Bacterial infection, especially with intestinal-type bacterial flora, is a common complication in patients with cirrhosis [1–4]. Spontaneous bacterial peritonitis (SBP), urinary tract infection, pneumonia, spontaneous bacterial empyema and bacteraemia are the most frequent infective complications 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 cirrhotics with nosocomial infection, especially those admitted to intensive care units, presumably due to the high rate of requirement for invasive procedures in this group [4]. Recent data suggest that between 15% and 35% of cirrhotic patients admitted to hospital develop nosocomial bacterial infection, substantially higher than the infection rate in the order of 5% to 7% in the general hospital patient setting [5]. Excluding sepsis related to invasive procedures in hospitalised patients, several factors likely predispose to the increased potential for bacterial infection in patients with cirrhosis. Increasing evidence points to a key role for bacterial translocation of intestinal flora from the intestinal lumen, in combination with failure of anti-bacterial defence mechanisms to efficiently clear these translocating microorganisms [6]. The latter 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 [7–10]. The high prevalence of associated malnutrition in cirrhotic patients [11] exacerbates the potential for infection. Impairment of mucosal immunity may also be important [12].

Complicating bacterial infection may have severe adverse clinical consequences in cirrhotic patients. The associated pro-inflammatory cytokine response exacerbates hepatic dysfunction, encephalopathy and the haemodynamic disturbances that underlie the development of portal hypertension and hepatorenal syndrome [2,6,13]. Increasing evidence suggests that bacterial infection is an important trigger for variceal re-bleeding in patients with cirrhosis [14–16], possibly as a consequence of activation of hepatic stellate cells, leading to increased intrahepatic vascular resistance, worsening of splanchnic vasodilatation with increased portal venous flow and pressure and precipitation or exacerbation of coagulopathy resulting from prostacyclin-related inhibition of platelet aggregation, the consumption of clotting factors by the extrinsic coagulation pathway and the production of endogenous heparinoids [14,17,18]. Variceal haemorrhage in turn predisposes to bacterial infection with gut-derived flora [19–24], setting up a vicious cycle between gastrointestinal bleeding and bacterial infection in this group. This is a phenomenon of substantial clinical importance, as complicating infection has recently been shown to be independently associated with early mortality in bleeding cirrhotic patients [20,21,23]. Sepsis is a common cause of death in patients with cirrhosis [2,3,25–27], with the mortality rate associated with bacterial infection in this group more than 20 times higher than that in the general population [28]. Recent studies suggest that, in addition to their role in the pathogenesis of overt infective episodes, the intestinal flora or their cellular components may contribute to cytokine production in cirrhosis even in the absence of overt infection [29]. Furthermore, treatment with either a Gram-positive synbiotic (probiotic and fermentable fibre) regimen, probiotics alone or fermentable fibre alone, with the aim of augmenting the intestinal content of lactic acid-type bacteria at the expense of other bacterial species with more pathogenic potential, has been
shown to improve liver function in cirrhotic patients [30,31]. Here we review current concepts regarding inter-relationships between the intestinal flora, bacterial translocation, risk of bacterial infection, pro-inflammatory cytokine production and liver function in this group.

1. 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 [32]. Gram-negative members of the Enterobacteriaceae family (such as *Escherichia coli* and *Klebsiella* spp.), enterococci and other streptococci species are the most effective at bacterial translocation to mesenteric lymph nodes [33]. These bacterial flora, the organisms most commonly implicated in community-acquired infective episodes in patients with cirrhosis [34], can translocate across even histologically normal intestinal mucosa [33,35]. Certain strains of *E. coli* are especially efficient at translocation, possibly as a result of their greater ability to adhere to the intestinal mucosal surface [36]. While obligate anaerobic bacterial flora outnumber aerobic species by more than 100-fold, these flora only rarely translocate from the intestinal lumen [33]. Conversely, these anaerobes limit the growth of other species with higher translocation potential and their selective elimination has been shown to promote translocation of such aerobic flora [37].

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 enteric flora in surgically removed mesenteric lymph nodes [38]. The prevalence of bacterial translocation to mesenteric lymph nodes is around 40% in cirrhotic rats with ascites and around 80% in such animals with SBP [39–43]. 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 [44].

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 (over 30%) 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 [45]. In another study, almost 20% of cirrhotic patients were found to have positive mesenteric lymph node cultures for enteric bacteria following partial hepatectomy, with bacteria responsible for instances of post-operative infection the same as those recovered from mesenteric lymph nodes in most cases [46]. The presence of bacterial DNA in serum and ascitic fluid has recently been proposed as a marker of bacterial translocation [47]. Using such molecular techniques, bacterial translocation may be present in as many as one-third of cirrhotic patients with non-neutrocytic and culture-negative ascites, with *E. coli* the most frequently identified bacterial species [47].

