

# Intestinal permeability in a patient with liver cirrhosis

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**Abstract:** Liver cirrhosis is a worldwide public health problem, and patients with this disease are at high risk of developing complications, bacterial translocation from the intestinal lumen to the mesenteric nodes, and systemic circulation, resulting in the development of severe complications related to high mortality rate. The intestinal barrier is a structure with a physical and biochemical activity to maintain balance between the external environment, including bacteria and their products, and the internal environment. Patients with liver cirrhosis develop a series of alterations in different components of the intestinal barrier directly associated with the severity of liver disease that finally increased intestinal permeability. A “leaky gut” is an effect produced by damaged intestinal barrier; alterations in the function of tight junction proteins are related to bacterial translocation and their products. Instead, increasing serum proinflammatory cytokines and hemodynamics modification, which results in the appearance of complications of liver cirrhosis such as hepatic encephalopathy, variceal hemorrhage, bacterial spontaneous peritonitis, and hepatorenal syndrome. The intestinal microbiota plays a fundamental role in maintaining the proper function of the intestinal barrier; bacterial overgrowth and dysbiosis are two phenomena often present in people with liver cirrhosis favoring bacterial translocation. Increased intestinal permeability has an important role in the genesis of these complications, and treating it could be the base for prevention and partial treatment of these complications.

**Keywords:** liver cirrhosis, hepatic encephalopathy, spontaneous bacterial peritonitis, variceal hemorrhage, bacterial translocation, intestinal permeability

## Introduction

Liver cirrhosis is a worldwide public health problem, and patients with this disease are at high risk of developing complications associated with bacterial infections, particularly spontaneous bacterial peritonitis (SBP) and hepatic encephalopathy (HE).<sup>1-5</sup>

There is a very close link between the liver and the gastrointestinal tract; the liver is constantly exposed to nutrients, toxins, food-derived antigens, microbial products, and gastrointestinal tract microorganisms.

Susceptibility to the development of bacterial infections in this population is due to various abnormalities in defense mechanisms, including deficient bactericidal capacity and opsonization, abnormal monocytic cell activity, decreased reticuloendothelial phagocytic potential, deficient chemotaxis, decreased complement levels, increased intestinal transit time resulting in bacterial overgrowth, and an increase in intestinal permeability leading to bacterial translocation.

In liver cirrhosis, bacterial translocation is a common denominator in the genesis of several of its complications: upper gastrointestinal bleeding, HE, and SBP.

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Increased intestinal permeability may precede and promote translocation of bacteria (migration of microbes or their products into mesenteric lymph nodes),<sup>6</sup> endotoxins (such as lipopolysaccharides), and pathogen-associated molecular patterns (PAMPs) into the portal venous system and extraintestinal sites; their presence has been reported in animal models of cirrhosis with ascites (45%–78%) versus normal animals (0%–4%) and in patients with cirrhosis undergoing laparotomy, particularly in those with the greatest liver function compromise.<sup>7</sup>

Bacterial translocation leads to a systemic inflammatory response with subsequent increases in portal hypertension, exacerbating the characteristic hyperdynamic circulation in these patients, all negatively impacting liver function.<sup>6,8</sup>

Intestinal bacterial overgrowth plays an important role in the pathogenesis of bacterial translocation in cirrhotic patients; this hypothesis has been supported by the results of clinical and experimental investigation, in which growth inhibition of intestinal Gram-negative aerobic flora decreases the incidence of SBP in these patients.<sup>9</sup> This practice is recommended in the international clinical practice guidelines to prevent the development and recurrence of SBP<sup>10</sup> and the incidence of intestinal bacterial overgrowth, bacterial translocation, and bacterial peritonitis in animal models.

Another key event in bacterial translocation and overgrowth is the delay in intestinal transit time in patients with cirrhosis and experimental cirrhosis models; some prokinetic drugs such as cisapride have been shown to decrease bacterial translocation and overgrowth in patients with liver cirrhosis.<sup>11</sup> This effect has also been described with the use of nonselective beta-blockers (propranolol) in animal models of cirrhosis, accelerating intestinal transit time and decreasing bacterial translocation.<sup>12</sup>

Likewise, there are structural and functional changes in the intestinal barrier that may be secondary to vascular stasis resulting from portal hypertension and that can lead to increased intestinal permeability to bacterial migration and their products. Structural changes such as mucosal congestion and edema have been observed in cirrhotic patients in whom broadening of intercellular spaces has also been described.<sup>13</sup>

In this population, there is also recent evidence of probable susceptibility to the development of increased intestinal permeability. Patients with the nucleotide-binding oligomerization domain containing 2 (*NOD2*) and Toll-like receptor-2 (TLR2) are also at greater risk of developing SBP.<sup>14,15</sup>

## Intestinal barrier

A defensive function is also necessary to prevent the passage of potentially noxious substances, such as pathogenic

microorganisms, antigens, and proinflammatory factors, from the intestinal lumen into the internal milieu and that may simultaneously allow selective passage of substances favoring the development of the intestinal immune system; these functions depend on the intestinal barrier.<sup>16</sup>

The intestinal barrier is a physical and functional separation between the environment and the organism's interior. It is formed by a mucinous component secreted by intestinal epithelial cells and by the intestinal epithelium per se, creating an intercellular junction layer that allows selective passage of substances. The gastrointestinal epithelium constitutes the longest interphase with the external environment and allows nutrient absorption while also acting as a physical barrier to proinflammatory molecules such as pathogens, toxins, and antigens. In the intestinal lumen, bacteria and antigens are degraded by gastric acid and pancreatic juice and promote a microenvironment fostering commensalism and the generation of antibacterial products.<sup>12,17–19</sup> The most important components of the intestinal barrier are hereby described.

