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EDITORIAL

Gut flora and bacterial translocation in chronic liver disease

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Abstract

Increasing evidence suggests that derangement of gut flora is of substantial clinical relevance to patients with cirrhosis. Intestinal bacterial overgrowth and increased bacterial translocation of gut flora from the intestinal lumen, in particular, predispose to an increased potential for bacterial infection in this group. Recent studies suggest that, in addition to their role in the pathogenesis of overt infective episodes and the clinical consequences of sepsis, gut flora contributes to the pro-inflammatory state of cirrhosis even in the absence of overt infection. Furthermore, manipulation of gut flora to augment the intestinal content of lactic acid-type bacteria at the expense of other gut flora species with more pathogenic potential may favourably influence liver function in cirrhotic patients. Here we review current concepts of the various inter-relationships between gut flora, bacterial translocation, bacterial infection, pro-inflammatory cytokine production and liver function in this group.

Key words: Gut flora; Bacterial translocation; Cirrhosis

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INTRODUCTION

Disturbances of the microecology of the indigenous gut flora are prevalent in patients with chronic liver disease. Significantly increased viable counts of both Gram-positive and negative bacterial species have been recovered from faeces of cirrhotic patients[1]. A high prevalence of small intestinal bacterial overgrowth, in particular, has been demonstrated in this group[2-5]. This has been attributed, at least in part, to a decrease in small intestinal motility associated with increased adrenergic activity and portal hypertension[4-8]. Studies performed in experimental animals demonstrating important effects of gut flora on intestinal motility[9,10] suggest that the development of small intestinal bacterial overgrowth in the setting of intestinal stasis may further impair intestinal motility, thereby creating a self-perpetuating vicious cycle.

In addition to the range of symptoms and consequences of bacterial overgrowth *per se*[11], increasing evidences suggest that derangement of gut flora is of substantial clinical relevance to patients with cirrhosis. Intestinal bacterial overgrowth and increased bacterial translocation of gut flora from the intestinal lumen, in combination with failure of immune defence mechanisms to efficiently remove these translocating microorganisms, predispose to an increased potential for bacterial infection in this group[12]. Impaired immune defences include reduced opsonic activity due to low hepatic synthesis of complement, deranged function of macrophage Fc gamma receptors and reduced phagocytic and killing capacity of neutrophils[13-16]. Impairment of mucosal immunity may also be important. The high prevalence of associated malnutrition in cirrhotic patients[17] exacerbates the potential for infection to occur.

Recent studies suggest that, in addition to their role in the pathogenesis of overt infective episodes and the clinical consequences of sepsis, gut flora contributes to the pro-inflammatory state of cirrhosis even in the absence of overt infection[18]. Furthermore, manipulation of gut-flora using either a Gram-positive synbiotic (probiotic and fermentable fibre) regimen or fermentable fibre alone to augment the intestinal content of lactic acid-type bacteria at the expense of other gut flora species with more pathogenic potential may favourably influence liver function in cirrhotic patients[19]. Here we review current concepts of the various inter-relationships between gut flora, bacterial translocation, bacterial infection, pro-inflammatory cytokine production and liver function in this group.

BACTERIAL TRANSLOCATION IN CIRRHOSIS

Bacterial translocation is defined as the migration of bacteria from the intestinal lumen to mesenteric lymph nodes or other extra-intestinal sites[20]. Gram-negative

members of the Enterobactereaceae family (such as *Escherichia coli* and *Klebsiella spp*), enterococci and other streptococci species are the most effective at bacterial translocation to mesenteric lymph nodes[21]. These bacterial flora, the organisms most commonly implicated in community-acquired infective episodes in patients with cirrhosis[22], can translocate across even histologically normal intestinal mucosa[21,23]. Certain strains of *Escherichia coli* are especially efficient at translocation, possibly as a result of a greater ability to adhere to the intestinal mucosal surface[24]. While obligate anaerobic bacterial flora outnumber aerobic species by more than 100-fold, these flora only rarely translocate from the intestinal lumen[21]. Conversely, anaerobic strains limit the growth of other species with higher translocation potential and their selective elimination has been shown to promote translocation of such aerobic flora[25].

Most data in support of the occurrence of increased bacterial translocation in cirrhosis come from studies performed in experimental animals, in which bacterial translocation was defined by the presence of positive bacteriological cultures for gut flora in surgically-removed mesenteric lymph nodes[26]. These studies demonstrate that the prevalence of bacterial translocation to mesenteric lymph nodes is around 40% in cirrhotic rats with ascites and around 80% in such animals with spontaneous bacterial peritonitis (SBP)[27-31]. The concept of bacterial translocation predisposing to infection in experimental cirrhosis is further supported by data showing that bacteria isolated from mesenteric lymph nodes are genetically identical to strains causing SBP in the same animal[32].

Clinical studies of bacterial translocation in cirrhosis have been limited by the lack of non-invasive methods to detect its presence. Nonetheless, available evidence suggests that increased translocation of gut flora does occur in cirrhotic patients. A high rate of positive mesenteric lymph node cultures for enteric bacteria (30.8%) has been reported in patients with Child-Pugh class C cirrhosis undergoing liver transplantation or hepatic resection, with the incidence in the order of five times higher in this group than in Child-Pugh class A or B patients[33]. In another study, almost 20% of cirrhotic patients were found to have positive mesenteric lymph node cultures following partial hepatectomy, with bacteria responsible for instances of post-operative infection the same as those recovered from mesenteric lymph nodes in most cases[34].

The presence of bacterial DNA in serum and ascitic fluid has recently been proposed as a relatively non-invasive surrogate marker of bacterial translocation in the clinical setting[35]. Using such molecular techniques, bacterial translocation may be present in as many as one third of cirrhotic patients with portal hypertension and culture-negative ascites, with *Escherichia coli* the most frequently identified bacterial species[35]. The clinical relevance of the presence of bacterial DNA in culture-negative ascites has been suggested by the finding of increased local levels of pro-inflammatory cytokines[36], although the validity of this approach and the full clinical relevance of a positive result remain to be firmly established. Increased serum levels of lipopolysaccharide-binding protein, a phenomenon attributed to translocation of bacteria from the gut to the circulation, have been documented in a proportion of cirrhotic patients without overt infection and to predict the later development of severe bacterial infection in this group[37].

Pathogenesis of bacterial translocation in cirrhosis

Intestinal bacterial overgrowth Intestinal bacterial overgrowth is a major factor promoting bacterial translocation[38-41] and available evidence suggests that factors that promote intestinal bacterial overgrowth in particular, such as impaired intestinal motility, are important in facilitating bacterial translocation in cirrhosis. In experimental animals with cirrhosis, strategies to increase the intestinal transit rate, such as treatment with propranolol or cisapride, have been shown to reduce bacterial overgrowth and bacterial translocation[38,40,41]. In the clinical setting, cisapride has been shown to increase small intestinal motility and reduce bacterial overgrowth in patients with cirrhosis, with a trend towards a lower incidence of infection in treated patients[3,42].

