita S, Matsumoto M, Kitano H, Hoppou K, Morimura M, Takaya A, Okamoto S, Tsujii T, Bode C, Bode JC. Endotoxemia in chronic hepatitis and cirrhosis: Epiphenomenon or of pathological relevence? In: Blum H, Bode C, Bode JC, Sartor RB, editors. Gut and the Liver. Dordrecht, Boston, London: Kluwer Academic Publishers, 1988

19    **Fukui H**, Matsumoto M, Tsujita S, Takaya A, Kojima H, Matsumura M, Tsujii T. Plasma endotoxin concentration and endotoxin binding capacity of plasma acute phase proteins in cirrhotics with variceal bleeding: an analysis by new methods. *J Gastroenterol Hepatol* 1994; **9**: 582-586 [PMID: 7532449 DOI: 10.1111/j.1440-1746.1994.tb01565.x]

20    **Bode C**, Fukui H, Bode JC. "Hidden" endotoxin in plasma of patients with alcoholic liver disease. *Eur J Gastroenterol Hepatol*

1993; **5**: 257-262 [DOI: 10.1097/00042737-199304000-00011]

21    **Obayashi T**, Tamura H, Tanaka S, Ohki M, Takahashi S, Arai M, Masuda M, Kawai T. A new chromogenic endotoxin-specific assay using recombined limulus coagulation enzymes and its clinical applications. *Clin Chim Acta* 1985; **149**: 55-65 [PMID: 3896576 DOI: 10.1016/0009-8981(85)90273-6]

22    **Lumsden AB**, Henderson JM, Kutner MH. Endotoxin levels measured by a chromogenic assay in portal, hepatic and peripheral venous blood in patients with cirrhosis. *Hepatology* 1988; **8**: 232-236 [PMID: 3281884 DOI: 10.1002/hep.1840080207]

23    **Tachiyama G**, Sakon M, Kambayashi J, Iijima S, Tsujinaka T, Mori T. Endogenous endotoxemia in patients with liver cirrhosis--a quantitative analysis of endotoxin in portal and peripheral blood. *Jpn J Surg* 1988; **18**: 403-408 [PMID: 3172582 DOI: 10.1007/BF02471464]

24    **Bigatello LM**, Broitman SA, Fattori L, Di Paoli M, Pontello M, Bevilacqua G, Nespoli A. Endotoxemia, encephalopathy, and mortality in cirrhotic patients. *Am J Gastroenterol* 1987; **82**: 11-15 [PMID: 3799574]

25    **Fukui H**, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. *J Hepatol* 1991; **12**: 162-169 [PMID: 2050995 DOI: 10.1016/0168-8278(91)90933-3]

26    **Fukui H**, Matsumoto M, Bode C, Bode JC, Tsujita S, Tsujii T. Endotoxaemia in patients with liver cirrhosis and upper gastrointestinal bleeding: detection by the chromogenic assay with plasma Tween 80 pretreatment. *J Gastroenterol Hepatol* 1993; **8**: 577-581 [PMID: 8280847 DOI: 10.1111/j.1440-1746.1993.tb01656.x]

27    **Vallance P**, Moncada S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? *Lancet* 1991; **337**: 776-778 [PMID: 1706450 DOI: 10.1016/0140-6736(91)91384-7]

28    **Guarner C**, Soriano G, Tomas A, Bulbena O, Novella MT, Balanzo J, Vilardell F, Mourelle M, Moncada S. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. *Hepatology* 1993; **18**: 1139-1143 [PMID: 8225220 DOI: 10.1002/hep.1840180520]

29    **Lin RS**, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, Hsu WC, Huang CC, Wang SS, Lo KJ. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995; **22**: 165-172 [PMID: 7790704 DOI: 10.1016/0168-8278(95)80424-2]

30    **Campillo B**, Bories PN, Benvenuti C, Dupeyron C. Serum and urinary nitrate levels in liver cirrhosis: endotoxemia, renal function and hyperdynamic circulation. *J Hepatol* 1996; **25**: 707-714 [PMID: 8938549 DOI: 10.1016/S0168-8278(96)80242-X]

31    **Lee FY**, Lu RH, Tsai YT, Lin HC, Hou MC, Li CP, Liao TM, Lin LF, Wang SS, Lee SD. Plasma interleukin-6 levels in patients with cirrhosis. Relationship to endotoxemia, tumor necrosis factor-alpha, and hyperdynamic circulation. *Scand J Gastroenterol* 1996; **31**: 500-505 [PMID: 8734349 DOI: 10.3109/00365529609006772]

32    **Bhimani EK**, Serracino-Inglott F, Sarela AI, Batten JJ, Mathie RT. Hepatic and mesenteric nitric oxide synthase expression in a rat model of CCl(4)-induced cirrhosis. *J Surg Res* 2003; **113**: 172-178 [PMID: 12943827]

33    **Rasaratnam B**, Connelly N, Chin-Dusting J. Nitric oxide and the hyperdynamic circulation in cirrhosis: is there a role for selective intestinal decontamination? *Clin Sci* (Lond) 2004; **107**: 425-434 [PMID: 15270715 DOI: 10.1042/CS20040157]

34    **Trebicka J**, Krag A, Gansweid S, Appenrodt B, Schiedermaier P, Sauerbruch T, Spengler U. Endotoxin and tumor necrosis factor-receptor levels in portal and hepatic vein of patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt. *Eur J Gastroenterol Hepatol* 2011; **23**: 1218-1225 [PMID: 21971377 DOI: 10.1097/MEG.0b013e32834a75dc]

35    **Miller AM**, Masrorpour M, Klaus C, Zhang JX. LPS exacerbates endothelin-1 induced activation of cytosolic phospholipase A2 and thromboxane A2 production from Kupffer cells of the prefibrotic rat liver. *J Hepatol* 2007; **46**: 276-285 [PMID: 17161492 DOI:

10.1016/j.jhep.2006.08.026]

36	**Steib CJ**, Hartmann AC, v Hesler C, Benesic A, Hennenberg M, Bilzer M, Gerbes AL. Intraperitoneal LPS amplifies portal hypertension in rat liver fibrosis. *Lab Invest* 2010; **90**: 1024-1032 [PMID: 20212458 DOI: 10.1038/labinvest.2010.60]

