Liver disease is associated with qualitative and quantitative changes in the intestinal microbiota. In cirrhotic patients the alteration in gut microbiota is characterized by an overgrowth of potentially pathogenic bacteria (i.e., gram negative species) and a decrease in autochthonous families. Here we summarize the available literature on the risk of gut dysbiosis in liver cirrhosis and its clinical consequences. We therefore described the features of the complex interaction between gut microbiota and cirrhotic host, the so called “gut-liver axis”, with a particular attention to the acquired risk of bacterial translocation, systemic inflammation and the relationship with systemic infections. Such knowledge might help to develop novel and innovative strategies for the prevention and therapy of gut dysbiosis and its complication in liver cirrhosis.
its consequences, mainly due to the passage of bacteria (or bacterial products) towards the liver and systemic circulation via the portal circulation or lymphatic system. The growing attention in the last years to this issue, the so-called “gut-liver axis”, is explained by the fact that the liver is in fact chronically exposed to gut-derived factors including bacteria and bacterial components. The passage of bacteria, or a part of them, has been recently associated with systemic inflammation and the complications of cirrhosis, such as hepatic encephalopathy.

This review aims at describing the evolution on the understanding of gut microbiota in end-stage liver disease over the last decades, the development of techniques to study gut microbiota, the characterization of gut dysbiosis and the clinical consequences of it.

**LITERATURE RESEARCH**

Bibliographic searches were performed in MEDLINE and EMBASE for the following words (all fields): (“Gut Microbiota” or “Bacterial Translocation” or “Inflammation” or “dysbiosis” or “rifaximin” or “lactulose”) and (“cirrhosis” [MeSH]) and (“cirrhosis decompensation” [MeSH] or “hepatic encephalopathy” or “hyperdynamic circulation”). Other relevant trials were identified by hand searched of the reference lists of the clinical trials identified during the electronic search. A first review was performed on the abstracts of the articles selected and if the inclusion criteria were satisfied, articles were included in the analysis. The information extracted from each of the selected publications included study design details, patient and microbiota characteristics, animal models of dysbiosis, methods of gut bacterial investigation and clinical consequences of dysbiosis. Studies published in abstract form only, or in non-English language were excluded.

**ARE WE MADE OF BACTERIA?**

The human intestinal tract harbors one of the most densely populated ecosystems on earth. The number of microorganisms living in the intestine range between $10^{11}$-$10^{13}$ CFU/mL. The gastrointestinal (GI) tract is the largest epithelial surface of the body and is in constant exposure to bacteria. More than 70% of all microorganisms associated with the human body live in the intestine, probably related to the richness of nutrients in this area. The total genome of microorganisms that are part of the flora, called microbiome (60000 genes), exceeds that encoded by the human genome by a factor of approximately 100, providing the expression of functions which have not evolved in humans. We can therefore be considered a superorganism consisting of microbial and human cells, and our genome is the sum of our genes and those belonging to the billions of microorganisms that are part of our microbiota. The distribution of the micro-organisms varies from $10^5$ to $10^8$ bacteria/mL between stomach and duodenum, to $10^7$ to $10^8$ bacteria/mL between jejunum and ileum, culminating in $10^{12}$-$10^{13}$ bacteria/mL in the colon. Most bacteria of faecal origin belong to two major phylogenetic lineages: *Firmicutes* and *Bacteroidetes*. In minor proportion *Actinobacteria* and *Proteobacteria* are also present. In healthy subjects, species
belonging to these phyla live in peaceful coexistence[13]. In the last decade, several pathologies have been correlated to alterations in the intestinal ecosystem equilibrium - this is called dysbiosis. In such a condition there is an increase of potentially pathogenic bacteria species/groups [Enterobacteriaceae, Escherichia coli (E. coli)], instead of potentially beneficial bacterial species/groups [Clostridiales, Fusobacterium prausnitzii (F. prausnitzii)]. E. coli is a commensal living in the large intestine; several strains within this species, harboring a set of virulent genes, cause a wide range of diseases[14]. F. prausnitzii is copiously present in the human gut, and it is one of the main producers of butyrate, which is the energy source for cells of the colonic mucosa. A decrease in Fusobacterium spp., in adult patients has been associated with inflammatory bowel disease (IBD)[15,16]. Recently the ratio of F. prausnitzii/E. coli expressed as relative abundances, has been proposed as a possible indicator of intestinal dysbiosis in adult subjects[15].

Gut microbiome in liver disease: Stable component or changing companion

A legitimate question arises when we begin to approach the gut microbiota in liver disease; is it the liver disease which modifies the gut bacteria or is it the gut dysbiosis which causes liver disease? Before answering these questions we need to discuss the stability of gut microbiota over time. The concept that the individual “fingerprint” of gut microbiota does not change within the host during his or her life is now under debate[17]. In their study Li et al[18] found a general microbiome signature conserved among body habitats of the same body region and over time suggesting that a relatively stable microbial community structure is maintained throughout the human microbiota. Analyses of the variation for the abundances of phyla across the cohort reveal that the detected core taxa are also stable. However this observation changed when the attention was shifted to oral and stool habitats which, in contrast to the rest of the body, tended to vary over time[18].

It is also important to emphasize that a phylum-based analysis is not suitable in cirrhosis, especially since Firmicutes includes several pathogenic taxa such as Staphylococcaceae and Enterococcaceae, which indeed are over-abundant in the sickest population and are very different from its other constituents such as Lachnospiraceae and Ruminococcaceae in their ultimate impact[19].

Indeed recent studies on gut microbiota in cirrhosis have shown that microbiota changed when the underlying disease worsened[10]. In advanced liver cirrhosis there appears to be an increase in dysbiosis, with a greater abundance of gram-negative taxa (Enterobacteriaceae, Bacteroidaceae). The consistent pattern of microbiota change and its association with the severity of cirrhosis cross-sectionally and longitudinally indicates that the underlying severity of cirrhosis may be a stronger determinant of microbial abundance. The influence of liver disease on gut bacteria composition is also confirmed by another study conducted in Asian cirrhotic hosts. Chen et al[9], by analyzing fecal samples of 36 cirrhotic patients and 24 healthy people, found that patients with cirrhosis had a reduced concentration of Bacteroidetes and Lachnospiraceae, whereas Proteobacteria, Fusobacterium spp., Enterobacteriaceae, Veillonellaceae and Streptococcaceae were increased. Interestingly, in their work the authors found that the alteration of microbiota composition correlated with the severity of liver disease.