Structural changes in intestinal mucosa Most clinical studies performed to investigate structural changes of intestinal mucosa in patients with cirrhosis have focussed on the small intestine. Shorter and thicker microvilli have been described. Morphologically intact tight junctions, which join together the apical poles of enterocytes and represent the first line of mucosal defence against paracellular absorption, have been reported in a small cohort of clinically stable cirrhotic patients with no prior history of infection with gut-derived bacteria[43]. Whether tight junctions are also intact in cirrhotic patients with a history of such infection and those with elevated nitric oxide levels may be more relevant, since nitric oxide, which is discussed in more detail below in relation to the clinical course of SBP, has been shown to reversibly dilate tight junctions in cultured intestinal epithelial cells[44]. Notably, dilatation of the intercellular space below tight junctions, the second line of defence against paracellular absorption, has been documented in patients with cirrhosis[43]. Mucosal alterations attributed to oxidative stress, including disturbed enterocyte mitochondrial function and increased lipid peroxidation of brush border membranes, have been reported in experimental animal models of cirrhosis[45], although the relevance of these changes to the pathogenesis of bacterial translocation is uncertain.

Thick-walled, dilated capillaries along with oedema of the lamina propria, fibromuscular proliferation, a reduced villus/crypt ratio and thickened muscularis mucosa in the small bowel have been found in cirrhotic patients with portal hypertension[46] and it has been proposed that an increased potential for bacterial translocation may exist in this setting[47]. However, in a study of cirrhotic patients with an elevated mean portal venous pressure in the order of 25 mm Hg undergoing liver transplantation, portal venous pressure was not significantly different in 8 cirrhotic patients with bacterial translocation and 71 with negative mesenteric lymph node culture results, implying that additional factors are required for bacterial translocation to occur in this group[33].

Luminal factors contributing to intestinal barrier function Other factors that contribute to the normal intestinal barrier against bacterial translocation (BT), including luminal factors, such as levels of bile acids, secretory immunoglobulin A, mucins, defensins, lysozyme and phopholipase A2, have been little studied in cirrhotic patients. Bile acids exert a trophic effect on intestinal mucosa[48] and inhibit intestinal bacterial overgrowth, especially of Gram-positive species[49]. An increased incidence of bacterial translocation in patients with obstructive jaundice has been reported[50]. However, whether the reduced intestinal luminal levels of bile acids that may be found in cirrhotic patients, as a consequence of reduced hepatic secretion and luminal deconjugation by overgrowth bacteria[12], contribute importantly to the potential for increased bacterial translocation in this group has not been established.

Intestinal immunity Little is known concerning the functional capacity of intestinal immunity in patients with cirrhosis and whether any disturbance of intestinal immune mechanisms contributes importantly to bacterial translocation, especially in the clinical setting. An increased number of intraepithelial lymphocytes with markedly impaired proliferative activity and capacity for production of interferon-γ have been reported in a murine model, with these changes correlating with increased bacterial translocation[51]. Whether this mechanism is important in the clinical situation is unknown. Notably, modest increases in small intestinal intraepithelial lymphocyte counts are found in patients with small intestinal bacterial overgrowth[52], raising the possibility that the increased levels found in cirrhotic animals may be explained on this basis. Whether the number or function of dendritic cells and other mononuclear cells in intestinal mucosa are deranged and contribute to the pathogenesis of bacterial translocation in patients with cirrhosis remains to be defined.

Anatomical site of bacterial translocation in cirrhosis

The anatomical site(s) from which bacterial translocation in cirrhosis occurs remains to be determined. Data in the non-cirrhotic setting suggest that the small intestine rather than the colon may be the major site of bacterial translocation. In particular, experimental studies involving inoculation of various regions of the gastrointestinal tract with equal quantities of *Escherichia coli* suggest that translocation occurs preferentially from the small bowel rather than the colon[53]. Similarly, in experimental animals with impaired intestinal motility, treatment with the prokinetic agent, cisapride, is associated with a reduced rate of bacterial translocation that parallels a reduction in jejunal but not caecal bacterial counts[3].

Intestinal permeability and its relation to bacterial translocation in cirrhosis

Functional studies have demonstrated that increased intestinal permeability, as reflected by dual sugar absorption tests or absorption of other test substances, occurs in patients with cirrhosis, especially in those with advanced liver disease[54-58]. Factors predisposing to this increased permeability in cirrhosis remain to be defined. In particular, any relationships with the structural changes of intestinal mucosa described above are uncertain. Enhanced intestinal permeability, likely via the paracellular route, has been reported in association with small intestinal bacterial overgrowth with Gram-negative gut flora in the non-cirrhotic setting[52], raising the possibility that this entity may at least contribute to the increased intestinal permeability in patients with cirrhosis. The relationship between intestinal permeability and the potential for bacterial translocation is uncertain, as increased permeability results have been found to correlate with a history of septic complications in some, but not all, reports.

GUT FLORA AND BACTERIAL INFECTION IN CIRRHOSIS

Bacterial infection, especially with intestinal-type flora, is a common complication in patients with cirrhosis[59-62]. Spontaneous bacterial peritonitis (SBP), urinary tract infection, respiratory tract sepsis (pneumonia and spontaneous bacterial empyema) and bacteraemia are the most frequent infective complications occurring in this group. The incidence of infection with Gram-positive cocci, in particular, has increased in recent years, with such flora now the most frequent isolates in hospitalised cirrhotic patients with nosocomial infection, especially those admitted to intensive care units, presumably due to the high rate of invasive procedures, including placement of indwelling vascular and bladder catheters, performed in this group[62]. Recent data suggest that between 15% and 35% of cirrhotic patients admitted to hospital develop nosocomial bacterial infection, substantially higher than the rate of hospital-acquired infection in the order of 5% to 7% in the general hospital setting[63].

Complicating infection may have severe adverse clinical consequences in cirrhotic patients. The associated pro-inflammatory response exacerbates hepatic dysfunction, encephalopathy and the haemodynamic disturbances that underlie the development of portal hypertension and hepatorenal syndrome[60,64,65]. Increasing evidence suggests that bacterial infection is a trigger for variceal haemorrhage in patients with cirrhosis[66,67], possibly as a consequence of both activation of hepatic stellate cells, leading to increased intrahepatic vascular resistance, and prostacyclin-related inhibition of platelet aggregation[66]. Conversely, variceal haemorrhage predisposes cirrhotic patients to bacterial infection with gut-derived flora[68-73], setting up a vicious cycle between gastrointestinal bleeding and infection in this group. This is a phenomenon of substantial clinical importance, as complicating infection is independently associated with early mortality in bleeding cirrhotic patients[69,70,72]. Worsening coagulopathy, due to the consumption of clotting factors by the extrinsic coagulation pathway[74] and the production of endogenous heparinoids[75], occurs in patients with cirrhosis and complicating bacterial infection. Sepsis is a common cause of death in cirrhotic patients[60,61,76-78]. The mortality rate associated with bacterial infection in this group is more than twenty times higher than in the general population[79].