37	**Wright G**, Davies NA, Shawcross DL, Hodges SJ, Zwingmann C, Brooks HF, Mani AR, Harry D, Stadlbauer V, Zou Z, Williams R, Davies C, Moore KP, Jalan R. Endotoxemia produces coma and brain swelling in bile duct ligated rats. *Hepatology* 2007; **45**: 1517-1526 [PMID: 17523148 DOI: 10.1002/hep.21599]

38	**Bajaj JS**, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, Sikaroodi M, Gillevet PM. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G168-G175 [PMID: 21940902 DOI: 10.1152/ajpgi.00190.2011]

39	**Coltart I**, Tranah TH, Shawcross DL. Inflammation and hepatic encephalopathy. *Arch Biochem Biophys* 2013; **536**: 189-196 [PMID: 23583306 DOI: 10.1016/j.abb.2013.03.016]

40	**O'Hair DP**, Adams MB, Tunberg TC, Osborn JL. Relationships among endotoxemia, arterial pressure, and renal function in dogs. *Circ Shock* 1989; **27**: 199-210 [PMID: 2650915]

41	**Wang W**, Faubel S, Ljubanovic D, Mitra A, Falk SA, Kim J, Tao Y, Soloviev A, Reznikov LL, Dinarello CA, Schrier RW, Edelstein CL. Endotoxemic acute renal failure is attenuated in caspase-1-deficient mice. *Am J Physiol Renal Physiol* 2005; **288**: F997-F1004 [PMID: 15644489 DOI: 10.1152/ajprenal.00130.2004]

42	**Huang LT**, Hung JF, Chen CC, Hsieh CS, Yu HR, Hsu CN, Tain YL. Endotoxemia exacerbates kidney injury and increases asymmetric dimethylarginine in young bile duct-ligated rats. *Shock* 2012; **37**: 441-448 [PMID: 22193869 DOI: 10.1097/SHK.0b013e318244b787]

43	**Uchihara M**, Izumi N, Sato C, Marumo F. Clinical significance of elevated plasma endothelin concentration in patients with cirrhosis. *Hepatology* 1992; **16**: 95-99 [PMID: 1535610 DOI: 10.1002/hep.1840160117]

44	**Wilkinson SP**, Moodie H, Stamatakis JD, Kakkar VV, Williams R. Endotoxaemia and renal failure in cirrhosis and obstructive jaundice. *Br Med J* 1976; **2**: 1415-1418 [PMID: 795499 DOI: 10.1136/bmj.2.6049.1415]

45	**Wilkinson SP**, Davies MH, Portmann B, Williams R. Renal failure in otherwise uncomplicated acute viral hepatitis. *Br Med J* 1978; **2**: 338-341 [PMID: 687905 DOI: 10.1136/bmj.2.6133.338]

46	**Shah N**, Dhar D, El Zahraa Mohammed F, Habtesion A, Davies NA, Jover-Cobos M, Macnaughtan J, Sharma V, Olde Damink SW, Mookerjee RP, Jalan R. Prevention of acute kidney injury in a rodent model of cirrhosis following selective gut decontamination is associated with reduced renal TLR4 expression. *J Hepatol* 2012; **56**: 1047-1053 [PMID: 22266601 DOI: 10.1016/j.jhep.2011.11.024]

47	**Tarao K**, Moroi T, Hirabayashi Y, Ikeuchi T, Endo O, Takamura Y. Effect of paromomycin sulfate on endotoxemia in patients with cirrhosis. *J Clin Gastroenterol* 1982; **4**: 263-267 [PMID: 7096960 DOI: 10.1097/00004836-198206000-00013]

48	**Kalambokis GN**, Mouzaki A, Rodi M, Pappas K, Fotopoulos A, Xourgia X, Tsianos EV. Rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites. *Clin Gastroenterol Hepatol* 2012; **10**: 815-818 [PMID: 22391344 DOI: 10.1016/j.cgh.2012.02.025]

49	**Such J**, Francés R, Pérez-Mateo M. Nitric oxide in patients with cirrhosis and bacterial infections. *Metab Brain Dis* 2002; **17**: 303-309 [PMID: 12602507 DOI: 10.1023/A: 1021905801401]

50	**Garcia-Martinez R**, Noiret L, Sen S, Mookerjee R, Jalan R. Albumin infusion improves renal blood flow autoregulation in patients with acute decompensation of cirrhosis and acute kidney injury. *Liver Int* 2015; **35**: 335-343 [PMID: 24620819 DOI: 10.1111/liv.12528]

51	**Alqahtani SA**, Fouad TR, Lee SS. Cirrhotic cardiomyopathy. *Semin Liver Dis* 2008; **28**: 59-69 [PMID: 18293277 DOI: 10.1055/s-2008-1040321]

52	**Karagiannakis DS**, Vlachogiannakos J, Anastasiadis G, Vafiadis-Zouboulis I, Ladas SD. Frequency and severity of cirrhotic cardiomyopathy and its possible relationship with bacterial endotoxemia. *Dig Dis Sci* 2013; **58**: 3029-3036 [PMID: 23907333 DOI: 10.1007/s10620-013-2693-y]

53	**Krag A**, Bendtsen F, Henriksen JH, Møller S. Low cardiac output predicts development of hepatorenal syndrome and survival in patients with cirrhosis and ascites. *Gut* 2010; **59**: 105-110 [PMID: 19837678 DOI: 10.1136/gut.2009.180570]

54	**Lata J**. Hepatorenal syndrome. *World J Gastroenterol* 2012; **18**: 4978-4984 [PMID: 23049205 DOI: 10.3748/wjg.v18.i36.4978]

55	**Grace JA**, Angus PW. Hepatopulmonary syndrome: update on recent advances in pathophysiology, investigation, and treatment. *J Gastroenterol Hepatol* 2013; **28**: 213-219 [PMID: 23190201 DOI: 10.1111/jgh.12061]

56	**Zhang HY**, Han de W, Su AR, Zhang LT, Zhao ZF, Ji JQ, Li BH, Ji C. Intestinal endotoxemia plays a central role in development of hepatopulmonary syndrome in a cirrhotic rat model induced by multiple pathogenic factors. *World J Gastroenterol* 2007; **13**: 6385-6395 [PMID: 18081228 DOI: 10.3748/wjg.v13.i25.3500]

57	**Zhang HY**, Han DW, Wang XG, Zhao YC, Zhou X, Zhao HZ. Experimental study on the role of endotoxin in the development of hepatopulmonary syndrome. *World J Gastroenterol* 2005; **11**: 567-572 [PMID: 15641147 DOI: 10.3748/wjg.v11.i4.567]