But liver disease per se is not the only variable which could influence the gut microbiota. In fact, cirrhosis are frequently exposed to health care structures and hospital admissions, among patients with decompensated cirrhosis, are common. The increase of certain bacterial taxa related to hospitalization such as Propionibacteriaceae and Halomonadaceae have been recently identified as markers of the underlying abnormal intestinal milieu and these bacterial taxa have been described as potential pathogens in humans[20,21]. The use of antibiotics during hospitalization is also frequent and might interact with the composition of gut microbiota. In the study of Pérez-Cobas et al[22], in the first days of antibiotic treatment the majority of the microbiota included species from the phylum Firmicutes. A drastic shift occurred 4-6 d after the start of antibiotic treatment (based on betalactam antibiotics), in fact, after one week of antibiotic therapy the predominantly active taxa were mainly members of the Streptococcaceae, Clostridiaceae and Bacteroidaceae (which are considered to belong to the “non autochthonous” group of bacteria)[21,22].

Dietary modifications may also affect the stability of gut microbiota in cirrhotic patients over time. For instance, restriction of dietary protein was considered a mainstay in the therapy of hepatic encephalopathy for a long time. More recently, it has been recognized that protein energy malnutrition is frequent in advanced liver disease and may adversely affect patient outcome[23]. So far there are no data on the effect of diet on gut microbiota in liver disease, however an interesting study comparing healthy subjects on a “western diet” with those on an high fiber diet with low protein and carbohydrate content, clearly showed changes in gut microbiota. De Filippo et al[24] compared the fecal microbiota of European children and that of children from a rural African village, where the diet was high in fiber content and low in animal derived protein and carbohydrates. The author found significant differences in gut microbiota between the two groups. The group with a low protein and carbohydrate diet showed a significant enrichment in Bacteroidetes and a depletion in Firmicutes, with a unique abundance of bacteria from the genus Prevotella and Xylanibacter, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, which was completely lacking in the subjects on a Western diet. Also, Enterobacteriaceae (Shigella and Escherichia) were significantly lower in the “Fiber-based diet” as opposed to the “protein and polysaccharide-rich diet”[25].

It is therefore possible that the gut microbiome is a
changing companion, which coevolved with the host and varies also according to his health and diet.

Liver-gut axis: Microbiota modification in cirrhosis

In the past, the description of the qualitative and quantitative characteristics of gut microflora composition in cirrhotic patients, has been limited by the scarce reliability of the culture techniques performed on fecal samples or on intestinal lumen aspirates. The advent of molecular techniques, mainly based on Polymerase Chain Reaction (PCR) of the bacterial ribosomal 16S rDNA sequence are now allowing to perform a complete description of the entire bacterial community of a sample. By means of techniques such as Temporal Temperature Gradient Gel Electrophoresis (TTGE), Real-time PCR and the recent pyrosequencing, it is becoming clear that patients suffering from chronic liver disease could harbor an unbalanced gut microbiota composition.

Cirrhotic patients are exposed to a higher risk of dysbiosis because of a variety of pathological interactions between the liver and the gastrointestinal tract. The alteration in intestinal motility, the higher gastric pH and the reduced bile acid concentration in the colon seen in patients with cirrhosis, may lead to a failure in the control of bacterial intestinal growth[26-28]. The clinical implications of this failure in the cirrhotic host might be represented by the occurrence of pathological bacterial translocation, a higher risk of intestinal bacterial infections and the risk of decompensation of liver disease[29].

When the gastric acid barrier fails (drug-induced hypochlorhydria, Helicobacter Pylori colonization, autoimmune atrophic gastritis, gastric surgery, etc.), “oropharyngeal flora” including mainly Gram-positive bacteria (Streptococcus spp., Staphylococcus spp., Micrococcus spp., Lactobacillus spp., Neisseria spp., Veillonella spp., Stomatococcus spp., Gemella spp., Corynebacterium spp., Actinomyces spp., Fusobacterium spp.) increases in the stomach, duodenum and proximal jejunum. At the same time, when the intestinal clearance is impaired (due to a reduction of small bowel motility), the concentration of the “colonial flora” (including Enterobacteriaceae, Enterococcus spp., Pseudomonas spp. and Bacteroides spp.) increases in the small intestine[30]. The pathogenesis of the decreased intestinal motility in cirrhotic patients is not completely clear, but is probably multifactorial: the main involved mechanism is the presence of an autoinflammatory process[31] which contributes to a delayed oro-cecal transit time (OCTT)[32]. Moreover, frequent comorbidities, such as diabetes, and long term pharmacological therapies may also prolong the OCTT. Beta-blockers, by improving intestinal motility and by lowering intestinal permeability, may reduce the small intestinal bacterial overgrowth (SIBO) and decrease the rates of spontaneous bacterial peritonitis[33,34]. In addition, the decrease in bile acids entering the intestine appears to favor the overgrowth of pathogenic and pro-inflammatory members of the microbiome including Porphyromonadaceae and Enterobacteriaceae[34], in fact, due to reduced concentration of bile acid, the bacterial population relying on $\alpha$-$\beta$-dehydroxylation for their energy supply collapses[35].

Kakiyama et al[36] showed that dysbiosis in patients with cirrhosis is partially due to low bile acid excretion into the gut. The total amount of bile acids in feces of patients with advanced cirrhosis decreased roughly 5-fold compared to controls and the ratio of secondary to primary biliary acid decreased significantly. The relative scarcity of secondary biliary acids significantly correlates with the reduction of the Clostridium cluster XIVa group; this is probably due to the high proportion of $\alpha$-$\beta$-dehydroxylation bacteria within this cluster.