SPONTANEOUS BACTERIAL PERITONITIS(SBP)

SBP, an infection of ascitic fluid typically with a single bacterial species in the absence of any other primary intra-abdominal source, is the most characteristic and serious infection occurring in patients with cirrhosis. Prospective studies suggest that SBP, including culture-negative and non-neutrocytic cases, is present in up to 23% of all cirrhotic patients with ascites admitted to hospital[80]. Gram-negative gut flora, especially *Escherichia coli* and *Klebsiella* species, are isolated in approximately 70% of culture-positive cases of community-acquired SBP. Aerobic Gram-positive bacteria belonging to *Streptococcus* and *Staphylococcus* spp constitute most of the remaining isolates. Pathogens belonging to *Aeromonas, Plesimonas, Listeria, Salmonella* and *Neisseria* spp are occasionally responsible. In keeping with their reduced potential for translocation from the intestine discussed above, obligate anaerobes are isolated in fewer than 5% of cases[81]. In hospitalised cirrhotic patients with nosocomial SBP, Gram-positive pathogens are predominant, accounting for over 70% of isolates, with methicillin-resistant *Staphylococcus aureus* accounting for nearly 25% of cases in one recent series[82].

The overall likelihood of a cirrhotic patient with ascites developing SBP at one year is in the order of 10%[80]. Clinical studies have identified several sub-groups of patients with cirrhotic ascites at particularly high risk for SBP. Over 70% of cases occur in those classified as Child-Pugh C[83]. An increased prevalence of SBP has been reported in patients with small intestinal bacterial overgrowth compared to their non-overgrowth counterparts, presumably due to the increased propensity for bacterial translocation[84]. Gastrointestinal haemorrhage is another important precipitant of SBP in this group. Bacteraemia and/or SBP, typically with Gram-negative enteric-type flora, develop within 48 hours of gastrointestinal haemorrhage in nearly 50% of Child-Pugh C patients with ascites[85]. The likelihood of SBP is related to the functional activity of Kupffer cells, which is impaired in patients with advanced liver disease[86]. However, the most powerful predictive factor identified in several series is an ascitic fluid total protein level $\leqslant$10 g/L, which reflects a low complement protein C3 concentration and, hence, opsonisation capacity. Patients with such low levels are at a six- to ten-fold increased risk of a first episode of SBP compared to cirrhotic counterparts with ascitic fluid total protein levels > 10 g/L[87,88].

CLINICAL COURSE

Despite a trend towards earlier diagnosis and commencement of antibiotic treatment, along with overall improvements in the general medical care of cirrhotic patients, the in-hospital mortality rate associated with SBP remains 30-50%[80]. This is largely related to underlying hepatic decompensation and a high prevalence of complicating hepatorenal syndrome. The latter occurs in approximately 30% of patients with SBP, predominantly in those with pre-existing renal impairment, and is progressive despite cure of infection in half of these cases[89]. Ascitic levels of nitric oxide, which are significantly increased in cirrhotic patients with SBP and persist for over two weeks despite appropriate antibiotic treatment[90,91], independently predict renal impairment in this setting[92]. Use of nephrotoxic antibiotics adds to the risk of renal failure in this setting[93]. The development of renal failure is the most important predictor of in-hospital mortality associated with SBP. This is in the order of 50% in those with complicating renal failure compared to only 6% in those without[89]. Albumin infusion improves systemic haemodynamics, prevents renal failure and improves survival compared to antibiotic treatment alone in patients with SBP. Beneficial effects are related to reduction in arterial vasodilatation and improved cardiac function[94]. Other correlates of poor outcome include increased ascitic levels of the pro-inflammatory cytokines, interleukin-6 and tumor necrosis factor-α (TNF-α)[95]. A recent report suggests a higher mortality rate in patients with nosocomial infection with staphylococcal species compared to that in patients with community-acquired SBP[82]. A trend towards a higher mortality rate when infection is with encapsulated strains of *Escherichia coli* associated with tissue invasiveness has also been reported[96].

The medium term prognosis for cirrhotic patients who have recovered from an episode of SBP is similarly poor, with a mortality rate at one year in the order of 30-80%[80]. Approximately 20% of patients who die within one year of an episode of SBP succumb to a further episode of spontaneous peritoneal infection, the remainder dying of causes, such as variceal haemorrhage, hepatorenal syndrome or hepatocellular carcinoma[97-99]. The risk of SBP recurrence within one year ranges from 40-70% and, as for an initial episode, is influenced mainly by the degree of underlying liver dysfunction and the ascitic fluid total protein level[83,98,99]. Survivors of an episode of SBP should be evaluated for liver transplantation in view of the high risk of recurrence and poor overall prognosis.

PROPHYLAXIS

Antibiotic prophylaxis against SBP may be beneficial in three high risk groups of cirrhotic patients, namely those who have survived a previous episode, those with low ascitic fluid total protein levels and those presenting with gastrointestinal haemorrhage[67-71,73,100-103]. Most prophylaxis studies have aimed to reduce or eradicate aerobic Gram-negative bacilli from the intestine using norfloxacin, a poorly absorbed quinolone with activity against these flora. The importance of antibiotic prophylaxis in patients with cirrhosis presenting with variceal haemorrhage is increasingly recognised. Treatment with norfloxacin, 400 mg twice daily either orally or via a nasogastric tube for seven days commencing immediately after emergency endoscopy, was associated with a significantly reduced in-hospital incidence of bacteraemia and/or SBP, especially with aerobic Gram-negative flora, compared to patients receiving no prophylactic antibiotics (3% *vs* 17%)[68]. Ciprofloxacin, 500 mg twice daily either orally or via nasogastric tube for 7 d immediately following endoscopy, has similarly been shown to significantly reduce the incidences of bacteraemia, SBP and urinary tract infection compared to placebo (0% *vs* 23%, 3% *vs*

13% and 5% *vs* 18%, respectively)[103]. Implementation of treatment protocols that incorporate antibiotic prophylaxis led to a fall in post-variceal bacterial infection rates from 38% to 14% over the past two decades in a large French centre[69]. Antibiotic prophylaxis has been shown to reduce the incidence of not only bacterial infection, but also early re-bleeding following variceal haemorrhage, especially in Child-Pugh class C patients, those requiring ventilatory support and those initially treated with balloon tamponade[67,70,71,73].

Several cost analysis studies have shown that prophylactic treatment with norfloxacin in each of these high risk groups is cost effective, as a consequence of the reduced incidence of SBP and its associated resource utilisation[67,104,105]. Short-term prophylaxis with trimethoprim-sulphamethoxazole is also cost-effective, especially in those at high risk for SBP[105]. Most studies report a reduction in overall mortality associated with prophylactic antibiotic treatment, although a statistically significant survival benefit has been more difficult to demonstrate. This is not surprising, as the likelihood of dying as a result of progressive liver failure, variceal haemorrhage, hepatocellular carcinoma or other causes is unaffected by the use of prophylactic antibiotics. More recently, a meta-analysis of 13 randomized controlled trials has suggested that antibiotic prophylaxis of hospitalized cirrhotic in-patients is efficacious in reducing the relative risk of in-hospital death (relative risk of dying 0.70), irrespective of underlying risk factors[107]. In addition, a meta-analysis of 534 cirrhotic patients with gastrointestinal bleeding has shown that short-term antibiotic prophylaxis significantly increases short-term survival rates by a mean 9% in this setting[107].