58	**Añel RM**, Sheagren JN. Novel presentation and approach to management of hepatopulmonary syndrome with use of antimicrobial agents. *Clin Infect Dis* 2001; **32**: E131-E136 [PMID: 11317264 DOI: 10.1086/320149]

59	**Violi F**, Ferro D, Basili S, Saliola M, Quintarelli C, Alessandri C, Cordova C. Association between low-grade disseminated intravascular coagulation and endotoxemia in patients with liver cirrhosis. *Gastroenterology* 1995; **109**: 531-539 [PMID: 7615203 DOI: 10.1016/0016-5085(95)90342-9]

60	**Villa E**, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, Tata C, Zecchini R, Gitto S, Petta S, Lei B, Bernabucci V, Vukotic R, De Maria N, Schepis F, Karampatou A, Caporali C, Simoni L, Del Buono M, Zambotto B, Turola E, Fornaciari G, Schianchi S, Ferrari A, Valla D. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* 2012; **143**: 1253-1260.e1-e4 [PMID: 22819864 DOI: 10.1053/j.gastro.2012.07.018]

61	**Ferro D**, Quintarelli C, Lattuada A, Leo R, Alessandroni M, Mannucci PM, Violi F. High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia. *Hepatology* 1996; **23**: 1377-1383 [PMID: 8675154 DOI: 10.1002/hep.510230613]

62	**Ferro D**, Basili S, Lattuada A, Mantovani B, Bellomo A, Mannucci PM, Violi F. Systemic clotting activation by low-grade endotoxaemia in liver cirrhosis: a potential role for endothelial procoagulant activation. *Ital J Gastroenterol Hepatol* 1997; **29**: 434-440 [PMID: 9494853]

63	**Violi F**, Ferro D, Basili S, Lionetti R, Rossi E, Merli M, Riggio O, Bezzi M, Capocaccia L. Ongoing prothrombotic state in the portal circulation of cirrhotic patients. *Thromb Haemost* 1997; **77**: 44-47 [PMID: 9031447]

64	**Saliola M**, Lorenzet R, Ferro D, Basili S, Caroselli C, Santo AD, Sallese M, Violi F. Enhanced expression of monocyte tissue factor in patients with liver cirrhosis. *Gut* 1998; **43**: 428-432 [PMID: 9863491]

65	**Uemura M**, Fujimura Y, Matsumoto M, Ishizashi H, Kato S, Matsuyama T, Isonishi A, Ishikawa M, Yagita M, Morioka C, Yoshiji H, Tsujimoto T, Kurumatani N, Fukui H. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. *Thromb Haemost* 2008; **99**: 1019-1029 [PMID: 18521503 DOI: 10.1160/TH08-01-0006]

66	**Takaya H**, Uemura M, Fujimura Y, Matsumoto M, Matsuyama T, Kato S, Morioka C, Ishizashi H, Hori Y, Fujimoto M, Tsujimoto T, Kawaratani H, Toyohara M, Kurumatani N, Fukui H. ADAMTS13 activity may predict the cumulative survival of patients with liver cirrhosis in comparison with the Child-Turcotte-Pugh score and the Model for End-Stage Liver Disease score. *Hepatol Res* 2012; **42**: 459-472 [PMID: 22292786 DOI: 10.1111/j.1872-034X.2011.00950.x]

67 **Ishikawa M**, Uemura M, Matsuyama T, Matsumoto M, Ishizashi H, Kato S, Morioka C, Fujimoto M, Kojima H, Yoshiji H, Tsujimoto T, Takimura C, Fujimura Y, Fukui H. Potential role of enhanced cytokinemia and plasma inhibitor on the decreased activity of plasma ADAMTS13 in patients with alcoholic hepatitis: relationship to endotoxemia. *Alcohol Clin Exp Res* 2010; **34** Suppl 1: S25-S33 [PMID: 19094193 DOI: 10.1111/j.1530-0277.2008.00850.x]

68 **Zeuzem S**. Gut-liver axis. *Int J Colorectal Dis* 2000; **15**: 59-82 [PMID: 10855547]

69 **Ilan Y**. Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis. *World J Gastroenterol* 2012; **18**: 2609-2618 [PMID: 22690069 DOI: 10.3748/wjg.v18.i21.2609]

70 **Pinzone MR**, Celesia BM, Di Rosa M, Cacopardo B, Nunnari G. Microbial translocation in chronic liver diseases. *Int J Microbiol* 2012; **2012**: 694629 [PMID: 22848224]

71 **Pijls KE**, Jonkers DM, Elamin EE, Masclee AA, Koek GH. Intestinal epithelial barrier function in liver cirrhosis: an extensive review of the literature. *Liver Int* 2013; **33**: 1457-1469 [PMID: 23879434 DOI: 10.1111/liv.12271]

72 **Bauer TM**, Schwacha H, Steinbrückner B, Brinkmann FE, Ditzen AK, Aponte JJ, Pelz K, Berger D, Kist M, Blum HE. Small intestinal bacterial overgrowth in human cirrhosis is associated with systemic endotoxemia. *Am J Gastroenterol* 2002; **97**: 2364-2370 [PMID: 12358257]

73 **Pérez-Paramo M**, Muñoz J, Albillos A, Freile I, Portero F, Santos M, Ortiz-Berrocal J. Effect of propranolol on the factors promoting bacterial translocation in cirrhotic rats with ascites. *Hepatology* 2000; **31**: 43-48 [PMID: 10613726 DOI: 10.1002/hep.510310109]

74 **Casafont Morencos F**, de las Heras Castaño G, Martín Ramos L, López Arias MJ, Ledesma F, Pons Romero F. Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Dig Dis Sci* 1996; **41**: 552-556 [PMID: 8617135]

75 **Chang CS**, Chen GH, Lien HC, Yeh HZ. Small intestine dysmotility and bacterial overgrowth in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 1998; **28**: 1187-1190 [PMID: 9794900 DOI: 10.1002/hep.510280504]

76 **Sánchez E**, Casafont F, Guerra A, de Benito I, Pons-Romero F. Role of intestinal bacterial overgrowth and intestinal motility in bacterial translocation in experimental cirrhosis. *Rev Esp Enferm Dig* 2005; **97**: 805-814 [PMID: 16438624]