In animal models the production of secondary bile acids by this group of bacteria cause a positive regulation of bile acid synthesis in the liver. In fact, the higher concentration of secondary bile acids in the ileum implies a lower concentration of tauro-β-muricholic acid, which is an inhibitor of hepatic bile acids synthesis (through the inhibition of FXR signaling)[37]. This interaction unveils another part of the complex interaction between gut microbiota and liver function. In this “liver-gut axis perspective”, as the severity of cirrhosis progresses, less secondary bile acids reach the large bowel. In particular, among the secondary bile acids, deoxycholic acid (DCA) is the one which displays the most potent antimicrobial activity[38]. Thus, the consequence of its reduced concentration is a higher risk of bacterial overgrowth in the small bowel, often characterized by a reduced F. prausnitzii/E. coli ratio[35-37] because of the relative abundance of gram-negative members of the oral and gut microbiota.

The alteration of the immune response is another relevant player within this context. To date, very little is known about the intestinal immune system in patients with cirrhosis. It could be possible that in patients with cirrhosis a higher stimulation of Toll Like Receptors system occurs due to the higher exposure to pro-inflammatory cytokines such as TNF-a, but, despite the state of activation of mononuclear cells, the innate immune response does not seem to be as effective as in controls because of the reduced phagoctytic and killing capacity[40].

Liver-gut axis: Quantitative changes of gut microbiota in cirrhosis

Several studies have demonstrated that patients with cirrhosis frequently have a “quantitative” alteration of gut microbiota the so called small intestinal bacterial overgrowth, SIBO[38-40]. Therefore, in cirrhosis we have not only qualitative differences in microbial communities, compared with people without cirrhosis, but also an increased intestinal burden of bacteria. SIBO has been documented to correlate with the severity of liver disease and to be a risk factor for clinical decompensation of liver cirrhosis, due to the fact that it favors encephalopathy and spontaneous bacterial peritonitis (SBP)[41]. The pathological mechanism sustaining the correlation between SIBO and decompensation of cirrhosis is most likely bacterial translocation. As the reasons for qualitative dysbiosis are multifactorial, also bacterial overgrowth is likely to be mul-
tificial including changes in intestinal motility, absence or decreased intestinal levels of bile acids, and altered mucosal innate immunity. Alterations in gastrointestinal motility in cirrhosis have been reported and ascribed to the effects of autonomic dysfunction, altered levels of neuropeptides and the effects of inflammatory mediators on bowel muscle and nerve[43]. The prevalence of SIBO, assessed by the quantification of the bacterial density in small intestinal aspirate, ranges from 30% to 73%[42-48]. In contrast, indirect estimates of SIBO obtained by glucose breath hydrogen test range from 30% to 49% because of the limited sensitivity of this test in the general population as well as in cirrhotic patients[46,49].

The methods used to analyze the presence of SIBO are still unsatisfying. In the last decade the diagnostic gold standard for SIBO was considered the microbiological culture of jejunal secretions. This test is the oldest method used to investigate the small intestinal gut flora. However, the invasive nature of this test and the belief that the cultures are often falsely negative because of the difficulties to cultivate obligate anaerobes, or falsely positive because of the possible contamination by the oral flora, led to the introduction of indirect, non-invasive substitutes. Hydrogen breath tests (HBT) are widely used to explore SIBO, however conclusions drawn from various studies are controversial: these tests seem to have some limitations, which may determine numerous types of bias particularly in cirrhotic patients.

HBTs are based on the fact that in humans the only source for hydrogen gas is the bacterial metabolism of carbohydrates. For these tests, different carbohydrates are orally administered to the patients and the concentration of hydrogen is measured in expired air before and after the administration of the chosen substrate[50]. Glucose and lactulose are the most commonly sugar used for diagnosis of SIBO.

The main limitation of glucose HBT is that the hydrogen peak can be due not only to bacterial overgrowth, but also to a slow intestinal transit, which can determine the presence of a residue of complex carbohydrates in the colon even after the usual 12 h fasting period; besides, an inadequate diet before the test may also influence the results (such as a diet rich in fiber). An example of the difficulties to use this test is given by Bauer et al[50] who demonstrated that in cirrhotic patients, the glucose HBT correlates poorly with the diagnostic gold standard for SIBO.

Lactulose is a carbohydrate which is not absorbed neither in the small intestine nor in the colon. The limitation of this test is that a rapid transit, which may be due also to the effect of the lactulose itself, is able to blunt a clear second peak making it impossible to distinguish SIBO from the physiologic colonic fermentation[50].

For these reasons, the sensibility and specificity for detecting SIBO of lactulose HBT are 68% and 44%, and for glucose HBT 62% and 83%, compared with jejunal cultures[50].

To date, these tests would need to be customized for the cirrhotic population and new methods to detect the quantitative and qualitative characteristics of gut microbiota should be derived by molecular biology (PCR, TTGE, Real-time PCR and pyrosequencing).

Other factors to be taken into account: Aetiology of liver disease and site of sampling

In studies that have assessed the taxonomic composition of the intestinal microbiota in patients with cirrhosis due to different etiologies, the fecal microbial communities did not differ depending on the different aetiology of liver disease[18,51-54]. Therefore, it is conceivable that the condition of end-stage liver disease itself may determine the content of the intestinal microbiome. Some differences have however been reported by some studies, patients with alcoholic cirrhosis had a significantly higher abundance of Enterobacteriaceae and Halomonadaceae, lower abundance of Lachnospiraceae, Ruminoococcaceae, and Clostridiales XIV, high endotoxin and lower F. prausnitzii/E. coli ratio despite statistically similar model for end-stage liver disease (MELD) score and BMI compared to cirrhotic patients of non alcoholic etiology. Whereas, in patients with cirrhosis due to nonalcoholic steatohepatitis (NASH), a higher abundance of Porphyromonadaceae, Bacteroidiaceae, and lower Veillonellaceae was found compared to their non-NASH counterparts[18].