A particular concern, especially with long-term antibiotic prophylaxis, is the potential for the development of infection with antibiotic-resistant bacteria. Initial studies of norfloxacin prophylaxis against SBP reported a low incidence of infection with quinolone-resistant Gram-negative bacterial species. More recent studies, however, suggest an increased prevalence of infection with such microorganisms. Over 20% of cases of SBP due to *Escherichia coli* were with a norfloxacin-resistant strain in a recent series from Spain[108], while 50% of cases of culture-positive SBP in patients receiving long-term norfloxacin prophylaxis and 16% of instances in patients not treated with this drug were caused by norfloxacin-resistant Gram-negative bacteria in another Spanish report[62]. A high rate of SBP due to trimethoprim-sulphamethoxazole-resistant Gram-negative flora in excess of 40% was also documented in norfloxacin-treated patients. An increased proportion of infections with Gram-positive bacterial species has also been reported in this setting, including instances of severe hospital-acquired staphylococcal infections[109]. An increased incidence of carriage of methicillin-resistant *Staphylococcus aureus* has also been documented in cirrhotic patients treated with norfloxacin prophylaxis[110].

Such experiences highlight the need for non-antibiotic-based strategies to prevent intestinal bacterial overgrowth, bacterial translocation and SBP in patients with cirrhosis. As alluded to earlier, a six-month trial of the pro-motility agent, cisapride, was associated with improved small intestinal motility, reversal of bacterial overgrowth and a tendency towards a reduced incidence of infection with gut-derived flora in a cohort of cirrhotic patients[3,42]. Unfortunately, cisapride has been withdrawn from use in some countries because of potential for cardiac arrhythmia. Alternatively, treatment with either symbiotics (in the form of four species of lactic acid bacteria and fermentable fibre) or fermentable fibre alone has recently been shown to significantly reduce viable counts of potentially pathogenic Gram-positive and Gram-negative gut flora in patients with cirrhosis[19]. In another study, a lower rate of post-operative bacterial infection was documented in liver transplant recipients treated with an early enteral supply of a synbiotic regimen that included *Lactobacillus plantarum* and fermentable fibre than in patients receiving selective intestinal decontamination[111]. A follow-up randomised double-blind trial in liver transplant recipients showed that early enteral nutrition supplemented with a mixture of lactic acid bacteria and fermentable fibre significantly reduced the incidence of post-operative bacterial infection compared to supplementation with fermentable fibre alone[112]. Based on these reports, the possible use of synbiotics for prophylaxis against SBP in cirrhotic patients warrants investigation. Conversely, probiotic treatment with a lactobacillus strain without additional fermentable fibre was ineffective in preventing bacterial translocation and SBP in rats with experimental cirrhosis, despite successful intestinal colonisation[113].

GUT FLORA AND PRO-INFLAMMATORY STATE OF CIRRHOSIS IN ABSENCE OF OVERT INFECTION

Pro-inflammatory cytokines, such as TNF-α, have been shown to be critically involved in the development and/or exacerbation of liver injury in animal models[115,116]. Activation of macrophages by endotoxin, a cell wall component of Gram-negative bacteria, plays a key role in the over-production of TNF- α and liver injury in these settings[117,118]. Increased translocation from the gut lumen and reduced hepatic clearance have each been proposed to predispose to endotoxaemia in this situation[119,120]. In the clinical setting, increased circulating levels of both endotoxin and pro-inflammatory cytokines have been documented in patients with chronic liver disease, even in the absence of overt infection[120-125]. Based on experiences in experimental animals, it has, until recently, been assumed that endotoxaemia causes the raised pro-inflammatory cytokine levels in this group[121,126-128]. However, a significant correlation between circulating endotoxin and pro-inflammatory cytokine levels has generally not been show n[18,120,121,124,126], raising the possibility that, unlike in animal models, stimuli other than endotoxin may be important.

Recent analyses of the expression of Toll-like receptors (TLRs) in patients with cirrhosis have helped to clarify this issue. These receptors are the human homologues of the Drosophila Toll protein and comprise a family of at least ten transmembrane receptor proteins, the intracellular domains of which show distinct sequence homology with the interleukin-1 receptor. TLRs play a critical role in the

induction of innate immunity to microbial pathogens via recognition of conserved molecular patterns. In particular, it is known that TLR4, in association with CD14 and MD-2, is responsible for signal transduction leading to TNF-α production in response to endotoxin. In contrast, TLR2 is required for signaling in response to a number of Gram-positive microbial stimuli, including whole bacteria and cell wall components, such as peptidoglycan and lipoteichoic acid[129-131]. Indeed, significantly increased expression on peripheral blood mononuclear cells (PBMCs) of TLR2 but not of TLR4 has recently been demonstrated in patients with cirrhosis but no overt infection[18]. Furthermore, PBMC expression of TLR2 but not that of TLR4 was shown to correlate significantly with circulating levels of both TNF-α and anti-inflammatory soluble TNF receptors[18]. These novel findings suggest that signaling via TLR2 but not TLR4 contributes to the increased circulating levels of TNF-α levels found in cirrhosis and imply, contrary to previous assumptions, an important role for Gram-positive microbial stimuli rather than endotoxin in this process. This contention is supported by *in vitro* PBMC stimulation data consistent with presensitisation by Gram-positive microbial stimuli but not endotoxin *in vivo*. In addition, supplementation of cirrhotic patients with a synbiotic regimen including four Gram-positive gut flora and fermentable fibre led to further increases in PBMC expression of TLR2 and circulating TNF-α levels in most cases, suggesting that Gram-positive stimuli derived from the gut, in particular, may be important in promoting the increased circulating levels of both pro-inflammatory and anti-inflammatory TNF-related molecules found in patients with cirrhosis[18].

GUT FLORA AND HEPATIC FUNCTION IN CIRRHOSIS

Reversal of minimal hepatic encephalopathy has been reported in 50% of predominantly Child-Pugh class B or C cirrhotic patients treated with a lactic acid bacteria-based synbiotic regimen for 30 d, an efficacy comparable to that of treatment with lactulose[19]. In addition, the Child-Pugh class improves in nearly 50% of initially Child-Pugh class B or C synbiotic-treated patients, a proportion significantly higher than that in placebo-treated counterparts (8%). Treatment is associated with a significant increase in the faecal content of lactobacilli at the expense of potentially pathogenic Gram-positive and negative bacterial species. Bacterial overgrowth is also reversed and improvements in minimal hepatic encephalopathy and the Child-Pugh class occurs in 50% and 29%, respectively, of cirrhotic patients treated with fermentable fibre alone. Both in synbiotic- and fermentable fibre alone-treated patients, improvement in the Child-Pugh class occurs as a result of significant improvements in the serum bilirubin and albumin levels and in prothrombin activity. Significantly reduced hepatic necro-inflammatory activity, as reflected by serial ALT levels, is documented in both groups. Improvement in clearance of indocyanine green has also been reported in cirrhotic patients treated with synbiotics[132]. These findings are in keeping with reported experiences in experimental animals' suggesting that oral supplementation with Gram-positive probiotics can protect against hepatocellular damage. In particular, rats fed lactobacilli have been shown to be protected against alcohol-induced liver damage[133], while studies of probiotic use in a murine model of non-alcoholic fatty liver disease have found reductions in the intra-hepatic expression of various molecular markers of inflammation, including nuclear factor kappa-B and TNF-α[134,135].