1.1. Pathogenesis

Several factors contribute to the pathogenesis of bacterial translocation in cirrhosis (Table 1), the most important of which is intestinal bacterial overgrowth [48–51]. As in experimental animals, intestinal bacterial overgrowth occurs commonly in patients with cirrhosis, especially in those with more advanced degrees of hepatic functional impairment. Significantly increased viable fecal counts of both Gram-positive and Gram-negative bacterial species have been reported [30,52]. A high prevalence of small intestinal bacterial overgrowth (SIBO) has been demonstrated [53–57]. Small intestinal hypomotility, which is especially pronounced in those patients with more advanced degrees of hepatic dysfunction [58] and reversible after successful liver transplantation [59], is a major factor predisposing to SIBO in this group. The pathogenesis of small intestinal hypomotility in cirrhosis is multifactorial, with increased adrenergic activity, enhanced nitric oxide production and structural intestinal damage due to oxidative stress and portal hypertension, especially prior to the development of collaterals, each considered important [54,55,60–64]. Studies performed in experimental animals demonstrating an influence of gut flora on intestinal motility [65,66] suggest that the development of SIBO in the setting of intestinal stasis may further impair intestinal motility, thereby creating a vicious cycle. Indeed, in the clinical setting, small intestinal motility is especially reduced in those cirrhotic patients with SIBO [62], while eradication of SIBO significantly improves small intestinal motility [66]. In experimental animals with cirrhosis,

| Table 1 |
| Factors that may contribute to the pathogenesis of bacterial translocation in patients with cirrhosis |
|-----------------|------------------|
| Intestinal bacterial overgrowth |
| Structural abnormalities of intestinal wall |
| Disturbance of luminal factors |
| Bile acids |
| Secretory immunoglobulin A |
| Mucins |
| Defensins |
| Lysozyme |
| Phospholipase A2 |
| Disturbance of mucosal immunity |
strategies to improve intestinal motility, such as treat-
ment with propranolol or cisapride, have been shown
to reduce bacterial overgrowth and bacterial translo-
caiton [48,50,51]. 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 bacterial infections
in treated patients [53,67].

Structural abnormalities in the intestinal wall may
also contribute to an increased potential for bacterial
translocation in cirrhosis. Most clinical studies per-
formed to investigate this issue 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 repre-
sent the first line of mucosal defence against paracellular
absorption, have been reported in a small cohort of clin-
ically stable cirrhotic patients with no prior history of
infection with gut-derived bacteria [68]. Whether tight
junctions are also intact in cirrhotic patients with a
history of infection with enteric bacteria and those with
elevated nitric oxide levels may be more relevant, since
nitric oxide, the relationship of which to the intestinal
flora in cirrhosis is discussed below, has been shown to
reversibly dilate tight junctions in cultured intestinal epi-
thelial cells [69]. Notably, dilatation of the intercellular
space below tight junctions, the second line of defence
against paracellular absorption, has been documented
in patients with cirrhosis [67]. Mucosal alterations
attributed to oxidative stress, including disturbed
enterocyte mitochondrial function and increased lipid
peroxidation of brush border membranes, have also
been reported in experimental animal models of cirrho-
sis [70–72], with a recent study in cirrhotic rats suggest-
ing that anti-oxidant treatment may prevents both
intestinal mucosal damage and bacterial translocation
[72].

Functional studies have demonstrated that increased
intestinal permeability, as reflected by dual sugar
absorption tests or absorption of other test substances,
occur in patients with cirrhosis, especially in those with
advanced liver disease [73–77,73]. Factors predisposing
to this increased permeability in cirrhosis remain to be
defined. In particular, any relationship with the structur-
al changes of intestinal mucosa described above is uncer-
tain. Enhanced intestinal permeability, likely via the
paracellular route, has been reported in association with
SIBO with Gram-negative gut flora in the non-cirrhotic
setting [78], raising the possibility that this entity may at
least contribute to the increased intestinal permeability
in patients with cirrhosis, as suggested in experimental
animals [50]. The relationship between intestinal perme-
ability 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.