More recently, also the definition of dysbiosis based on qualitative and quantitative DNA analysis of stool specimens is under debate. In fact, studies in non-cirrhotic populations have demonstrated differences between the microbiome of the intestinal mucosa compared with the microbiome of the stool[50].

In the cirrhotic host one study has reported a significant difference in mucosa and stool's bacteria composition[50]. Prominent bacterial genera found at a higher abundance in the mucosal microbiome belonged to Firmicutes (Blautia, Incertae Sedis XI), Actinobacteria (Propionibacterium and Streptomyces), and Proteobacteria (Vibrio). Interestingly most bacteria found in higher abundance in stool microbiome were Firmicutes (Lactobacillus, Roseburia, Veillonella, and Incertae Sedis XIV), Propionibacterium and Vibrio genera were significantly more abundant in the mucosa than in the stool.

In cirrhotic patients taking rifaximin or lactulose for HE, the stool microbiome did not show significant changes, by converse the mucosal microbiome in the rifaximin group showed a significantly decreased abundance of autochthonous bacteria (Blautia and Roseburia) and Veillonellaceae, but an increased abundance of Propionibacterium[50].

Route of bacteria: From gut to liver

The term bacterial translocation (BT) was first coined by Berg and Garlington in 1979 as the passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes (MLN) and possibly other organs[57].

However this definition, along with the advances in
DNA and microbiome-associated molecular patterns (MAMPs) detecting technology, has been expanded and BT is now defined as the migration of viable microorganisms but also of microbial products [endotoxins such as lipopolysaccharide (LPS), lipoteichoic acid, bacterial DNA, peptidoglycans, and fragments, e.g., muramylidipeptide, etc] across an anatomically intact intestinal barrier from the intestinal lumen to MLN and other extraintestinal organs and sites\(^{[80]}\).

The mechanisms implicated in this phenomenon have not been completely clarified because of the difficulties in detecting BT in humans. The data on the pathogenesis of BT are derived mainly from animal studies and, to date, only few studies have described it in healthy subjects and in pathological conditions.

Bacteria present in the autochthonous flora, in healthy people, are able to translocate in low numbers from the gut lumen, but are physiologically killed during their passage through the epithelial barrier or in the MLN, contributing to important immunological functions\(^{[80]}\). These events mean that MLN are normally sterile. In cirrhosis, alterations of physiological mechanisms may lead to translocation and replication of the endogenous gut flora in MLN\(^{[66]}\). The species that more frequently translocate are Enterobacteriaceae, Enterococcus spp and Proteus spp. On the other hand, obligate anaerobes are rarely able to cross the gastrointestinal barrier\(^{[81]}\). In some cases, the translocation of viable bacteria may induce “spontaneous” bacterial infections while the translocation of bacterial fragments may produce a pro-inflammatory state due to the release of cytokines and nitric oxide. It has been suggested that only BT with clinical consequences might be defined as “pathological”\(^{[89]}\). The growing attention to this phenomenon is motivated by the high impact of BT in terms of its frequency and its clinical consequences.

The presence of enteric-derived bacteria in mesenteric lymph nodes and in the venous blood stream occurs more frequently in patient with cirrhosis compared with controls, and among cirrhotic patients BT is approximately 5-6 folds more frequent in Child C compared to Child A and B. Cirera et al\(^{[62]}\), based on the culture of mesenteric lymph node in one hundred cirrhotic patients undergoing liver transplantation and in thirty-five patients without cirrhosis undergoing laparotomy, found that enteric-derived bacteria were grown in approximately 8.1% Child B and in 31% Child C patients. Mesenteric lymph nodes of controls without cirrhosis were positive for bacterial growth in 8% of cases. Although the methodology of bacterial culture used by Cirera et al\(^{[62]}\) is now replaced by PCR technology because of its higher sensibility in detecting BT, their study has the value of firstly demonstrating two important issues in liver cirrhosis: (1) the rate and degree of pathological BT depend on the severity of liver disease; and (2) the translocation of entire and viable bacteria to MLN is a feature of decompensated cirrhosis. On the contrary, if we consider the translocation of nonvital gut-derived bacteria (such as bacterial DNA or LPS) the scenario is rather different. In fact, this latter variant of translocation has been demonstrated to occur also in non ascitic mice and the detection of bacterial DNA in the systemic circulation and/or in the mesenteric lymph node seems to be independent from the severity of liver disease\(^{[83]}\).

Current data suggest two major pathways of gastrointestinal permeability, which might contribute to translocation: transcellular, through the enterocytes, under the control of specific enterocyte channels and membrane pumps, and paracellular via the tight junctions holding epithelial cells together. More commonly, translocation generally occurs transcellularly and directly\(^{[29]}\). This route is more important than the paracellular route, as it can happen also in an intestine with a healthy mucosa. In the same way, there are two major routes by which bacterial compounds might gain access to the systemic circulation: via the enteric venous system to the portal vein or following the enteric lymphatic drainage\(^{[80]}\). Convincing evidence suggests that the lymphatic route might be the principal pathway of translocation. Experimental and clinical studies detected viable bacteria in MLN. Animal studies demonstrated the MLN are the first station where translocated bacteria can be found\(^{[39,49,65]}\). The identification of intestinal bacteria in normally sterile MLN is considered direct evidence of BT. As indirect marker, any detection of intestinal bacteria in cultures of the portal or peripheral blood may suggest BT, as well as the detection of endotoxin in peripheral blood. More recent methods involving PCR have been introduced for detecting microbial DNA in blood; these methods have a higher sensitivity than blood cultures for assessing BT\(^{[84]}\).

Another limiting aspect of many studies coming from the last decades is represented by the use of cultures of the MLN to investigate this phenomenon: the presence of cultivable microorganisms in the MLN was considered proof of BT. This assumption implicates an underestimation of the possibility to translocate for the bacteria which are “difficult to culture”, such as the anaerobic bacteria.

Because of the invasiveness to obtain MLN in human subjects, these techniques are seldom employed in studying BT in humans. Bacteria more frequently present in experimental models of BT, such as Enterobacteriaceae, are present in human MLN in only 25% of cases\(^{[82]}\). However, more recent studies applying PCR detection of bacterial human DNA found that about 40% of patients with advanced cirrhosis had pathological BT\(^{[87]}\).