77 **Zhang W**, Gu Y, Chen Y, Deng H, Chen L, Chen S, Zhang G, Gao Z. Intestinal flora imbalance results in altered bacterial translocation and liver function in rats with experimental cirrhosis. *Eur J Gastroenterol Hepatol* 2010; **22**: 1481-1486 [PMID: 20739895 DOI: 10.1097/MEG.0b013e32833eb8b0]

78 **De Minicis S**, Rychlicki C, Agostinelli L, Saccomanno S, Candelaresi C, Trozzi L, Mingarelli E, Facinelli B, Magi G, Palmieri C, Marzioni M, Benedetti A, Svegliati-Baroni G. Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. *Hepatology* 2014; **59**: 1738-1749 [PMID: 23959503 DOI: 10.1002/hep.26695]

79 **Xie YR**, Liu SL, Liu X, Luo ZB, Zhu B, Li ZF, Li LJ, He Y, Jiang L, Li H, Ruan B. Intestinal microbiota and innate immunity-related gene alteration in cirrhotic rats with liver transplantation. *Transplant Proc* 2011; **43**: 3973-3979 [PMID: 22172882 DOI: 10.1016/j.transproceed.2011.08.113]

80 **Artis D**. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol* 2008; **8**: 411-420 [PMID: 18469830 DOI: 10.1038/nri2316]

81 **Bäckhed F**, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005; **307**: 1915-1920 [PMID: 15790844 DOI: 10.1126/science.1104816]

82 **Seo YS**, Shah VH. The role of gut-liver axis in the pathogenesis of liver cirrhosis and portal hypertension. *Clin Mol Hepatol* 2012; **18**: 337-346 [PMID: 23323248 DOI: 10.3350/cmh.2012.18.4.337]

83 **Kalaitzakis E**. Gastrointestinal dysfunction in liver cirrhosis. *World J Gastroenterol* 2014; **20**: 14686-14695 [PMID: 25356031 DOI: 10.3748/wjg.v20.i40.14686]

84 **Choi Y**, Jeon WK, Hwang SJ, Kim BI, Sohn CI, Park DI, Cho YK, Kim HJ, Park JH. The role of the gut barrier function in the

85 **Scarpellini E**, Valenza V, Gabrielli M, Lauritano EC, Perotti G, Merra G, Dal Lago A, Ojetti V, Ainora ME, Santoro M, Ghirlanda G, Gasbarrini A. Intestinal permeability in cirrhotic patients with and without spontaneous bacterial peritonitis: is the ring closed? *Am J Gastroenterol* 2010; **105**: 323-327 [PMID: 19844200 DOI: 10.1038/ajg.2009.558]

86 **Campillo B**, Pernet P, Bories PN, Richardet JP, Devanlay M, Aussel C. Intestinal permeability in liver cirrhosis: relationship with severe septic complications. *Eur J Gastroenterol Hepatol* 1999; **11**: 755-759 [PMID: 10445796 DOI: 10.1097/00042737-199907000-00013]

87 **Pascual S**, Such J, Esteban A, Zapater P, Casellas JA, Aparicio JR, Girona E, Gutiérrez A, Carnices F, Palazón JM, Sola-Vera J, Pérez-Mateo M. Intestinal permeability is increased in patients with advanced cirrhosis. *Hepatogastroenterology* 2003; **50**: 1482-1486 [PMID: 14571769]

88 **Kalaitzakis E**, Johansson JE, Bjarnason I, Björnsson E. Intestinal permeability in cirrhotic patients with and without ascites. *Scand J Gastroenterol* 2006; **41**: 326-330 [PMID: 16497621 DOI: 10.1080/00365520510024278]

89 **Ersöz G**, Aydin A, Erdem S, Yüksel D, Akarca U, Kumanlioglu K. Intestinal permeability in liver cirrhosis. *Eur J Gastroenterol Hepatol* 1999; **11**: 409-412 [PMID: 10321758 DOI: 10.1097/00042737-199904000-00009]

90 **Huglo D**, De Botton S, Canva-Delcambre V, Colombel JF, Wallaert B, Steinling M, Marchandise X. Simultaneous determination of pulmonary and intestinal permeability in patients with alcoholic liver cirrhosis. *Eur J Nucl Med* 2001; **28**: 1505-1511 [PMID: 11685493 DOI: 10.1007/s002590100589]

91 **Zuckerman MJ**, Menzies IS, Ho H, Gregory GG, Casner NA, Crane RS, Hernandez JA. Assessment of intestinal permeability and absorption in cirrhotic patients with ascites using combined sugar probes. *Dig Dis Sci* 2004; **49**: 621-626 [PMID: 15185867 DOI: 10.1023/B: DDAS.0000026307.56909.21]

92 **Dastych M**, Dastych M, Novotná H, Cíhalová J. Lactulose/mannitol test and specificity, sensitivity, and area under curve of intestinal permeability parameters in patients with liver cirrhosis and Crohn's disease. *Dig Dis Sci* 2008; **53**: 2789-2792 [PMID: 18320320]

93 **Perera L**, Shao L, Patel A, Evans K, Meresse B, Blumberg R, Geraghty D, Groh V, Spies T, Jabri B, Mayer L. Expression of nonclassical class I molecules by intestinal epithelial cells. *Inflamm Bowel Dis* 2007; **13**: 298-307 [PMID: 17238179 DOI: 10.1002/ibd.20026]

94 **Hossain MI**, Nahar B, Hamadani JD, Ahmed T, Roy AK, Brown KH. Intestinal mucosal permeability of severely underweight and nonmalnourished Bangladeshi children and effects of nutritional rehabilitation. *J Pediatr Gastroenterol Nutr* 2010; **51**: 638-644 [PMID: 20871416 DOI: 10.1097/MPG.0b013e3181eb3128]

95 **Fleming SC**, Kapembwa MS, Laker MF, Levin GE, Griffin GE. Rapid and simultaneous determination of lactulose and mannitol in urine, by HPLC with pulsed amperometric detection, for use in studies of intestinal permeability. *Clin Chem* 1990; **36**: 797-799 [PMID: 2110873]

96 **Keshavarzian A**, Holmes EW, Patel M, Iber F, Fields JZ, Pethkar S. Leaky gut in alcoholic cirrhosis: a possible mechanism for alcohol-induced liver damage. *Am J Gastroenterol* 1999; **94**: 200-207 [PMID: 9934756 DOI: 10.1111/j.1572-0241.1999.00797.x]

97 **Feld JJ**, Meddings J, Heathcote EJ. Abnormal intestinal permeability in primary biliary cirrhosis. *Dig Dis Sci* 2006; **51**: 1607-1613 [PMID: 16937077]