REFERENCES

1 **Floch MH**, Katz J, Conn HO. Qualitative and quantitative relationships of the fecal flora in cirrhotic patients with portal systemic encephalopathy and following portacaval anastomosis. *Gastroenterology* 1970; **59**: 70-75
2 **Bauer TM**, Schwacha H, Steinbruckner 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 endotoxaemia. *Am J Gastroenterol* 2002; **97**: 2364-2370
3 **Pardo A,** Bartoli R, Lorenzo-Zuniga V, Planas R, Vinado B, Riba J, Cabre E, Santos J, Luque T, Ausina V, Gassull MA. Effect of cisapride on intestinal bacterial overgrowth and bacterial translocation in cirrhosis. *Hepatology* 2000; **31**: 858-863
4 **Bauer TM**, Steinbruckner B, Brinkmann FE, Ditzen AK, Schwacha H, Aponte JJ, Pelz K, Kist M, Blum HE. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. *Am J Gastroenterol* 2001; **96**: 2962-2967
5 **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
6 **Chesta J**, Defilippi C, Defilippi C. Abnormalities in proximal small bowel motility in patients with cirrhosis. *Hepatology* 1993; **17**: 828-832
7 **Stewart JJ**, Battarbee HD, Farrar GE, Betzing KW. Intestinal myoelectrical activity and transit time in chronic portal hypertension. *Am J Physi*ol 1992; **263**: G474-G479
8 **Sadik R,** Abrahamsson H, Bjornsson E, Gunnarsdottir A, Stotzer PO. Etiology of portal hypertension may influence gastrointestinal transit. *Scand J Gastroenterol* 2003; **38**: 1039-1044
9 **Husebye E**, Hellstrom PM, Sundler F, Chen J, Midtvedt T. Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G368-G380
10 **Liu MT,** Rothstein JD, Gershon MD, Kirchgessner AL. Glutamatergic enteric neurons. *J Neurosci* 1997; **17**: 4764-4784
11 **Riordan SM**. A critical appraisal of diagnostic tests for small intestinal bacterial overgrowth. *Gastroenterology International* 1996; **9**: 110-118
12 **Wiest R**, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005; **41**: 422-433
13 **Gomez F**, Ruiz P, Schreiber AD. Impaired function of macrophage Fc gamma receptors and bacterial infection in alcoholic cirrhosis. *N Engl J Med* 1994; **331**: 1122-1128
14 **Hassner A**, Kletter Y, Jedvab M, Aronson M, Shibolet S. Impaired monocyte function in liver cirrhosis. *Lancet* 1979; **1**: 329-330
15 **Ono Y,** Watanabe T, Matsumoto K, Ito T, Kunii O, Goldstein E. Opsonophagocytic dysfunction in patients with liver cirrhosis and low responses to tumor necrosis factor-alpha and lipopolysaccharide in patients' blood. *J Infect Chemother* 2004; **10**: 200-207
16 **Rajkovic IA**, Williams R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity in alcoholic cirrhosis and hepatitis. *Hepatology* 1986; **6**: 252-262
17 **Riordan SM**, Williams R. Nutrition and liver transplantation. *J Hepatol* 1999; **31**; 955-962
18 **Riordan SM**, Skinner N, Nagree A, McCallum H, McIver CJ,

Kurtovic J, Hamilton JA, Bengmark S, Williams R, Visvanathan K. Peripheral blood mononuclear cell expression of toll-like receptors and relation to cytokine levels in cirrhosis. *Hepatology* 2003; **37**: 1154-1164

19 **Liu Q,** Duan ZP, Ha da K, 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

20 **Berg RD**, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. *Infect Immun* 1979; **23**: 403-411

21 **Steffen EK**, Berg RD, Deitch EA. Comparison of translocation rates of various indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node. *J Infect Dis* 1988; **157**: 1032-1038

22 **Garcia-Tsao G**. Spontaneous bacterial peritonitis. *Gastroenterol Clin North Am* 1992; **21**: 257-275

23 **Wells CL**. Colonization and translocation of intestinal bacterial flora. *Transplant Proc* 1996; **28**: 2653-2656

24 **Ljungdahl M**, Lundholm M, Katouli M, Rasmussen I, Engstrand L, Haglund U. Bacterial translocation in experimental shock is dependent on the strains in the intestinal flora. *Scand J Gastroenterol* 2000; **35**: 389-397

25 **Wells CL**, Maddaus MA, Reynolds CM, Jechorek RP, Simmons RL. Role of anaerobic flora in the translocation of aerobic and facultatively anaerobic intestinal bacteria. *Infect Immun* 1987; **55**: 2689-2694

26 **Garcia-Tsao G**, Wiest R. Gut microflora in the pathogenesis of the complications of cirrhosis. *Best Pract Res Clin Gastroenterol* 2004; **18**: 353-372

27 **Llovet JM,** Bartoli R, Planas R, Cabre E, Jimenez M, Urban A, Ojanguren I, Arnal J, Gassull MA. Bacterial translocation in cirrhotic rats. Its role in the development of spontaneous bacterial peritonitis. *Gut* 1994; **35**: 1648-1652

28 **Runyon BA**, Squier S, Borzio M. Translocation of gut bacteria in rats with cirrhosis to mesenteric lymph nodes partially explains the pathogenesis of spontaneous bacterial peritonitis. *J Hepatol* 1994; **21**: 792-796

29 **Guarner C**, Runyon BA, Young S, Heck M, Sheikh MY. Intestinal bacterial overgrowth and bacterial translocation in cirrhotic rats with ascites. *J Hepatol* 1997; **26**: 1372-1378

30 **Garcia-Tsao G**, Lee FY, Barden GE, Cartun R, West AB. Bacterial translocation to mesenteric lymph nodes is increased in cirrhotic rats with ascites. *Gastroenterology* 1995; **108**: 1835-1841

31 **Runyon BA**, Borzio M, Young S, Squier SU, Guarner C, Runyon MA. Effect of selective bowel decontamination with norfloxacin on spontaneous bacterial peritonitis, translocation, and survival in an animal model of cirrhosis. *Hepatology* 1995; **21**: 1719-1724

32 **Llovet JM**, Bartoli R, March F, Planas R, Vinado B, Cabre E, Arnal J, Coll P, Ausina V, Gassull MA. Translocated intestinal bacteria cause spontaneous bacterial peritonitis in cirrhotic rats: molecular epidemiologic evidence. *J Hepatol* 1998; **28**: 307-313

33 **Cirera I**, Bauer TM, Navasa M, Vila J, Grande L, Taura P, Fuster J, Garcia-Valdecasas JC, Lacy A, Suarez MJ, Rimola A, Rodes J. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001; **34**: 32-37

34 **Yeh DC**, Wu CC, Ho WM, Cheng SB, Lu IY, Liu TJ, Peng FK. Bacterial translocation after cirrhotic liver resection: a clinical investigation of 181 patients. *J Surg Res* 2003; **111**: 209-214

35 **Such J**, Frances R, Munoz C, Zapater P, Casellas JA, Cifuentes A, Rodriguez-Valera F, Pascual S, Sola-Vera J, Carnicer F, Uceda F, Palazon JM, Perez-Mateo M. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, nonneutrocytic ascites. *Hepatology* 2002; **36**: 135-141