This percentage is similar to that reported previously\(^{[83]}\) and is also similar to the percentage of cirrhotic patients having a positive serum level of LPS and lipopolysaccharide-binding protein\(^{[80]}\). More interestingly, the origin of the detected bacterial DNA is that typical of bacteria that usually are reported to cause episodes of infection, such as SBP.

Pathological factors leading to BT in cirrhotic patients are SIBO, increased intestinal permeability and alterations of the local host immune system. Bacterial overgrowth is probably a prerequisite for the development of BT; in

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cirrhotic rats the absence of SIBO prevents the development of BT and cirrhotic animals without BT had a similar count of cecal aerobic bacteria as healthy rats. Indeed, bacteria that translocate to MLN are the same ones overgrowing in the intestinal lumen, although not all the bacteria present in large quantity are found in MLN. These data suggest that other important factors are involved in BT.

The intestinal mucosal barrier has secretory and anatomical means of preventing the adhesion and penetration of microorganisms. Increases in intestinal permeability (IP) in patients with cirrhosis have been reported using various methods, and involving the assessment of structural changes, oxidative stress, and alteration in enterocyte function. If the epithelium is not physically damaged, endogenous bacteria translocate by an intracellular route through the epithelial lining cells and then travel via the lymph to the mesenteric lymph nodes. If the epithelium is physically damaged, bacteria translocate via the intercellular route between the epithelial cells to directly access the blood and lymph nodes. The increasing interest in intestinal permeability derives from the hypothesis that a leaky gut may lead to the passage of toxins, antigens, or bacteria into the body, and may play a pathogenic role in the development of chronic liver injury. Some studies have shown an association between increased intestinal permeability and severity of cirrhosis. Nevertheless, an increased IP alone is not able to determine pathological BT; in an experimental model, Perez-Paramo et al. showed that BT occurred only in rats with associated SIBO and increased IP, whereas it did not occur in rats with either increased IP or SIBO.

Both systemic and local immune defenses are impaired in cirrhosis. Gut microbiota interact with both the innate and the adaptive immune system. Cross talk between the mucosal immune system and the endogenous microflora favours the mutual growth, survival and inflammatory control of the intestinal ecosystem.

In cirrhosis, the impairment of the immune system may affect BT in two ways: the impairment of the local immune system (the gut associated lymphoid tissue) increases the ability of bacteria to translocate to MLN, the impairment of the adaptive immune system affects the capability of these microorganism to replicate and reach the systemic circulation, therefore causing the pathological consequences of BT.

In addition, impaired antimicrobial mechanisms may further contribute to the development of bacterial translocation in cirrhosis. In a recent study performed on rats, Teltschik et al. found that bacterial translocation was detectable in 40% of rats with cirrhosis. Compared with the group without translocation, these animals exhibited diminished intestinal Paneth cell producer antimicrobial factors (α-cryptdin 5 and 7 expression). In contrast, animals with portal vein ligation without cirrhosis had no alteration in antimicrobial peptides expression. The decrease in Paneth cell antimicrobial protein expression was most pronounced in the ileum and the caecum. In keeping with these results, in cirrhotic rats the antimicrobial activity toward different commensal strains was reduced, especially in the distal ileum and the cecum, where most of the bacterial translocation it is thought to occur.

**Clinical consequences of changes in the intestinal microbiome**

The growing attention to the role of the gut microbiome in cirrhosis is justified by its key role in bacterial translocation, and by the role of this and other bacterial products such as endotoxin in the pathogenesis of complications of cirrhosis, including hepatic encephalopathy (HE), SBP, and other infections. The inflammatory response secondary to the higher levels of circulating bacteria or microbiome-associated molecular patterns (MAMP) is among the leading causes of multi-organ failure, acute-on-chronic liver failure (ACLF), and death in cirrhosis. In Table 1 we summarize the clinical consequences of dysbiosis in the cirrhotic host.

**Dysbiosis and infection:** In hospitalized cirrhotic patients with nosocomial SBP, Gram-positive pathogens are the main isolated species (70% of all isolates), with methicillin-resistant Staphylococcus aureus in almost 25% of cases. In two recent studies performed on cirrhotic patients, the authors found an increased abundance of members of the Enterobacteriaceae family, which includes important Gram-negative pathogens such as Salmonella, Shigella, Yersinia, Klebsiella and E. coli. Interestingly, the abovementioned species, especially E. coli, are those that most frequently cause infections and spontaneous bacteremia in patients with cirrhosis.

There are few data on the role of dysbiosis as a risk factor for the development of infections. However, a significantly preponderance of gram negative species has been found in stool samples from cirrhotic patients with SIRS. Within this group a lower ratio of E. faecium/E. coli and higher endotoxin levels have been found compared with cirrhotics without SIRS. Also, in patients who developed SIRS and/or died from ACLF, endotoxin levels were higher and the microbiota ratio was lower with a significantly higher abundance of gram negative species. It is conceivable that the reduction in autochthonous taxa can be deleterious for the intestinal mucosa given that they produce short-chain fatty acids which reduce colonic inflammation and nourish colonocytes, compete with pathogenic bacteria for nutrients, produce anti-bacterial peptides and may improve the intestinal barrier. Therefore, the gut dysbiosis seems to participate in the disruption of intestinal epithelial tight junctions and the imbalance of proliferation and apoptosis of intestinal epithelial cells. The resulting intestinal mucosal atrophy and edema associated with portal hypertension might explain the higher concentration of serum endotoxin, which has been demonstrated in cirrhotic patients with dysbiosis.
Table 1  Changes in intestinal microbiota and clinical consequence in cirrhosis