98 **Parlesak A**, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000; **32**: 742-747 [PMID: 10845660]

99 **Lee S**, Son SC, Han MJ, Kim WJ, Kim SH, Kim HR, Jeon WK, Park KH, Shin MG. Increased intestinal macromolecular permeability and urine nitrite excretion associated with liver

pathophysiology of viral liver cirrhosis. *Hepatogastroenterology* 2011; **58**: 1244-1247 [PMID: 21937387 DOI: 10.5754/hge10338]

cirrhosis with ascites. *World J Gastroenterol* 2008; **14**: 3884-3890 [PMID: 18609714]

100  **Kim BI**, Kim HJ, Park JH, Park DI, Cho YK, Sohn CI, Jeon WK, Kim HS, Kim DJ. Increased intestinal permeability as a predictor of bacterial infections in patients with decompensated liver cirrhosis and hemorrhage. *J Gastroenterol Hepatol* 2011; **26**: 550-557 [PMID: 21332551 DOI: 10.1111/j.1440-1746.2010.06490.x]

101  **Assimakopoulos SF**, Tsamandas AC, Tsiaoussis GI, Karatza E, Triantos C, Vagianos CE, Spiliopoulou I, Kaltezioti V, Charonis A, Nikolopoulou VN, Scopa CD, Thomopoulos KC. Altered intestinal tight junctions' expression in patients with liver cirrhosis: a pathogenetic mechanism of intestinal hyperpermeability. *Eur J Clin Invest* 2012; **42**: 439-446 [PMID: 22023490 DOI: 10.1111/j.1365-2362.2011.02609.x]

102  **Assimakopoulos SF**, Tsamandas AC, Tsiaoussis GI, Karatza E, Zisimopoulos D, Maroulis I, Kontogeorgou E, Georgiou CD, Scopa CD, Thomopoulos KC. Intestinal mucosal proliferation, apoptosis and oxidative stress in patients with liver cirrhosis. *Ann Hepatol* 2013; **12**: 301-307 [PMID: 23396742]

103  **Ridlon JM**, Alves JM, Hylemon PB, Bajaj JS. Cirrhosis, bile acids and gut microbiota: unraveling a complex relationship. *Gut Microbes* 2013; **4**: 382-387 [PMID: 23851335 DOI: 10.4161/gmic.25723]

104  **Bertók L**. Bile acids in physico-chemical host defence. *Pathophysiology* 2004; **11**: 139-145 [PMID: 15561510 DOI: 10.1016/j.pathophys.2004.09.002]

105  **Clements WD**, Parks R, Erwin P, Halliday MI, Barr J, Rowlands BJ. Role of the gut in the pathophysiology of extrahepatic biliary obstruction. *Gut* 1996; **39**: 587-593 [PMID: 8944570]

106  **Lorenzo-Zúñiga V**, Bartolí R, Planas R, Hofmann AF, Viñado B, Hagey LR, Hernández JM, Mañé J, Alvarez MA, Ausina V, Gassull MA. Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats. *Hepatology* 2003; **37**: 551-557 [PMID: 12601352 DOI: 10.1053/jhep.2003.50116]

107  **Guo J**, Friedman SL. Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. *Fibrogenesis Tissue Repair* 2010; **3**: 21 [PMID: 20964825 DOI: 10.1186/1755-1536-3-21]

108  **Seki E**, Schnabl B. Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut. *J Physiol* 2012; **590**: 447-458 [PMID: 22124143 DOI: 10.1113/jphysiol.2011.219691]

109  **Huang H**, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, Rowland CM, Catanese JJ, Leong DU, Sninsky JJ, Layden TJ, Wright TL, White T, Cheung RC. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. *Hepatology* 2007; **46**: 297-306 [PMID: 17461418 DOI: 10.1002/hep.21695]

110  **Paik YH**, Schwabe RF, Bataller R, Russo MP, Jobin C, Brenner DA. Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. *Hepatology* 2003; **37**: 1043-1055 [PMID: 12717385 DOI: 10.1053/jhep.2003.50182]

111  **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332 [PMID: 17952090 DOI: 10.1038/nm1663]

112  **Seki E**, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. *Hepatology* 2008; **48**: 322-335 [PMID: 18506843 DOI: 10.1002/hep.22306]

113  **Messingham KA**, Faunce DE, Kovacs EJ. Alcohol, injury, and cellular immunity. *Alcohol* 2002; **28**: 137-149 [PMID: 12551755 DOI: 10.1016/S0741-8329(02)00278-1]

114  **Szabo G**, Mandrekar P, Girouard L, Catalano D. Regulation of human monocyte functions by acute ethanol treatment: decreased tumor necrosis factor-alpha, interleukin-1 beta and elevated interleukin-10, and transforming growth factor-beta production. *Alcohol Clin Exp Res* 1996; **20**: 900-907 [PMID: 8865966 DOI: 10.1111/j.1530-0277.1996.tb05269.x]

115  **Vreugdenhil AC**, Snoek AM, van 't Veer C, Greve JW, Buurman WA. LPS-binding protein circulates in association with apoB-containing lipoproteins and enhances endotoxin-LDL/VLDL interaction. *J Clin Invest* 2001; **107**: 225-234 [PMID: 11160139

DOI: 10.1172/JCI10832]

116  **Vreugdenhil AC**, Rousseau CH, Hartung T, Greve JW, van 't Veer C, Buurman WA. Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. *J Immunol* 2003; **170**: 1399-1405 [PMID: 12538700]

117  **Berbée JF**, Havekes LM, Rensen PC. Apolipoproteins modulate the inflammatory response to lipopolysaccharide. *J Endotoxin Res* 2005; **11**: 97-103 [PMID: 15949136 DOI: 10.1179/096805105X35215]

118  **Szabo G**, Bala S. Alcoholic liver disease and the gut-liver axis. *World J Gastroenterol* 2010; **16**: 1321-1329 [PMID: 20238398 DOI: 10.3748/wjg.v16.i11.1321]

119  **Roth RI**, Levin FC, Levin J. Distribution of bacterial endotoxin in human and rabbit blood and effects of stroma-free hemoglobin. *Infect Immun* 1993; **61**: 3209-3215 [PMID: 8335351]