36 **Frances R**, Munoz C, Zapater P, Uceda F, Gascon I, Pascual S, Perez-Mateo M, Such J. Bacterial DNA activates cell mediated immune response and nitric oxide overproduction in peritoneal macrophages from patients with cirrhosis and ascites. *Gut* 2004; **53**: 860-864

37 **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

38 **Zhang SC**, Wang W, Ren WY, Zhou K, He BM, Zhu WN. Effects of cisapride on intestinal bacterial and endotoxin translocation in cirrhotic rats. *Zhonghua Ganzangbing Zazhi* 2003; **11**: 539-541

39 **Zhang S,** Wang W, Ren W, Dai Q, He B, Zhou K. Effects of lactulose on intestinal endotoxin and bacterial translocation in cirrhotic rats. *Chin Med J (Engl)* 2003; **116**: 767-771

40 **Zhang SC**, Wang W, Ren WY, He BM, Zhou K, Zhu WN. Effect of cisapride on intestinal bacterial and endotoxin translocation in cirrhosis. *World J Gastroenterol* 2003; **9**: 534-538

41 **Perez-Paramo M**, Munoz 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

42 **Madrid AM**, Hurtado C, Venegas M, Cumsille F, Defilippi C. Long-Term treatment with cisapride and antibiotics in liver cirrhosis: effect on small intestinal motility, bacterial overgrowth, and liver function. *Am J Gastroenterol* 2001; **96**: 1251-1255

43 **Such J**, Guardiola JV, de Juan J, Casellas JA, Pascual S, Aparicio JR, Sola-Vera J, Perez-Mateo M. Ultrastructural characteristics of distal duodenum mucosa in patients with cirrhosis. *Eur J Gastroenterol Hepatol* 2002; **14**: 371-376

44 **Salzman AL**, Menconi MJ, Unno N, Ezzell RM, Casey DM, Gonzalez PK, Fink MP. Nitric oxide dilates tight junctions and depletes ATP in cultured Caco-2BBe intestinal epithelial monolayers. *Am J Physiol* 1995; **268**: G361-G373

45 **Ramachandran A**, Prabhu R, Thomas S, Reddy JB, Pulimood A, Balasubramanian KA. Intestinal mucosal alterations in experimental cirrhosis in the rat: role of oxygen free radicals. *Hepatology* 2002; **35**: 622-629

46 **Misra V**, Misra SP, Dwivedi M, Gupta SC. Histomorphometric study of portal hypertensive enteropathy. *Am J Clin Pathol* 1997; **108**: 652-657

47 **Hashimoto N**, Ohyanagi H. Effect of acute portal hypertension on gut mucosa. *Hepatogastroenterology* 2002; **49**: 1567-1570

48 **Levi AC,** Borghi F, Petrino R, Bargoni A, Fronticelli CM, Gentilli S. Modifications of the trophism of intestinal mucosa after intestinal and bilio-pancreatic diversion in the rat. *Ital J Gastroenterol* 1991; **23**: 202-207

49 **Simon GL**, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology* 1984; **86**: 174-193

50 **Sakrak O**, Akpinar M, Bedirli A, Akyurek N, Aritas Y. Short and long-term effects of bacterial translocation due to obstructive jaundice on liver damage. *Hepatogastroenterology* 2003; **50**: 1542-1546

51 **Inamura T**, Miura S, Tsuzuki Y, Hara Y, Hokari R, Ogawa T, Teramoto K, Watanabe C, Kobayashi H, Nagata H. Ishii H. Alteration of intestinal intraepithelial lymphocytes and increased bacterial translocation in a murine model of cirrhosis. *Immunol Lett* 2003; **90**: 3-11

52 **Riordan SM,** McIver CJ, Thomas DH, Duncombe VM, Bolin TD, Thomas MC. Luminal bacteria and small-intestinal permeability. *Scand J Gastroenterol* 1997; **32**: 556-563

53 **Koh IH,** Guatelli R, Montero EF, Keller R, Silva MH, Goldenberg S, Silva RM. Where is the site of bacterial translocation--small or large bowel? *Transplant Proc* 1996; **28**: 2661

54 **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

55 **Pascual S**, Such J, Esteban A, Zapater P, Casellas JA, Aparicio JR, Girona E, Gutierrez A, Carnices F, Palazon JM, Sola-Vera J, Perez-Mateo M. Intestinal permeability is increased in patients with advanced cirrhosis. *Hepatogastroenterology* 2003; **50**: 1482-1486

56 **Di Leo V**, Venturi C, Baragiotta A, Martines D, Floreani A. Gastroduodenal and intestinal permeability in primary biliary cirrhosis. *Eur J Gastroenterol Hepatol* 2003; **15**: 967-973