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 [79] and it has been
proposed that an increased potential for bacterial trans-
location may exist in this setting [80]. 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 bacte-
rial translocation and 71 counterparts with negative
mesenteric lymph node cultures, implying that addition-
al factors are required for bacterial translocation to
occur in this group [45]. Conversely, a sustained reduct-
ion in portal hypertension, as reflected by a decrease
in the hepatic-portal venous gradient by >20% of base-
line or to <12 mm Hg following treatment with pro-
pranolol with or without isosorbide mononitrate, has
recently been shown to reduce the risk of infective com-
lications in cirrhotic patients, presumably by reducing
bacterial translocation [81]. Notably, portal hyperten-
sion induced acutely, rather than chronic portal
hypertension, has been associated with bacterial translo-
cation in experimental rodent models.

Luminal factors that contribute to the normal intesti-
nal barrier against bacterial translocation in health,
including bile acids, secretory immunoglobulin A, muc-
ins, defensins, lysozyme and phospholipase A2, have
been little studied in patients with cirrhosis. Bile acids
both exert a trophic effect on intestinal mucosa [82]
and inhibit intestinal bacterial overgrowth, especially
of Gram-positive species [83]. An increased incidence
of bacterial translocation in patients with obstructive
jaundice has been reported [84]. However, whether the
reduced intestinal luminal levels of bile acids that may
be found in patients with cirrhosis, as a consequence
of reduced hepatic secretion and luminal deconjugation
by overgrowth bacteria [6], contribute importantly to
the potential for increased bacterial translocation in this
group has not been established. A recent study
performed in rats with cirrhosis induced by carbon
tetrachloride found that the administration of conjugat-
ed bile acids led to reversal of intestinal bacterial over-
growth, a reduced rate of bacterial translocation and
increased survival [85].

Little is known concerning the functional capacity of
intestinal immunity in patients with cirrhosis and
whether any disturbance of intestinal immune mecha-
nisms contributes importantly to bacterial translocation.
An increased number of intraepithelial lymphocytes
with markedly impaired proliferative activity and capac-
ity for production of interferon-γ have been reported in
a murine model, with these changes correlating with
increased bacterial translocation [12]. 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 [78], 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.

The anatomical site(s) from which bacterial translocation occurs in cirrhosis also remains to be determined. Data in the non-cirrhotic setting suggest that the small intestine 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 \textit{E. coli} suggest that translocation occurs preferentially from the small bowel rather than the colon [86]. Similarly, in experimental animals with impaired intestinal motility, treatment with the pro-kinetic agent, cisapride, was associated with a reduced rate of bacterial translocation that paralleled a reduction in jejunal but not caecal bacterial counts [53]. A recent study performed in cirrhotic rats found that animals with translocated bacteria had significantly higher caecal bacterial counts than counterparts without bacterial translocation; over 95% of translocating bacterial strains were found to be overgrown in the caecum. Concurrent analysis of the bacterial ecology of the small intestine was not performed [87].

2. 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. SBP is considered to be present if the ascitic neutrophil count is in excess of 250 per microlitre [88]. Prospective studies suggest that SBP is present in up to 23% of all cirrhotic patients with ascites undergoing paracentesis [89], although the incidence is notably low in recent outpatient-based series of asymptomatic patients [90–92]. Gram-negative intestinal flora, especially \textit{Escherichia coli} and \textit{Klebsiella} species, are isolated in approximately 70% of culture-positive cases of community-acquired SBP. Aerobic Gram-positive bacteria belonging to \textit{Streptococcus} and \textit{Staphylococcus} species constitute most of the remaining isolates. Pathogens belonging to \textit{Aeromonas}, \textit{Plesiomonas}, \textit{Listeria}, \textit{Salmonella} and \textit{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 [93]. In hospitalised cirrhotic patients with nosocomial SBP, Gram-positive pathogens are predominant, accounting for over 70% of isolates, with methicillin-resistant \textit{Staphylococcus aureus} accounting for nearly 25% of cases in one recent series [94].

The overall likelihood of a cirrhotic patient with ascites developing SBP at one year is in the order of 10% [89]. 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 [95]. An increased prevalence of SBP has been reported in patients with SIBO compared to their non-overgrowth counterparts, presumably due to the increased propensity for bacterial translocation [96]; the reverse is also the case, with patients with SBP shown to have a higher prevalence of bacterial overgrowth than those without SBP [54]. Gastrointestinal haemorrhage is another important precipitant of SBP in this group. A meta-analysis of five randomised controlled trials suggests that the risk of bacteraemia and/or SBP within 7–14 days of gastrointestinal haemorrhage is 27% [97]. The likelihood of SBP is related to the functional activity of Kupffer cells, which is impaired in patients with advanced liver disease [98]. However, the most powerful predictive factor identified in several series is an ascitic fluid total protein level \(\leq 10\) g/L, which reflects a low complement concentration and, hence, opsonisation capacity. Patients with such low ascitic protein levels are at a 6- to 10-fold increased risk of a first episode of SBP compared to cirrhotic counterparts with ascitic fluid total protein levels >10 g/L [99,100].