120  **Schäfer C**, Parlesak A, Schütt C, Bode JC, Bode C. Concentrations of lipopolysaccharide-binding protein, bactericidal/permeability-increasing protein, soluble CD14 and plasma lipids in relation to endotoxaemia in patients with alcoholic liver disease. *Alcohol Alcohol* 2002; **37**: 81-86 [PMID: 11825862 DOI: 10.1093/alcalc/37.1.81]

121  **Park CT**, Wright SD. Plasma lipopolysaccharide-binding protein is found associated with a particle containing apolipoprotein A-I, phospholipid, and factor H-related proteins. *J Biol Chem* 1996; **271**: 18054-18060 [PMID: 8663389 DOI: 10.1074/jbc.271.30.18054]

122  **Massamiri T**, Tobias PS, Curtiss LK. Structural determinants for the interaction of lipopolysaccharide binding protein with purified high density lipoproteins: role of apolipoprotein A-I. *J Lipid Res* 1997; **38**: 516-525 [PMID: 9101432]

123  **Thabut D**, Tazi KA, Bonnefont-Rousselot D, Aller M, Farges O, Guimont MC, Tellier Z, Guichard C, Ogier-Denis E, Poynard T, Moreau R, Lebrec D. High-density lipoprotein administration attenuates liver proinflammatory response, restores liver endothelial nitric oxide synthase activity, and lowers portal pressure in cirrhotic rats. *Hepatology* 2007; **46**: 1893-1906 [PMID: 17918268 DOI: 10.1002/hep.21875]

124  **Ramírez MJ**, Ibáñez A, Navasa M, Casals E, Morales-Ruiz M, Jiménez W, Arroyo V, Rodés J. High-density lipoproteins reduce the effect of endotoxin on cytokine production and systemic hemodynamics in cirrhotic rats with ascites. *J Hepatol* 2004; **40**: 424-430 [PMID: 15123356 DOI: 10.1016/j.jhep.2003.11.017]

125  **Galbois A**, Thabut D, Tazi KA, Rudler M, Mohammadi MS, Bonnefont-Rousselot D, Bennani H, Bezeaud A, Tellier Z, Guichard C, Coant N, Ogier-Denis E, Moreau R, Lebrec D. Ex vivo effects of high-density lipoprotein exposure on the lipopolysaccharide-induced inflammatory response in patients with severe cirrhosis. *Hepatology* 2009; **49**: 175-184 [PMID: 19053046 DOI: 10.1002/hep.22582]

126  **Fukui H**, Tsujita S, Matsumoto M, Morimura M, Kitano H, Kinoshita K, Kikuchi E, Okamoto Y, Tsujii T. Endotoxin inactivating action of plasma in patients with liver cirrhosis. *Liver* 1995; **15**: 104-109 [PMID: 7791538 DOI: 10.1111/j.1600-0676.1995.tb00115.x]

127  **Zieve L**. Hepatic encephalopathy: Summary of present knowledge with an elaboration on recent developments. In: Popper HS, editor. Progress in Liver Diseases (Volume Vol VI). New York: Grune and Stratton, 1979: 327-341

128  **Sorrentino D**, Potter B, Berk P. From albumin to the cytoplasm: The hepatic uptake of organic anions. In: Popper HS, editor. Progress in Liver Diseases (Volume Vol IX). New York: Grune and Stratton, 1990: 203-235

129  **Kikkert R**, Bulder I, de Groot ER, Aarden LA, Finkelman MA. Potentiation of Toll-like receptor-induced cytokine production by (1--&gt; 3)-beta-D-glucans: implications for the monocyte activation test. *J Endotoxin Res* 2007; **13**: 140-149 [PMID: 17621556 DOI: 10.1177/0968051907080024]

130  **Rice PJ**, Adams EL, Ozment-Skelton T, Gonzalez AJ, Goldman MP, Lockhart BE, Barker LA, Breuel KF, Deponti WK, Kalbfleisch JH, Ensley HE, Brown GD, Gordon S, Williams DL. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *J Pharmacol Exp Ther* 2005; **314**: 1079-1086 [PMID: 15976018 DOI: 10.1124/jpet.105.085415]

131  **Marshall JC**, Walker PM, Foster DM, Harris D, Ribeiro M, Paice

J, Romaschin AD, Derzko AN. Measurement of endotoxin activity in critically ill patients using whole blood neutrophil dependent chemiluminescence. *Crit Care* 2002; **6**: 342-348 [PMID: 12225611]

132   **Albillos A**, de la Hera A, González M, Moya JL, Calleja JL, Monserrat J, Ruiz-del-Arbol L, Alvarez-Mon M. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. *Hepatology* 2003; **37**: 208-217 [PMID: 12500206 DOI: 10.1053/jhep.2003.50038]

133   **Albillos A**, de-la-Hera A, Alvarez-Mon M. Serum lipopolysaccharide-binding protein prediction of severe bacterial infection in cirrhotic patients with ascites. *Lancet* 2004; **363**: 1608-1610 [PMID: 15145636 DOI: 10.1016/S0140-6736(04)16206-5]

134   **Shimizu T**, Tani T, Endo Y, Hanasawa K, Tsuchiya M, Kodama M. Elevation of plasma peptidoglycan and peripheral blood neutrophil activation during hemorrhagic shock: plasma peptidoglycan reflects bacterial translocation and may affect neutrophil activation. *Crit Care Med* 2002; **30**: 77-82 [PMID: 11902292]

135   **Such J**, Francés R, Muñoz C, Zapater P, Casellas JA, Cifuentes A, Rodríguez-Valera F, Pascual S, Sola-Vera J, Carnicer F, Uceda F, Palazón JM, Pérez-Mateo M. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, nonneutrocytic ascites. *Hepatology* 2002; **36**: 135-141 [PMID: 12085357 DOI: 10.1053/jhep.2002.33715]

136   **Guarner C**, González-Navajas JM, Sánchez E, Soriando G, Francés R, Chiva M, Zapater P, Benlloch S, Muñoz C, Pascual S, Balanzó J, Pérez-Mateo M, Such J. The detection of bacterial DNA in blood of rats with CCl4-induced cirrhosis with ascites represents episodes of bacterial translocation. *Hepatology* 2006; **44**: 633-639 [PMID: 16941689 DOI: 10.1002/hep.21286]

137   **Bellot P**, García-Pagán JC, Francés R, Abraldes JG, Navasa M, Pérez-Mateo M, Such J, Bosch J. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* 2010; **52**: 2044-2052 [PMID: 20979050 DOI: 10.1002/hep.23918]