57 **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
58 **Ersoz G**, Aydin A, Erdem S, Yuksel D, Akarca U, Kumanlioglu K. Intestinal permeability in liver cirrhosis. *Eur J Gastroenterol Hepatol* 1999; **11**: 409-412
59 **Planas R,** Balleste B, Alvarez MA, Rivera M, Montoliu S, Galeras JA, Santos J, Coll S, Morillas RM, Sola R. Natural history of decompensated hepatitis C virus-related cirrhosis. A study of 200 patients. *J Hepatol* 2004; **40**: 823-830
60 **Strauss E**, Gomes de Sa Ribeiro Mde F. Bacterial infections associated with hepatic encephalopathy: prevalence and outcome. *Ann Hepatol* 2003; **2**: 41-45
61 **Yoneyama K**, Miyagishi K, Kiuchi Y, Shibata M, Mitamura K. Risk factors for infections in cirrhotic patients with and without hepatocellular carcinoma. *J Gastroenterol* 2002; **37**: 1028-1034
62 **Fernandez J**, Navasa M, Gomez J, Colmenero J, Vila J, Arroyo V, Rodes J. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002; **35**: 140-148
63 **Navasa M**, Fernandez J, Rodes J. Bacterial infections in liver cirrhosis. *Ital J Gastroenterol Hepatol* 1999; **31**: 616-625
64 **Riordan SM**, Williams R. Mechanisms of hepatocyte injury, multiorgan failure, and prognostic criteria in acute liver failure. *Semin Liver Dis* 2003; **23**: 203-215
65 **Kantrow SP,** Taylor DE, Carraway MS, Piantadosi CA. Oxidative metabolism in rat hepatocytes and mitochondria during sepsis. *Arch Biochem Biophys* 1997; **345**: 278-288
66 **Goulis J**, Patch D, Burroughs AK. Bacterial infection in the pathogenesis of variceal bleeding. *Lancet* 1999; **353**: 139-142
67 **Soriano G**, Guarner C, Tomas A, Villanueva C, Torras X, Gonzalez D, Sainz S, Anguera A, Cusso X, Balanzo J. Norfloxacin prevents bacterial infection in cirrhotics with gastrointestinal hemorrhage. *Gastroenterology* 1992; **103**: 1267-1272
68 **Carbonell N**, Pauwels A, Serfaty L, Fourdan O, Levy VG, Poupon R. Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology* 2004; **40**: 652-659
69 **Pohl J**, Pollmann K, Sauer P, Ring A, Stremmel W, Schlenker T. Antibiotic prophylaxis after variceal hemorrhage reduces incidence of early rebleeding. *Hepatogastroenterology* 2004; **51**: 541-546
70 **Zhao C,** Chen SB, Zhou JP, Xiao W, Fan HG, Wu XW, Feng GX, He WX. Prognosis of hepatic cirrhosis patients with esophageal or gastric variceal hemorrhage: multivariate analysis. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 416-419
71 **Deschenes M**, Villeneuve JP. Risk factors for the development of bacterial infections in hospitalized patients with cirrhosis. *Am J Gastroenterol* 1999; **94**: 2193-2197
72 **Schachter M.** Heredofamilial tremors and sporadic "essential" tremors in the child and adolescent. *Pediatrie* 1975; **30**: 401-411
73 **Goulis J**, Armonis A, Patch D, Sabin C, Greenslade L, Burroughs AK. Bacterial infection is independently associated with failure to control bleeding in cirrhotic patients with gastrointestinal hemorrhage. *Hepatology* 1998; **27**: 1207-1212
74 **Plessier A,** Denninger MH, Consigny Y, Pessione F, Francoz C, Durand F, Francque S, Bezeaud A, Chauvelot-Moachon L, Lebrec D, Valla DC, Moreau R. Coagulation disorders in patients with cirrhosis and severe sepsis. *Liver Int* 2003; **23**: 440-448
75 **Montalto P**, Vlachogiannakos J, Cox DJ, Pastacaldi S, Patch D, Burroughs AK. Bacterial infection in cirrhosis impairs coagulation by a heparin effect: a prospective study. *J Hepatol* 2002; **37**: 463-470
76 **Jarcuska P,** Veseliny E, Orolin M, Takacova V, Hancova M. Infectious complications in patients with liver cirrhosis. *Klin Mikrobiol Infekc Lek* 2004; **10**: 176-180
77 **Brann OS.** Infectious complications of cirrhosis. *Curr Gastroenterol Rep* 2001; **3**: 285-292
78 **Borzio M,** Salerno F, Piantoni L, Cazzaniga M, Angeli P, Bissoli F, Boccia S, Colloredo-Mels G, Corigliano P, Fornaciari G, Marenco G, Pistara R, Salvagnini M, Sangiovanni A. Bacterial infection in patients with advanced cirrhosis: a multicentre prospective study. *Dig Liver Dis* 2001; **33**: 41-48
79 **Vilstrup H.** Cirrhosis and bacterial infections. *Rom J Gastroenterol* 2003; **12**: 297-302
80 **Navasa M**, Rodes J. Bacterial infections in cirrhosis. *Liver Int* 2004; **24**: 277-280
81 **Hoefs JC,** Canawati HN, Sapico FL, Hopkins RR, Weiner J, Montgomerie JZ. Spontaneous bacterial peritonitis. *Hepatology* 1982; **2**: 399-407
82 **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
83 **Andreu M**, Sola R, Sitges-Serra A, Alia C, Gallen M, Vila MC, Coll S, Oliver MI. Risk factors for spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Gastroenterology* 1993; **104**: 1133-1138
84 **Morencos FC,** de las Heras Castano G, Martin Ramos L, Lopez Arias MJ, Ledesma F, Pons Romero F. Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Dig Dis Sci* 1995; **40**: 1252-1256
85 **Bleichner G,** Boulanger R, Squara P, Sollet JP, Parent A. Frequency of infections in cirrhotic patients presenting with acute gastrointestinal haemorrhage. *Br J Surg* 1986; **73**: 724-726
86 **Bolognesi M**, Merkel C, Bianco S, Angeli P, Sacerdoti D, Amodio P, Gatta A. Clinical significance of the evaluation of hepatic reticuloendothelial removal capacity in patients with cirrhosis. *Hepatology* 1994; **19**: 628-634
87 **Runyon BA**. Low-protein-concentration ascitic fluid is predisposed to spontaneous bacterial peritonitis. *Gastroenterology* 1986; **91**: 1343-1346
88 **Llach J**, Rimola A, Navasa M, Gines P, Salmeron JM, Gines A, Arroyo V, Rodes J. Incidence and predictive factors of first episode of spontaneous bacterial peritonitis in cirrhosis with ascites: relevance of ascitic fluid protein concentration. *Hepatology* 1992; **16**: 724-727
89 **Follo A,** Llovet JM, Navasa M, Planas R, Forns X, Francitorra A, Rimola A, Gassull MA, Arroyo V, Rodes J. Renal impairment after spontaneous bacterial peritonitis in cirrhosis: incidence, clinical course, predictive factors and prognosis. *Hepatology* 1994; **20**: 1495-1501
90 **Jimenez W**, Ros J, Morales-Ruiz M, Navasa M, Sole M, Colmenero J, Sort P, Rivera F, Arroyo V, Rodes J. Nitric oxide production and inducible nitric oxide synthase expression in peritoneal macrophages of cirrhotic patients. *Hepatology* 1999; **30**: 670-676
91 **Bories PN,** Campillo B, Azaou L, Scherman E. Long-lasting NO overproduction in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 1997; **25**: 1328-1333
92 **Such J**, Hillebrand DJ, Guarner C, Berk L, Zapater P, Westengard J, Peralta C, Soriano G, Pappas J, Frances R, Munoz C, Runyon BA. Nitric oxide in ascitic fluid is an independent predictor of the development of renal impairment in patients with cirrhosis and spontaneous bacterial peritonitis. *Eur J Gastroenterol Hepatol* 2004; **16**: 571-577
93 **Hampel H,** Bynum GD, Zamora E, El-Serag HB. Risk factors for the development of renal dysfunction in hospitalized patients with cirrhosis. *Am J Gastroenterol* 2001; **96**: 2206-2210
94 **Fernandez J**, Navasa M, Garcia-Pagan JC, G-Abraldes J, Jimenez W, Bosch J, Arroyo V. Effect of intravenous albumin on systemic and hepatic hemodynamics and vasoactive neurohormonal systems in patients with cirrhosis and spontaneous bacterial peritonitis. *J Hepatol* 2004; **41**: 384-390
95 **Propst T**, Propst A, Herold M, Schauer G, Judmaier G, Braunsteiner H, Stoffler G, Vogel W. Spontaneous bacterial peritonitis is associated with high levels of interleukin-6 and its secondary mediators in ascitic fluid. *Eur J Clin Invest* 1993; **23**: 832-836
96 **Soriano G**, Coll P, Guarner C, Such J, Sanchez F, Prats G, Vilardell F. Escherichia coli capsular polysaccharide and spontaneous bacterial peritonitis in cirrhosis. *Hepatology* 1995; **21**: 668-673
97 **Runyon BA,** McHutchison JG, Antillon MR, Akriviadis EA, Montano AA. Short-course versus long-course antibiotic treatment of spontaneous bacterial peritonitis. A randomized