2.1. Clinical course

Despite the fact that resolution of SBP is usually achieved with only a short course of antibiotic treatment, the availability of effective non-nephrotoxic antibiotic options and overall improvements in the general medical care of cirrhotic patients, the in-hospital mortality rate associated with SBP in unselected patients remains in the order of 20–30% [101–103]. This is largely related to the generally advanced degree of underlying hepatic functional 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 [104]. Ascitic levels of nitric oxide, which are significantly increased in cirrhotic patients with SBP and persist for over two weeks despite appropriate antibiotic treatment [105,106], independently predict renal impairment in this setting [107]. Use of nephrotoxic antibiotics adds to the risk of renal failure in this setting [108]. The development of renal failure is the most important predictor of in-hospital mortality associated with SBP. In one recent series, this approximated 50% in those with complicating renal failure compared to
only 6% in those without [104]. Poorer outcome also correlates with exaggerated peritoneal production of the proinflammatory cytokines, interleukin-6 (IL-6) and tumour necrosis factor (TNF-α), in response to the infecting bacteria [109]. Notably, findings from a recent study performed in experimental animals raise the possibility that adjunctive anti-TNF-α treatment may reduce SBP-related mortality in this circumstance [110]. 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 [94]. A trend towards a higher mortality rate when infection is with encapsulated strains of *E. coli* associated with an increased propensity for tissue invasiveness has also been reported [111].

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% [89]. 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 [112–114]. The risk of SBP recurrence within one year ranges from 40% to 70% and, as for an initial episode, is influenced mainly by the degree of underlying liver dysfunction and the ascitic fluid total protein level [95,113,114].

### 2.2. Management

Empiric antibiotic treatment should be commenced immediately after diagnostic paracentesis in patients in whom SBP is clinically suspected or after the demonstration of a raised ascitic neutrophil count. It is inappropriate to wait for the result of ascitic culture in view not only of the suboptimal sensitivity of this test but also of the risk of rapid clinical deterioration. The antibiotic of choice is generally a third generation cephalosporin, such as cefotaxime, which has adequate penetration into ascitic fluid and offers acceptable cover against the bacteria most often responsible for SBP with low toxicity. Treatment with 2 g of cefotaxime intravenously every eight to 12 h is as effective as six hourly dosing [112]. Treatment for five days is as effective as a 10 day course [115]. Intravenous ceftriaxone [116], cefonicid [117], amoxicillin with clavulanic acid [118,119] and ceftazidime [120] are also effective. Randomised data suggest that oral ofloxacin (400 mg every 12 h) may be as effective as intravenous cefotaxime in patients with uncomplicated SBP [121]. Early switching to oral ciprofloxacin following initial treatment with intravenous ceftazidime has been shown to be more cost-effective than a full course of parenteral therapy, provided that patients were not receiving prophylactic quinolones prior to the onset of infection [120]. The appropriateness of any chosen antibiotic regimen may be reviewed when ascitic fluid culture and sensitivity results eventually become available. Albumin infusion improves systemic haemodynamics, reduces the incidence of renal failure and improves survival compared to antibiotic treatment alone in patients with SBP, with beneficial effects related to reduction in arterial vasodilatation and improved cardiac function [103,122]. Roles for *N*-acetylcysteine [123] and terlipressin [122], in combination with albumin infusion, have been proposed for patients with hepatorenal syndrome associated with SBP. Granulocyte–macrophage colony stimulating factor has been shown *in vitro* to reverse defects in neutrophil phagocytosis and chemotaxis in cirrhotic patients [124] and may have a role in the management of SBP in selected patients, such as those with severe infection and an incomplete initial response to antibiotics, although this remains to be properly assessed. Survivors of an episode of SBP should be evaluated for OLT in view of the high risk of recurrence and poor overall prognosis.