| Ref.                                                                 | Implicated dysbiosis                                                                 | Potential biological functions                                                                                                                                 |
|---------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| **Cirrhosis vs healthy people**                                      | Overgrowth of (family):                                                              | There is a reduction in autochthonous taxa that can be disruptive given that they produce short-chain fatty acids that reduce colonic inflammation and nourish colonocytes improving the intestinal barrier |
| Stool samples, Bajaj et al\(^{[5]}\)                                | Leuconostocaceae ↑↑↑                                                                  | There was a significantly lower abundance of autochthonous genera (Clostridium Incertae Sedis XIV) and a higher abundance of potentially pathogenic ones (Enterococcus, Proteus, Clostridium) in cirrhotic patients compared with the mucosa of healthy controls |
| Mucosal samples, Bajaj et al\(^{[5]}\)                              | Clostridiaceae - Clostridium ↑↑↑                                                     | There is an increase in abundance of pathogenic taxa, reduction in autochthonous taxa and higher endotoxemia compared to uninfected patients despite matching for MELD-score and medication confounders |
| **Cirrhosis with vs without infection**                              | Overgrowth of (family):                                                              | This relative overgrowth of Enterobacteriaceae can result in endotoxemia due to increased production with worsening intestinal permeability, which has been associated with worsening disease severity and complications in cirrhosis. The lower abundance of butyrate producing genera (such as Roseburia and Ruminococcaceae) might represent a trophic injury to colonocytes |
| Stool samples, Bajaj et al\(^{[18]}\)                               | Enterobacteriaceae ↑↑↑                                                                |                                                                                                                                                           |
| **Cirrhosis with vs without inflammation**                           | Overgrowth of (family):                                                              | Firmicutes such as members of genera Veillonella, Megasphaera, Bifidobacterium, and Enterococcus were higher in HE whereas Roseburia was more abundant in the no-HE group |
| Mucosal samples, Bajaj et al\(^{[23]}\)                             | Enterococcaceae - Enterococcus ↑↑                                                  | With the increase in cirrhosis severity, there was a significant increase in potentially pathogenic and decrease in autochthonous taxa |
| Higher MELD score                                                   | Overgrowth of (family):                                                              |                                                                                                                                                           |
| Stool samples, Bajaj et al\(^{[18]}\)                               | Staphylococcaceae                                                                   |                                                                                                                                                           |

\(^{[5]}\) Giannelli V et al. Dysbiosis and gut-liver axis in cirrhosis
Cirrhotics with vs without decompensated disease | Stool samples, Bajaj et al (2010) | Overgrowth of (family):
| Enterobacteriaceae ↑
| Alcaligenaceae ↑
| Reduction of (family):
| Clostridium Incertae sedis XIV ↓
| Lachnospiraceae ↓
| Ruminococcaceae ↓
| Veillonellaceae ↓
| With the increase in cirrhosis severity, there was a significant increase in potentially pathogenic and decrease in autochthonous taxa

Aetiology of cirrhosis

Alcoholic etiology vs others | Stool samples, Bajaj et al (2010) | Overgrowth of (family):
| Enterobacteriaceae ↑
| Halomonadaceae ↑
| Reduction of (family):
| Clostridiales Incertae sedis XIV ↓
| Lachnospiraceae ↓
| Ruminococcaceae ↓
| Bacteroidaceae ↑
| Porphyromonadaceae ↑
| Reduction of (family):
| Veillonellaceae ↓
| Alcoholic cirrhosis had a significantly higher abundance of Enterobacteriaceae and Halomonadaceae, lower Lachnospiraceae, Ruminococcaceae, and Clostridiales XIV, despite statistically similar MELD score and BMI compared to those without alcoholic etiology

NASH aetiology vs others | | Overgrowth of (family):
| Enterobacteriaceae ↑
| Halomonadaceae ↑
| Reduction of (family):
| Clostridiales Incertae sedis XIV ↓
| Lachnospiraceae ↓
| Ruminococcaceae ↓
| Bacteroidaceae ↑
| Porphyromonadaceae ↑
| Reduction of (family):
| Veillonellaceae ↓
| There is a higher abundance of Porphyromonadaceae, Bacteroidaceae, and lower Veillonellaceae in NASH patients than the non-NASH counterparts

### Bacterial translocation and infection in cirrhosis:

In addition to the potential influence of BT on the natural history of cirrhosis, another point of interest is represented by the higher risk of developing infections in patients once bacteria (or parts of bacteria) translocate to mesenteric lymphnodes and the systemic circulation. Animal studies demonstrate that the prevalence of bacterial translocation in animals with SBP is twice higher than in those without SBP (80% of BT vs 40%) [8]. Furthermore, it has been shown that bacteria isolated from mesenteric lymph nodes are genetically identical to those causing SBP in the same animal [64]. Even in cirrhotic patients, bacteria responsible for post-operative infections, are in most cases the same as those recovered from mesenteric lymph nodes cultures [5].

Whether the qualitative composition of the intestinal microbiota represent a risk factor for infection in cirrhotic patients has not been completely clarified. The higher preponderance in cirrhotic stool and intestinal mucosa of those species of gram negative and positive bacteria (Enterobacteriaceae, Streptococcus spp, and Enterococcus faecalis) [3,18] that are most widely involved in systemic infections, along with the loss of non pathogenic commensal bacteria, suggest a critical interaction of intestinal dysbiosis and risk of infection in cirrhosis.

### Dysbiosis and inflammation:

There is another clinically relevant consequence of gut bacteria dysbiosis and overgrowth which is the induction of inflammation with hemodynamic derangement caused by migration of intestinal bacteria into the peritoneal cavity and the systemic circulation [8].