controlled study of 100 patients. *Gastroenterology* 1991; **100**: 1737-1742
98 **Kurtovic J**, Riordan SM, Williams R. Liver transplantation for hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2005; **19**: 147-160
99 **McHutchison JG**, Runyon BA. Spontaneous bacterial peritonitis. In: Surawicz C, Owen CS (eds). *Gastrointestinal and Hepatic Infections*. Philadeplphia: WB Saunders Co; 1995; 455-475
100 **Gines P**, Rimola A, Planas R, Vargas V, Marco F, Almela M, Forne M, Miranda ML, Llach J, Salmeron JM. Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. *Hepatology* 1990; **12**: 716-724
101 **Soriano G**, Guarner C, Teixido M, Such J, Barrios J, Enriquez J, Vilardell F. Selective intestinal decontamination prevents spontaneous bacterial peritonitis. *Gastroenterology* 1991; **100**: 477-481
102 **Novella M**, Sola R, Soriano G, Andreu M, Gana J, Ortiz J, Coll S, Sabat 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
103 **Hsieh WJ**, Lin HC, Hwang SJ, Hou MC, Lee FY, Chang FY, Lee SD. The effect of ciprofloxacin in the prevention of bacterial infection in patients with cirrhosis after upper gastrointestinal bleeding. *Am J Gastroenterol* 1998; **93**: 962-966
104 **Younossi ZM**, McHutchison JG, Ganiats TG. An economic analysis of norfloxacin prophylaxis against spontaneous bacterial peritonitis. *J Hepatol* 1997; **27**: 295-298
105 **Inadomi J**, Sonnenberg A. Cost-analysis of prophylactic antibiotics in spontaneous bacterial peritonitis. *Gastroenterology* 1997; **113**: 1289-1294
106 **Soares-Weiser K**, Brezis M, Tur-Kaspa R, Paul M, Yahav J, Leibovici L. Antibiotic prophylaxis of bacterial infections in cirrhotic inpatients: a meta-analysis of randomized controlled trials. *Scand J Gastroenterol* 2003; **38**: 193-200
107 **Bernard B**, Grange JD, Khac EN, Amiot X, Opolon P, Poynard T. Antibiotic prophylaxis for the prevention of bacterial infections in cirrhotic patients with gastrointestinal bleeding: a meta-analysis. *Hepatology* 1999; **29**: 1655-1661
108 **Cereto F**, Molina I, Gonzalez A, Del Valle O, Esteban R, Guardia J, Genesca J. Role of immunosuppression in the development of quinolone-resistant Escherichia coli spontaneous bacterial peritonitis and in the mortality of E. coli spontaneous bacterial peritonitis. *Aliment Pharmacol Ther* 2003; **17**: 695-701
109 **Campillo B**, Dupeyron C, Richardet JP, Mangeney N, Leluan G. Epidemiology of severe hospital-acquired infections in patients with liver cirrhosis: effect of long-term administration of norfloxacin. *Clin Infect Dis* 1998; **26**: 1066-1070
110 **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
111 **Rayes N,** Seehofer D, Hansen S, Boucsein K, Muller AR, Serke S, Bengmark S, Neuhaus P. Early enteral supply of lactobacillus and fiber versus selective bowel decontamination: a controlled trial in liver transplant recipients. *Transplantation* 2002; **74**: 123-127
112 **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
113 **Bauer TM**, Fernandez J, Navasa M, Vila J, Rodes J. Failure of Lactobacillus spp. to prevent bacterial translocation in a rat model of experimental cirrhosis. *J Hepatol* 2002; **36**: 501-506
114 **Iimuro Y,** Gallucci RM, Luster MI, Kono H, Thurman RG. Antibodies to tumor necrosis factor alfa attenuate hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat. *Hepatology* 1997; **26**: 1530-1537
115 **Yin M**, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, Thurman RG. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology* 1999; **117**: 942-952
116 **Enomoto N**, Ikejima K, Bradford BU, Rivera CA, Kono H, Goto M, Yamashina S, Schemmer P, Kitamura T, Oide H, Takei Y, Hirose M, Shimizu H, Miyazaki A, Brenner DA, Sato N, Thurman RG. Role of Kupffer cells and gut-derived endotoxins in alcoholic liver injury. *J Gastroenterol Hepatol* 2000; **15 Suppl**: D20-D25
117 **French SW.** Intragastric ethanol infusion model for cellular and molecular studies of alcoholic liver disease. *J Biomed Sci* 2001; **8**: 20-27
118 **Nanji AA,** Khettry U, Sadrzadeh SM, Yamanaka T. Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E2, leukotriene B4, and thromboxane B2. *Am J Pathol* 1993; **142**: 367-373
119 **Rivera CA,** Bradford BU, Seabra V, Thurman RG. Role of endotoxin in the hypermetabolic state after acute ethanol exposure. *Am J Physiol* 1998; **275**: G1252-G1258
120 **Khoruts A**, Stahnke L, McClain CJ, Logan G, Allen JI. Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. *Hepatology* 1991; **13:** 267-276
121 **von Baehr V**, Docke WD, Plauth M, Liebenthal C, Kupferling S, Lochs H, Baumgarten R, Volk H-D. Mechanisms of endotoxin tolerance in patients with alcoholic liver cirrhosis: role of interleukin 10, interleukin 1 receptor antagonist, and soluble tumour necrosis factor receptors as well as effector cell desensitisation. *Gut* 2000; **47**: 281-287
122 **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
123 **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
124 **Chan CC,** Hwang SJ, Lee FY, Wang SS, Chang FY, Li CP, Chu CJ, Lu RH, Lee SD. Prognostic value of plasma endotoxin levels in patients with cirrhosis. *Scand J Gastroenterol* 1997; **32**: 942-946
125 **Hanck C**, Manigold T, Bocker U, Kurimoto M, Kolbel CB, Singer MV, Rossol S. Gene expression of interleukin 18 in unstimulated peripheral blood mononuclear cells of patients with alcoholic cirrhosis. *Gut* 2001; **49**: 106-111
126 **Tilg H,** Wilmer A, Vogel W, Herold M, Nolchen B, Judmaier G, Huber C. Serum levels of cytokines in chronic liver diseases. *Gastroenterology* 1992; **103**: 264-274
127 **Schafer C**, Schips I, Landig J, Bode JC, Bode C. Tumor-necrosis-factor and interleukin-6 response of peripheral blood monocytes to low concentrations of lipopolysaccharide in patients with alcoholic liver disease. *Z Gastroenterol* 1995; **33**: 503-508
128 **Deviere J**, Content J, Denys C, Vandenbussche P, Le Moine O, Schandene L, Vaerman JP, Dupont E. Immunoglobulin A and interleukin 6 form a positive secretory feedback loop: a study of normal subjects and alcoholic cirrhotics. *Gastroenterology* 1992; **103**: 1296-1301
129 **Medzhitov R,** Janeway C Jr. Innate immunity. *N Engl J Med* 2000; **343**: 338-344
130 **Yoshimura A,** Lien E, Ingalls RR, Tuomanen E, Dziarski R, Golenbock D. Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J Immunol* 1999; **163**: 1-5
131 **Akira S**, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001; **2**: 675-680
132 **Kurtovic J**, Ruettimann U, Adamson H, Bihari D, Bengmark S, Williams R, Riordan SM. Improvement in indocyanine green clearance following synbiotic treatment in cirrhosis. *Gut* 2003; **52 Suppl 1**: A3
133 **Nanji AA,** Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). *Proc Soc Exp Biol Med* 1994; **205**: 243-247

134 **Li Z**, Yang S, Lin H, Huang J, Watkins PA, Moser AB, Desimone C, Song XY, Diehl AM. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003; **37**: 343-350
135 **Solga SF**. Probiotics can treat hepatic encephalopathy. *Med Hypotheses* 2003; **61**: 307-313

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