#### 2.3. Prophylaxis

There is evidence that antibiotic prophylaxis for SBP may be of benefit in three high risk groups with cirrhotic ascites, namely those who have survived a previous episode, those with low ascitic fluid total protein levels and those presenting with gastrointestinal haemorrhage. Most secondary 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. A double-blind, placebo-controlled trial evaluating the long-term efficacy of norfloxacin (400 mg daily) in cirrhotic patients who had survived a previous episode of SBP found a significantly reduced rate of SBP recurrence in the treated group (12% versus 35%) during a mean follow-up period of six months [125]. The overall probability of SBP recurrence at one year of follow-up was 20% in the group receiving norfloxacin prophylaxis compared to 68% in the placebo group.

The efficacy of both short- and long-term primary prophylaxis with norfloxacin has been assessed in cirrhotic patients with low ascitic fluid protein concentrations. A randomised, controlled study in those with an ascitic fluid total protein concentration ≤10 g/L found that prophylactic norfloxacin (400 mg/day) during periods of hospitalisation was associated with a significantly reduced in-hospital incidence of SBP (0% versus 23%) [126]. Long-term primary prophylaxis with norfloxacin may be preferable to prophylaxis only during periods of hospitalisation in this group. In 109 cirrhotic patients with ascitic fluid total protein levels ≤10 g/L or serum bilirubin concentrations >2.5 mg/dL, the prevalence of SBP after a mean 43 weeks of follow-up was significantly lower in those randomised to receive continuous pro-
phylaxis (both as inpatients and outpatients) (2%) than in those receiving prophylaxis only while hospitalised (17%) [127].

Short-term prophylaxis with norfloxacin is indicated in patients with cirrhotic ascites presenting with variceal haemorrhage. For example, treatment with 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% versus 17%) [20]. Development of treatment protocols that included antibiotic prophylaxis led to a fall in post-variceal haemorrhage bacterial infection rates from 38% to 14% over the past two decades in a large French series [19]. 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 [21,22,24,128].

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 utilization [129–131]. 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, hepatocellular carcinoma or other causes is unaffected by the use of prophylactic antibiotics. More recently, a meta-analysis of 13 randomised controlled trials suggested that antibiotic prophylaxis of hospitalised cirrhotic patients is efficacious in reducing the relative risk of in-hospital death (relative risk of dying 0.70), irrespective of underlying risk factors [132]. 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 [97].

The efficacy of alternative antibiotic regimens to norfloxacin for SBP prophylaxis in a variety of clinical settings has been assessed in several studies. Ciprofloxacin, 500 mg twice daily either orally or via nasogastric tube for 7 days immediately following endoscopy for upper gastrointestinal haemorrhage, has been shown to significantly reduce the incidences of bacteraemia, SBP and urinary tract infection compared to placebo (0% versus 23%, 3% versus 13% and 5% versus 18%, respectively) [133]. A weekly dose of 750 mg of ciprofloxacin for the prevention of primary or recurrent SBP in 28 cirrhotic patients with ascitic fluid protein levels <15 g/L was associated with a significantly reduced incidence of SBP at six months compared to that in patients randomised to receive placebo (4% versus 22%) [134]. There were no instances of acquired resistance to ciprofloxacin or other side effects reported over this time. However, other data suggest that weekly quinolone dosages are associated with development of an increased rate of quinolone-resistant bacteria in stool [135] and more recent findings suggest less efficacy against Enterobacteriaceae than daily norfloxacin [136]. In a study in which the presence of cirrhotic ascites was the sole entry criterion, use of trimethoprim–sulphamethoxazole for five days per week was associated with a significantly reduced incidence of bacteraemia and/or SBP during a median follow-up of three months than occurred in non-treated patients (3% versus 27%) [137]. No adverse effects were reported. As with norfloxacin, prophylaxis with trimethoprim–sulphamethoxazole was cost-effective in patients with cirrhotic ascites, especially those at high risk for SBP [131].

As alluded to above, of particular concern 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 *E. coli* were with a norfloxacin-resistant strain in a recent series from Spain [138], 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 [4]. 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 [139,140].

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 [53,67]. Unfortunately, cisapride has been withdrawn from use in some countries because of potential for cardiac arrhythmia. Alternatively, treatment with symbiotics (probiotics and fermentable fibre) or fermentable fibre alone has recently been shown to significantly reduce viable fecal counts of potentially pathogenic gut flora in patients with cirrhosis [30]. 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 symbiotic regimen that included *Lactobacillus plantarum* and fermentable fibre than in patients receiving selective intestinal decontamination [141]. 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 [142]. These findings are compatible with a report in rats with liver failure following 90% hepatectomy in which treatment with a lactobacillus strain (*Lactobacillus reuteri* R2LC) in combination with fermentable oatmeal was associated with a significant reduction in bacterial translocation [143]. Based on these reports, the possible use of symbiotics for prophylaxis against SBP in cirrhotic patients warrants investigation in randomised trials.