138   **García-Tsao G**. Bacterial translocation: cause or consequence of decompensation in cirrhosis? *J Hepatol* 2001; **34**: 150-155 [PMID: 11211893]

139   **Adachi Y**, Enomoto M, Adachi M, Suwa M, Nagamine Y, Nanno T, Hashimoto T, Inoue H, Yamamoto T. Enteric coated polymyxin B in the treatment of hyperammonemia and endotoxemia in liver cirrhosis. *Gastroenterol Jpn* 1982; **17**: 550-557 [PMID: 6298048]

140   **Bode C**, Schäfer C, Fukui H, Bode JC. Effect of treatment with paromomycin on endotoxemia in patients with alcoholic liver disease--a double-blind, placebo-controlled trial. *Alcohol Clin Exp Res* 1997; **21**: 1367-1373 [PMID: 9394106]

141   **Ginés P**, Rimola A, Planas R, Vargas V, Marco F, Almela M, Forné M, Miranda ML, Llach J, Salmerón JM. Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. *Hepatology* 1990; **12**: 716-724 [PMID: 2210673]

142   **Novella M**, Solà R, Soriano G, Andreu M, Gana J, Ortiz J, Coll S, Sàbat M, Vila MC, Guarner C, Vilardell F. Continuous versus inpatient prophylaxis of the first episode of spontaneous bacterial peritonitis with norfloxacin. *Hepatology* 1997; **25**: 532-536 [PMID: 9049193 DOI: 10.1002/hep.510250306]

143   **Fernández J**, Navasa M, Gómez J, Colmenero J, Vila J, Arroyo V, Rodés J. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002; **35**: 140-148 [PMID: 11786970 DOI: 10.1053/jhep.2002.30082]

144   **Campillo B**, Dupeyron C, Richardet JP. Epidemiology of hospital-acquired infections in cirrhotic patients: effect of carriage of methicillin-resistant Staphylococcus aureus and influence of previous antibiotic therapy and norfloxacin prophylaxis. *Epidemiol Infect* 2001; **127**: 443-450 [PMID: 11811877]

145   **Fernández J**, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, Vila C, Pardo A, Quintero E, Vargas V, Such J, Ginès P, Arroyo V. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterology* 2007; **133**: 818-824 [PMID: 17854593 DOI:

10.1053/j.gastro.2007.06.065]

146   **Bass NM**, Mullen KD, Sanyal A, Poordad F, Neff G, Leevy CB, Sigal S, Sheikh MY, Beavers K, Frederick T, Teperman L, Hillebrand D, Huang S, Merchant K, Shaw A, Bortey E, Forbes WP. Rifaximin treatment in hepatic encephalopathy. *N Engl J Med* 2010; **362**: 1071-1081 [PMID: 20335583 DOI: 10.1056/NEJMoa0907893]

147   **Gerard L**, Garey KW, DuPont HL. Rifaximin: a nonabsorbable rifamycin antibiotic for use in nonsystemic gastrointestinal infections. *Expert Rev Anti Infect Ther* 2005; **3**: 201-211 [PMID: 15918778 DOI: 10.1586/14787210.3.2.201]

148   **Jiang ZD**, DuPont HL. Rifaximin: in vitro and in vivo antibacterial activity--a review. *Chemotherapy* 2005; **51** Suppl 1: 67-72 [PMID: 15855749 DOI: 10.1159/000081991]

149   **Debbia EA**, Maioli E, Roveta S, Marchese A. Effects of rifaximin on bacterial virulence mechanisms at supra- and sub-inhibitory concentrations. *J Chemother* 2008; **20**: 186-194 [PMID: 18467244 DOI: 10.1179/joc.2008.20.2.186]

150   **Di Piazza S**, Gabriella Filippazzo M, Valenza LM, Morello S, Pastore L, Conti A, Cottone S, Pagliaro L. Rifaximine versus neomycin in the treatment of portosystemic encephalopathy. *Ital J Gastroenterol* 1991; **23**: 403-407 [PMID: 1742538]

151   **Testa R**, Eftimiadi C, Sukkar GS, De Leo C, Rovida S, Schito GC, Celle G. A non-absorbable rifamycin for treatment of hepatic encephalopathy. *Drugs Exp Clin Res* 1985; **11**: 387-392 [PMID: 3836135]

152   **Bucci L**, Palmieri GC. Double-blind, double-dummy comparison between treatment with rifaximin and lactulose in patients with medium to severe degree hepatic encephalopathy. *Curr Med Res Opin* 1993; **13**: 109-118 [PMID: 8325041 DOI: 10.1185/03007993091115 39]

153   **Mas A**, Rodés J, Sunyer L, Rodrigo L, Planas R, Vargas V, Castells L, Rodríguez-Martínez D, Fernández-Rodríguez C, Coll I, Pardo A. Comparison of rifaximin and lactitol in the treatment of acute hepatic encephalopathy: results of a randomized, double-blind, double-dummy, controlled clinical trial. *J Hepatol* 2003; **38**: 51-58 [PMID: 12480560 DOI: 10.1016/S0168-8278(02)00350-1]

154   **Vlachogiannakos J**, Saveriadis AS, Viazis N, Theodoropoulos I, Foudoulis K, Manolakopoulos S, Raptis S, Karamanolis DG. Intestinal decontamination improves liver haemodynamics in patients with alcohol-related decompensated cirrhosis. *Aliment Pharmacol Ther* 2009; **29**: 992-999 [PMID: 19210289 DOI: 10.1111/j.1365-2036.2009.03958.x]

155   **Kalambokis GN**, Mouzaki A, Rodi M, Tsianos EV. Rifaximin for the prevention of spontaneous bacterial peritonitis. *World J Gastroenterol* 2012; **18**: 1700-1702 [PMID: 22529702 DOI: 10.3748/wjg.v18.i14.1700]

156   **Kalambokis GN**, Mouzaki A, Rodi M, Tsianos EV. Rifaximin improves thrombocytopenia in patients with alcoholic cirrhosis in association with reduction of endotoxaemia. *Liver Int* 2012; **32**: 467-475 [PMID: 22098272]

157   **Dănulescu RM**, Ciobică A, Stanciu C, Trifan A. The role of rifaximine in the prevention of the spontaneous bacterial peritonitis. *Rev Med Chir Soc Med Nat Iasi* 2013; **117**: 315-320 [PMID: 24340510]