Inflammatory cytokines contribute to the hyperdynamic circulation, portal hypertension [3], impaired liver function and impairment of coagulation [27]. A causal relationship between BT-mediated inflammation and portal hypertension has been demonstrated in studies performed on animal models where the administration of bacterial DNA or LPS leads to exacerbation of portal hypertension [8]. Steib et al infused LPS in the peritoneum of rats with bile duct ligation-induced cirrhosis and in sham-operate rats and found, after 3 h from infusion, an increased basal portal pressure only in fibrotic animals. They also found that, in cirrhotic rats, the LPS pretreatment after 3 h further up-regulated the expression of the pro-inflammatory pathway driven by TLR4 and by MyD88. With this regard, it is interesting to report the results of a recent trial performed on cirrhotic patients, which were randomly assigned to receive a selective intestinal decontamination with norfloxacin (400 mg twice daily) or placebo [60]. In the treated arm, the serum endotoxin levels were significantly reduced after 4 wk and only in these antibiotic-treated patients the authors demonstrated an amelioration of the hyperdynamic circulation compared with controls receiving placebo. This group of patients showed a reduced cardiac output and a reduced mean HVPG equal to 4 mmHg, whereas no effects were found on renal function (the glomerular filtration rate and the renin-angiotensin system did not change significantly after selective intestinal decontamination). These results in cirrhotic patients seem to indirectly demonstrate the vicious cycle existing between gut-dysbiosis, the pro-inflammatory state driven by bacterial translocation and the worsening of portal dysfunction.
hypothesis. The ability of norfloxacin to selectively alter the microbiota of the intestine could be responsible for the hemodynamic changes seen in these patients. Norfloxacin is incompletely absorbed from the intestine, is mostly active against aerobic gram-negative bacteria and rarely causes bacterial resistance.

In another recent study, the investigation was focused on the non-absorbable antibiotic rifaximin, and the authors found similar results to norfloxacin. In fact, treated mice with rifaximin had a reduction of portal pressure and fibrosis in vivo compared to the untreated group. This agent is concentrated in the gastrointestinal tract, thereby modulates the gram negative flora and reduces the production of intestinal bacteria-derived LPS with no systemic toxicity or resistance. In their elegant study, Zhu et al[8] went further in their analysis of mechanism of rifaximin on portal hypertension and inflammatory amelioration.

Since TLR4 is the canonical receptor for LPS, they used TLR4 mutant mice which were therefore unable to activate the TLR4 pathway in response to LPS presentation. In these TLR4 mutant animals, the exposure to LPS was not reflected by any change in portal hypertension, angiogenesis, fibrosis, and pro-inflammatory state even without rifaximin[87]. The TLR4 mutant mice showed comparable and, in some cases, less portal hypertension and inflammation than wild type cirrhotic mice who received rifaximin. Together, these data suggest that the beneficial effect of rifaximin on liver inflammatory state and portal hypertension are also mediated by the down regulation of the LPS-TLR4 pathway as a result of the reduced gram negative flora.

Rifaximin is of topical interest since it is FDA approved for treatment of hepatic encephalopathy, with an acceptable safety profile in patients with chronic liver disease. Therefore, the modulation of the microbiota might represent a potential therapeutic strategy in the management of liver disease. Intestinal decontamination with nonabsorbable antibiotics (i.e., rifaximin) is also an effective treatment for minimal and overt hepatic encephalopathy[88,89].

In this scenario, recent studies have utilized a simple ratio of “good vs bad” (Firmicutes/bacteroides) taxa abundance (termed the cirrhosis dysbiosis ratio) to identify patients at higher risk of cirrhosis complications. This ratio is based on prior studies in patients with cirrhosis, which includes the highly relevant taxon Enterobacteriaceae, which is important in complications of cirrhosis, and produces one of the most potent endotoxins[89].

Interestingly, Bajaj et al[9] found that patients with cirrhosis developed clinical decompensation (such as hepatic encephalopathy) more frequently in the presence of a relatively reduced abundance of taxa considered benign and autochthonous, including Lachnospiraceae, Ruminococcaceae, and Clostridiales, Incertae Sedis XIV (from now on called Clostridiales, XIV) and a relatively higher abundance of others, particularly Enterobacteriaceae and Bacteroidaceae[1,8,28]. Moreover they demonstrated again how the severity of liver disease per se negatively affects the composition of the microbiota; in fact the MELD score seemed to negatively correlated with “positive” bacteria such as Clostridiales XIV, Lachnospiraceae and Ruminococcaceae and with Rikenellaceae, and positively with potentially pathogenic taxa such as Staphylococcaceae, Enterococcaceae and Enterobacteriaceae. It is interesting to note that the “quality” of gut microbiota reflected the “quantity” of serum levels of endotoxin. In fact, in patients with lower concentration of faecal Clostridiales XIV, Lachnospiraceae and Ruminococcaceae, the levels of endotoxin were significantly higher. Among other species of interest, they also found a reduction in Veillonellaceae and Porphyromonadaceae with worsening liver disease compared to healthy controls. Considering the ratio between autochthonous and pathogenic bacteria (Firmicutes/bacteroides), this was about three times higher in controls compared to all cirrhotic patients.

The liver is the first extraintestinal organ encountered by the venous blood from the small and large intestines draining from the portal vein. For this reason, the liver is vulnerable to the exposure of bacterial products translocated from the gut lumen via the portal vein. In a healthy organism only minor quantities of translocated bacterial products reach the liver. In general, the hepatic immune system tolerates these bacterial products avoiding an uncontrolled immune responses, a phenomenon known as “liver tolerance”[90]. The first immunological response to the presence of bacteria (or MAMPs) is driven by the activation of innate immune system. In particular, translocated bacterial products augment the activation of hepatic immune cells through pattern recognition receptors including Toll-like receptors (TLRs). The activated TLR system induces the production of cytokine such as IL-1 and IL-6 and type I IFN. In recent studies, this kind of inflammation without infection has been defined as sterile inflammation and is caused by the release from damaged cells or tissues of some factors, such as alarmin, which trigger TLR signaling[91]. TLR system is a complex system which involves more than 10 members of the TLR family but the description of this intricate response is outside the scope of this review[92,93]. It is however of interest to mention the role of TLR4 (which binds lipopolysaccharide) because its single nucleotide polymorphism (SNP) seems to correlate to the risk of liver cirrhosis in patients with chronic hepatitis C infection[94].

In two different study performed on animal models using mice knockout for TLR4 co-receptors (i.e., CD14 and LPS-binding protein), the authors found a lower induction of liver fibrosis mediated by bile duct ligation[95,96]. Their results showed that TLR4-mutant mice have similar levels of elevated LPS in the blood compared to wild-type mice but a lower liver fibrosis[97]. In addition, in another study mice deficient of the TLR4, LPS binding protein and CD14 were shown to be resistant to alcohol-induced liver damage[97,98]. Finally, gut sterilization with antibiotics decreased plasma LPS levels and liver...
steatosis, inflammation and injury after chronic ethanol exposure.