Preliminary data are available in various *in vitro* and *in vivo* settings as to whether treatment with probiotics (intestinal bacterial flora without the additional fermentable fibre component) may be of benefit in reducing bacterial translocation and its infective complications. *In vitro*, the probiotic bacterium *Lactobacillus casei* GG has been shown to inhibit translocation of *E. coli* in a dose-dependent manner in a cell culture model [144]. *In vivo*, data in experimental animals with cirrhosis as to the possible efficacy of probiotics in preventing bacterial translocation and its infective complications. *In vitro*, treatment with the probiotic strain *Enterococcus faecium* M-74 may prevent episodes of febrile neutropoena in patients receiving antinecancer chemotherapy [149]. Reported experiences as to any clinically relevant reduction in risk of SBP and other bacterial infections in patients with cirrhosis are lacking. The possible value of anti-oxidant therapy in this regard, as suggested in an experimental animal model of cirrhosis in which treatment was associated with a reduced rate of bacterial translocation [72], also remains to be determined.

**3. Intestinal flora, pro-inflammatory cytokines and vasoactive mediators in cirrhosis in the absence of overt infection**

As discussed earlier, bacterial DNA may be present in ascitic fluid of as many as one-third of cirrhotic patients with non-neutrocytic ascites and negative bacterial culture [47]. Longitudinal analyses of ascites and peripheral blood of individual patients indicate that the presence of bacterial DNA in this circumstance reflects a single-species, dynamic manifestation of bacterial translocation [150]. Increasing evidence points to the presence of bacterial DNA in non-neutrocytic and culture-negative ascites as being of substantial pathophysiological relevance in cirrhotic patients. Peritoneal macrophages recovered from such patients have been found to produce significantly higher amounts of TNF-α, IL-2, IL-6, IL-12, inducible nitric oxide synthase and nitric oxide than cells from cirrhotic patients without bacterial DNA in ascitic fluid [151,152].

Activation of macrophages by endotoxin, a cell wall component of Gram-negative bacteria, plays a key role in the over-production of TNF-α in experimental animal models of liver injury [153–156]. Increased translocation from the gut lumen and reduced hepatic clearance have each been proposed to predispose to endotoxaemia in this situation [157,158]. In the clinical setting, increased circulating levels of endotoxin have been documented in patients with chronic liver disease, even in the absence of overt infection [29,159–164]. Based on experiences in experimental animals, it has been assumed that endotoxaemia is a major cause of the raised pro-inflammatory cytokine levels found in this group [157,165–167]. However, a significant correlation between circulating endotoxin and proinflammatory cytokine levels has generally not been shown [29,159,160,163,164]. On the other hand, elevated circulating levels of pro-inflammatory cytokines and nitric oxide metabolites, along with the degree of systemic vasodilatation, have been found in cirrhotic patients to correlate significantly with plasma concentrations of lipopolysaccharide-binding protein, which is produced in the liver in response to endotoxin and enhances the binding of endotoxin to the CD14/ Toll-like receptor 4 (TLR4) receptor complex [168]. Treatment with norfloxacin partially reversed the cytokine and haemodynamic derangements, further raising the possibility that endotoxin may contribute to the pathogenesis of these phenomena [168]. Such findings are in keeping with the earlier demonstration in cirrhotic rats with ascites that bacterial translocation to mesenteric lymph nodes was associated with over-production of both TNF-α and nitric oxide, leading in turn to increased vasodilatation of the mesenteric vasculature [169].

Conversely, significantly increased expression on peripheral blood mononuclear cells (PBMCs) of TLR2, responsible for signaling in response to Gram-positive microbial stimuli, but not of TLR4, has been demonstrated in patients with cirrhosis [29]. In this study, PBMC expression of TLR2, but not that of TLR4, was shown to correlate significantly with circulating levels of both TNF-α and soluble TNF receptors [29]. These findings suggested that signaling via TLR2 but not TLR4 may contribute to the increased circulating levels of TNF-α levels found in cirrhosis and imply, contrary to previous assumptions, an important role for
Table 2
Effects of probiotic or synbiotic treatment on hepatic function in liver injury models