158   **Bajaj JS**, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, Fuchs M, Ridlon JM, Daita K, Monteith P, Noble NA, White MB, Fisher A, Sikaroodi M, Rangwala H, Gillevet PM. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One* 2013; **8**: e60042 [PMID: 23565181 DOI: 10.1371/journal.pone.0060042]

159   **Ahluwalia V**, Wade JB, Heuman DM, Hammeke TA, Sanyal AJ, Sterling RK, Stravitz RT, Luketic V, Siddiqui MS, Puri P, Fuchs M, Lennon MJ, Kraft KA, Gilles H, White MB, Noble NA, Bajaj JS. Enhancement of functional connectivity, working memory and inhibitory control on multi-modal brain MR imaging with Rifaximin in Cirrhosis: implications for the gut-liver-brain axis. *Metab Brain Dis* 2014; **29**: 1017-1025 [PMID: 24590688 DOI: 10.1007/s11011-014-9507-6]

160   **Gorbach SL**. Probiotics and gastrointestinal health. *Am J Gastro-*

*enterol* 2000; **95**: S2-S4 [PMID: 10634218]

161  **Griffiths EA**, Duffy LC, Schanbacher FL, Qiao H, Dryja D, Leavens A, Rossman J, Rich G, Dirienzo D, Ogra PL. In vivo effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. *Dig Dis Sci* 2004; **49**: 579-589 [PMID: 15185861 DOI: 10.1023/B: DDAS.0000026302.92898.ae]

162  **Loguercio C**, Federico A, Tuccillo C, Terracciano F, D'Auria MV, De Simone C, Del Vecchio Blanco C. Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. *J Clin Gastroenterol* 2005; **39**: 540-543 [PMID: 15942443]

163  **Lata J**, Novotný I, Príbramská V, Juránková J, Fric P, Kroupa R, Stibůrek O. The effect of probiotics on gut flora, level of endotoxin and Child-Pugh score in cirrhotic patients: results of a double-blind randomized study. *Eur J Gastroenterol Hepatol* 2007; **19**: 1111-1113 [PMID: 17998837 DOI: 10.1097/MEG.0b013e3282efa40e]

164  **Stadlbauer V**, Mookerjee RP, Hodges S, Wright GA, Davies NA, Jalan R. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. *J Hepatol* 2008; **48**: 945-951 [PMID: 18433921 DOI: 10.1016/j.jhep.2008.02.015]

165  **Lata J**, Jurankova J, Kopacova M, Vitek P. Probiotics in hepatology. *World J Gastroenterol* 2011; **17**: 2890-2896 [PMID: 21734800 DOI: 10.3748/wjg.v17.i24.2890]

166  **McGee RG**, Bakens A, Wiley K, Riordan SM, Webster AC. Probiotics for patients with hepatic encephalopathy. *Cochrane Database Syst Rev* 2011; **(11)**: CD008716 [PMID: 22071855 DOI: 10.1002/14651858.CD008716.pub2]

167  **Liu Q**, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; **39**: 1441-1449 [PMID: 15122774 DOI: 10.1002/hep.20194]

168  **Rayes N**, Seehofer D, Theruvath T, Schiller RA, Langrehr JM, Jonas S, Bengmark S, Neuhaus P. Supply of pre- and probiotics reduces bacterial infection rates after liver transplantation--a randomized, double-blind trial. *Am J Transplant* 2005; **5**: 125-130 [PMID: 15636620 DOI: 10.1111/j.1600-6143.2004.00649.x]

169  **Wan XY**, Luo M, Li XD, He P, Wu MC. Inhibitory effects of taurine and oat fiber on intestinal endotoxin release in rats. *Chem*

*Biol Interact* 2010; **184**: 502-504 [PMID: 20056113 DOI: 10.1016/j.cbi.2009.12.031]

170  **Riggio O**, Varriale M, Testore GP, Di Rosa R, Di Rosa E, Merli M, Romiti A, Candiani C, Capocaccia L. Effect of lactitol and lactulose administration on the fecal flora in cirrhotic patients. *J Clin Gastroenterol* 1990; **12**: 433-436 [PMID: 2398251]

171  **Chen C**, Li L, Wu Z, Chen H, Fu S. Effects of lactitol on intestinal microflora and plasma endotoxin in patients with chronic viral hepatitis. *J Infect* 2007; **54**: 98-102 [PMID: 17049992 DOI: 10.1016/j.jinf.2005.11.013]

172  **Loguercio C**, Abbiati R, Rinaldi M, Romano A, Del Vecchio Blanco C, Coltorti M. Long-term effects of Enterococcus faecium SF68 versus lactulose in the treatment of patients with cirrhosis and grade 1-2 hepatic encephalopathy. *J Hepatol* 1995; **23**: 39-46 [PMID: 8530808 DOI: 10.1016/0168-8278(95)80309-2]

173  **Bajaj JS**, Saeian K, Christensen KM, Hafeezullah M, Varma RR, Franco J, Pleuss JA, Krakower G, Hoffmann RG, Binion DG. Probiotic yogurt for the treatment of minimal hepatic encephalopathy. *Am J Gastroenterol* 2008; **103**: 1707-1715 [PMID: 18691193 DOI: 10.1111/j.1572-0241.2008.01861.x]

174  **Mittal VV**, Sharma BC, Sharma P, Sarin SK. A randomized controlled trial comparing lactulose, probiotics, and L-ornithine L-aspartate in treatment of minimal hepatic encephalopathy. *Eur J Gastroenterol Hepatol* 2011; **23**: 725-732 [PMID: 21646910 DOI: 10.1097/MEG.0b013e32834696f5]

175  **Malaguarnera M**, Greco F, Barone G, Gargante MP, Malaguarnera M, Toscano MA. Bifidobacterium longum with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. *Dig Dis Sci* 2007; **52**: 3259-3265 [PMID: 17393330 DOI: 10.1007/s10620-006-9687-y]

176  **Kitchens RL**, Thompson PA. Modulatory effects of sCD14 and LBP on LPS-host cell interactions. *J Endotoxin Res* 2005; **11**: 225-229 [PMID: 16176659 DOI: 10.1179/096805105X46565]

177  **Gioannini TL**, Zhang D, Teghanemt A, Weiss JP. An essential role for albumin in the interaction of endotoxin with lipopolysaccharide-binding protein and sCD14 and resultant cell activation. *J Biol Chem* 2002; **277**: 47818-47825 [PMID: 12372833 DOI: 10.1074/jbc.M206404200]

# Baishideng®