The potential therapeutic options are fascinating because they might allow the possibility to interrupt the vicious cycle existing between gut dysbiosis and liver disease. To date, relative small randomized controlled trials have been addressed to evaluate the effects of gut flora manipulation in patient with cirrhosis. In Table 2 we summarize the most relevant studies, with greater attention to those aimed at verifying the clinical benefits of gut microbiota modification. Overall, they agree with the result that targeting the gut microbiota might be effective in reducing the proliferation of harmful gut flora and consequently it limited the complications of dysbiosis such as the pathological bacterial translocation.

In these previous studies, the “manipulation” of gut bacteria has been done by prebiotics (i.e., dietary manipulation/supplementation), probiotics and systemic or poorly absorbable antibiotics. We do not consider faecal transplantation in our review because there are no data in cirrhotic patients, so far. On the whole, these studies demonstrated the positive effect of microbiota modulation

| Ref. | Type of study | Category of patients | Therapy | Clinical outcome | Microbiota changing |
|------|---------------|----------------------|---------|------------------|---------------------|
| Albillos et al[9] | RCT | 102 Cirrhotics/30 controls | Norfloxacin 400 mg orally TD vs Placebo | Norfloxacin improved cardiac index in patients with elevated LBP; no improvement in portal pressure in the rest of patients | NA |
| Bass et al[10] | RCT | 299 cirrhotics | Rifaximin 550 mg twice daily (140 patients) vs placebo (159 patients) | Rifaximin group maintained remission from hepatic encephalopathy more effectively than did placebo | NA |
| Rayes et al[11] | RCT | 66 cirrhotics underwent LT | Pediococcus pentosaceus + Leuconostoc mesenteroides + Lactobacillus paracasei and L. plantarum vs Placebo | Significant reduction of post-operative bacterial infections (3% vs 48% of controls) | NA |
| Lata et al[12] | RCT | 39 cirrhotics | Cirrhotics were randomly allocated to treatment with E. coli Nissle or placebo for 42 d | In probiotic group, the authors found a trend of significant lowering of the endotoxemia ($P = 0.07$) and improvement of Child-Pugh score ($P = 0.06$) | Restoration of normal colonic colonization in pts treated with E. coli Nissle |
| Gupta et al[13] | RCT | 94 cirrhotic patients with large oesophageal varices without history of variceal bleeding | Patients were randomized to three treatment Groups: (1) propranolol plus placebo (2) propranolol Plus norfloxacin 400 mg BD (3) propranolol plus VSL#3, 900 billion/d | Group 2 and 3 showed a greater reduction in HVPG than Group 1. In addition, in Group 2 and 3 the TNF-a levels were significantly lower than Group 1 | NA |
| Bajaj et al[14] | RCT | 25 nonalcoholic MHE cirrhotics (defined by a standard psychometric battery) | Cirrhotics were randomized to receive yogurt containing Lactobacillus bulgaricus and Streptococcus Thermophilus or no treatment for 60 d in a 2:1 ratio | Twelve of 17 yogurt patients reversed MHE (71% on intention-to-treat and 86% on per-protocol analysis) compared to 0% in the no-treatment group ($P = 0.0030$). Levels of citokine were similar between groups | NA |
| Bajaj et al[15] | PS | 20 nonalcoholic MHE cirrhotics (defined by a standard psychometric battery) | Patients received rifaximin 550 mg BID for 8 wk and the psychometric tests, stool analysis and blood test were repeated at the end of the study | There was a significant improvement in cognition test performance and a reduction of endotoxemia after rifaximin | After rifaximin there was a significant reduction in the abundance of faecal Veillonellaceae ($P = 0.025$) and increase in the abundance of Eubacteriaceae ($P = 0.042$) |
| Bergheim et al[16] | Animal model | Mice with induced NAFLD | For 8 wk, CS7BL/ J6 mice had free access to solutions containing 30% glucose, fructose, sucrose, or water sweetened with artificial sweetener or plain water | The group treated with polymyxin B and Neomycin were protected against the fructose-induced NAFLD and had a lower level of endotoxin | NA |

RCT: Randomized controlled study; PS: Pilot study; LT: Liver transplanted; NA: Not applicable (the study did not provide microbiota characterization); BD: Bis in die; HVPG: Hepatic venus pressure gradient; NAFLD: Non-alcoholic fatty liver disease.

Table 2 Effects of the intervention on gut microbiota composition and its clinical and/or biochemical consequences
in term of reduction of: fibrogenesis, endotoxin plasma concentration, portal hypertension and pro inflammatory state.\textsuperscript{[2,3,10,11]}. There is however still a lack of data regarding the effect of these interventions on the specific gut bacteria composition in liver cirrhosis, with the exception of the recent studies conducted by Patidar et al.\textsuperscript{[12]}. which accurately studied the composition of microbiota after lactulose and/or rifaximin therapies. To date, the strongest evidence regarding the clinical benefit of gut modulation in cirrhosis is still based on the large literature on hepatic encephalopathy treatment, and rifaximin plus lactulose resulted to be superior to placebo in treating the overt and minimal hepatic encephalopathy. With this regard we refer the reader to the already published studies which extensively described this clinically relevant aspect of gut-liver axis\textsuperscript{[10,11]}. 

**CONCLUSION**

We have reviewed the large literature supporting the concept of the strict interplay between gut bacteria and liver disease. Gut microbiota alteration appears to be rather common in advanced liver disease and it sustains the concept of how gut microbiota phenotype and the presence of pathological bacterial translocation are determinant factors for liver function and hepatic chronic inflammation. Herein, we also described the relevance of the clinical consequences of microbiota alteration. The clinical implications of dysbiosis is that future therapeutic approaches to liver cirrhosis, especially in decompensated patients, should consider the gut-liver axis.

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