<table>
<thead>
<tr>
<th>Authors (Reference No.)</th>
<th>Study model</th>
<th>Probiotic or synbiotic regimen</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanj et al. (1994) [171]</td>
<td>Rats with alcohol-induced toxicity</td>
<td>Probiotic (Lactobacillus strain GG)</td>
<td>Liver injury significantly reduced in treated compared to untreated animals</td>
</tr>
<tr>
<td>Kasravi et al. (1996) [172]</td>
<td>Rats with d-galactosamine-induced liver injury</td>
<td>Probiotic (Lactobacillus reuteri R2LC)</td>
<td>No beneficial effect of treatment with probiotic on extent of liver injury</td>
</tr>
<tr>
<td>Kasravi et al. (1997) [173]</td>
<td>Rats with d-galactosamine-induced liver injury</td>
<td>Probiotic (Lactobacillus reuteri R2LC or Lactobacillus plantarum)</td>
<td>Treatment with Lactobacillus plantarum associated with reduced liver injury compared to controls</td>
</tr>
<tr>
<td>Adawi et al. (1997) [147]</td>
<td>Rats with d-galactosamine-induced liver injury</td>
<td>Probiotic (Lactobacillus reuteri R2LC or Lactobacillus rhamnosus or Lactobacillus plantarum or Lactobacillus reuteri strain 108)</td>
<td>Bacterial strain-related improvement in liver injury in treated animals compared to controls</td>
</tr>
<tr>
<td>Adawi et al. (1998) [174]</td>
<td>Rats with d-galactosamine-induced liver injury</td>
<td>Probiotic (Lactobacillus plantarum with/without N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide production)</td>
<td>Treatment with Lactobacillus plantarum associated with reduced liver injury compared to controls; protective effect abolished by L-NAME</td>
</tr>
<tr>
<td>Adawi et al. (2001) [148]</td>
<td>Rats with d-galactosamine-induced liver injury</td>
<td>Probiotic (Bifidobacterium animalis or Lactobacillus acidophilus or Lactobacillus rhamnosus or Lactobacillus plantarum or Lactobacillus fermentum)</td>
<td>Bacterial strain-related improvement in liver injury compared to controls</td>
</tr>
<tr>
<td>Li et al. (2003) [175]</td>
<td>Ob/Ob mice (non-alcoholic fatty liver disease model)</td>
<td>Probiotic (Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus bulgaricus and Lactobacillus plantarum)</td>
<td>Treatment associated with improved liver histology, reduced hepatocyte total fatty acid content, reduced serum ALT levels and improvement in insulin resistance</td>
</tr>
<tr>
<td>Jia and Zhang (2005) [176]</td>
<td>Rats with thioacetamide-induced liver injury</td>
<td>Probiotic (Bifidobacteria, lactobacilli and Streptococcus thermophilus)</td>
<td>Treatment associated with reduced histological liver injury compared to untreated controls</td>
</tr>
<tr>
<td>Marotta et al. (2005) [177]</td>
<td>Rats with liver injury associated with alcohol-induced pancreatitis</td>
<td>Synbiotic (Lactobacillus acidophilus, Lactobacillus helveticus or Bifidobacteria in an enriched medium)</td>
<td>Treatment associated with reduced liver damage compared to controls</td>
</tr>
<tr>
<td>Han et al. (2005) [178]</td>
<td>Mice with carbon tetrachloride-induced liver damage</td>
<td>Probiotic (Lactobacillus brevis, Lactobacillus acidophilus or Bifidobacterium longum)</td>
<td>Treatment associated with significant reduction in liver injury compared to controls; Lactobacillus acidophilus the most protective</td>
</tr>
<tr>
<td>Chen et al. (2005) [179]</td>
<td>Mice with carbon tetrachloride-induced liver damage</td>
<td>Probiotic (lactobacilli)</td>
<td>Treatment associated with reduced liver injury compared to controls</td>
</tr>
<tr>
<td>Liu et al. (2004) [30]</td>
<td>Patients with cirrhosis</td>
<td>Synbiotic (Pediacoccus pentosaceus 5-33:3, Leuconostoc mesenteroides 32-77:1, Lactobacillus paracasei subspecies paracasei 19, Lactobacillus plantarum 2592 and fermentable fibre)</td>
<td>Treatment associated with improvement in liver function and reduction in serum ALT levels; no improvements in placebo-treated patients</td>
</tr>
<tr>
<td>Loguerio et al. (2005) [31]</td>
<td>Patients with cirrhosis</td>
<td>Probiotic (Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii subspecies bulgaricus, Lactobacillus plantarum and Streptococcus salivarius subspecies thermophilus)</td>
<td>Treatment associated with improvement in liver function and reduction in serum ALT levels</td>
</tr>
</tbody>
</table>
Gram-positive microbial stimuli rather than endotoxin in this process. This contention is supported by in vitro PBMC stimulation data consistent with presensitization by Gram-positive microbial stimuli but not endotoxin in vivo. In addition, supplementation of cirrhotic patients with a symbiotic regimen including four Gram-positive gut flora and fermentable fibre that has been shown to increase the fecal content of Gram-positive lactic acid bacteria and reduce viable counts of Gram-negative species led to a significant further increase in PBMC expression of TLR2, along with increased circulating TNF-α levels in most cases, suggesting that Gram-positive stimuli derived from the intestine, in particular, may be important in promoting the increased circulating cytokine levels found in apparently uninfected patients with cirrhosis [29].

3.1. Intestinal flora and liver function in cirrhosis in the absence of overt infection

An important question that arises in view of these observations indicating that the intestinal bacterial flora or their products influence systemic cytokine levels in cirrhosis is whether the intestinal flora may have an impact on hepatic damage in this setting [170]. This issue has been addressed in experimental animal models of liver injury in which animals have been treated with various intestinal flora-based probiotic or symbiotic regimens [147,148,171–179]. These studies mostly demonstrated treatment-related improvement in liver injury, depending on the bacterial species used (Table 2).

In the clinical setting, initial interest in the potential of modulating the intestinal flora for therapeutic gain in cirrhotic patients centred on the possible efficacy of probiotics for the treatment of overt hepatic encephalopathy. Four small, non-placebo-controlled studies involving treatment with Lactobacillus acidophilus or Enterococcus faecium suggested possible benefit [180–183]. Of note, patients who responded to supplementation with Enterococcus faecium maintained clinical improvement during a two week period off-treatment, whereas encephalopathy grade typically returned to baseline following treatment with lactulose [183]. More recently, reversal of minimal hepatic encephalopathy has been reported in 50% of predominantly Child–Pugh class B or C cirrhotic patients, mostly due to hepatitis B virus infection, who were treated for 30 days with a lactic acid bacteria-based symbiotic regimen (comprising Pediacoccus pentoseceus 5-33:3, Leuconostoc mesenteroides 32-77:1, Lactobacillus paracasei subspecies paracasei 19 and Lactobacillus plantarum 2592, each at a dose of 1011 colony forming units) and fermentable fibre (Cocktail 2000; Medipharm, Kagerod, Sweden). The efficacy of symbiotic therapy was comparable to that of lactulose and substantially higher than that in placebo-treated controls [30]. In addition, the Child–Pugh class improved in nearly 50% of initially Child–Pugh class B or C symbiotic-treated patients, a proportion significantly higher than that in placebo-treated counterparts (8%). Treatment was associated with a significant increase in the fecal content of lactobacilli, while overgrowth of potentially pathogenic other Gram-positive and Gram-negative bacterial species was reversed. Such bacterial overgrowth was also reversed and improvements in minimal hepatic encephalopathy and the Child–Pugh class occurred in 50% and 29%, respectively, of cirrhotic patients treated with fermentable fibre alone. Both in symbiotic- and fermentable fibre alone-treated patients, improvement in the Child–Pugh class occurred 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 serum ALT levels, was documented in both groups [30] (Table 2).

In addition to this evidence that symbiotics and fermentable fibre may improve hepatic function in patients with cirrhosis, a beneficial effect of probiotic therapy has recently been documented. In particular, patients with cirrhosis due to alcohol or hepatitis C virus infection and elevated mean pre-treatment serum ALT concentrations demonstrated improvement in hepatic function and serum ALT levels following treatment with a combination of Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii subspecies bulgaricus, Lactobacillus plantarum and Streptococcus salivarius subspecies thermophilus at a dose of 5 × 1011 colony forming units (VSL#3; VSL Pharmaceuticals, USA) [31] (Table 2). Studies to elucidate molecular mechanisms by which modulation of the intestinal bacterial flora may afford hepatoprotection in the clinical setting are in progress. Such studies will be important if the various interactions between the intestinal flora, bacterial infection, intestinal function and hepatic function in cirrhosis discussed in this review are to be better understood for therapeutic gain.

References


Low-protein-concentration ascitic fluid is predisposed to spontaneous bacterial translocation due to obstructive jaundice on liver damage. Hepato-Gastroenterology 2003;50:1542–1546.


Role of intestinal bacterial overgrowth and intestinal motility in bacterial translocation due to obstructive jaundice on liver damage. Hepato-Gastroenterology 2003;50:1542–1546.


and presence of bacterial DNA. Eur J Gastroenterol Hepatol 2005;17:45–51.