## Intestinal mucosa

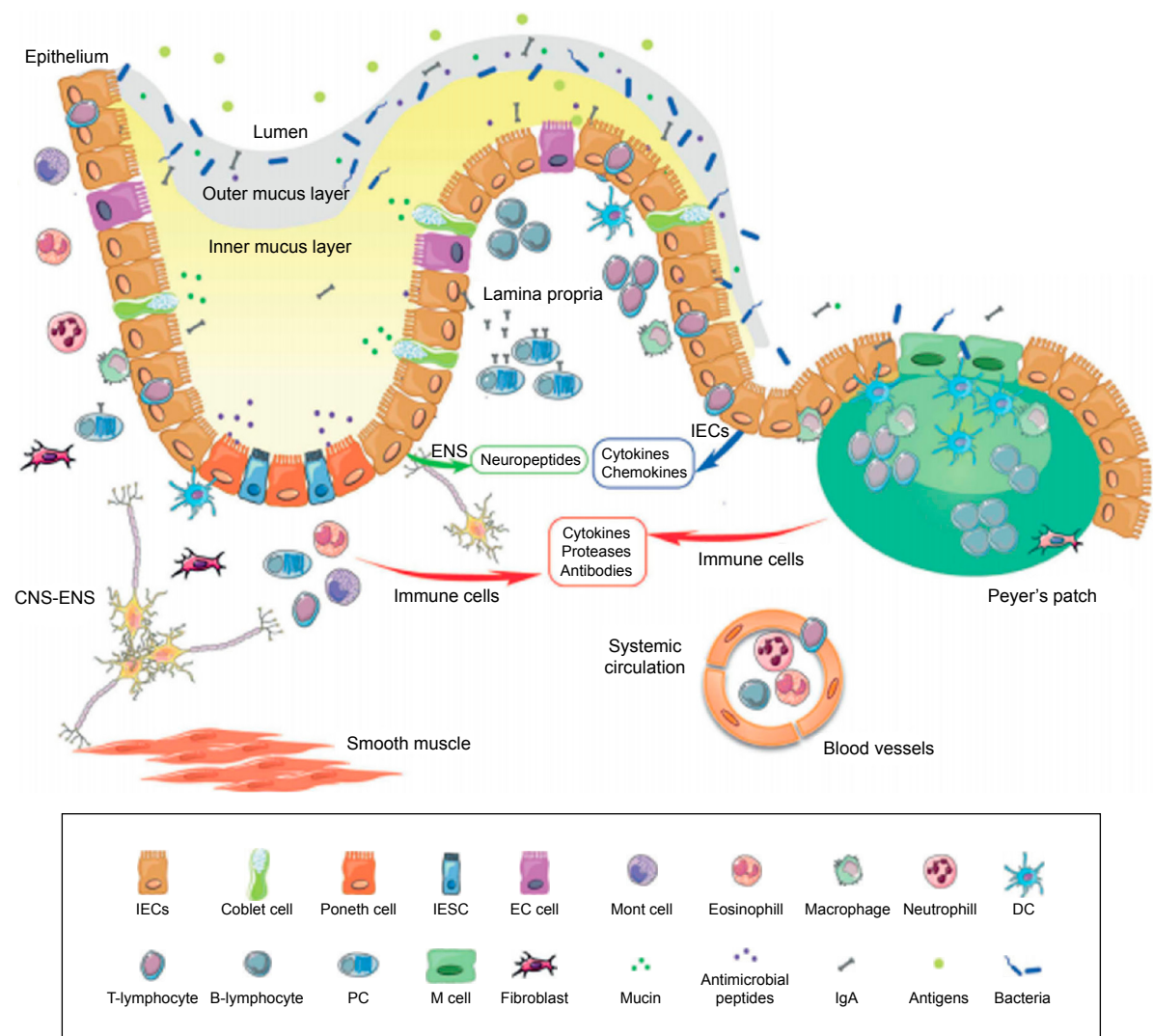
The intestinal epithelium is covered by a microclimate consisting of a nonagitated mucus layer 100  $\mu\text{m}$  in thickness, rich in mucin, water, and glycocalyx, mainly secreted by goblet cells with hydrophobic and tensioactive properties, which prevent the adhesion of enteric bacteria to the intestinal epithelium.<sup>19</sup> Externally, there is a layer of agitated mucus composed of mucin and antimicrobial substances; the thickness and composition of the mucus layer vary depending on its location in the intestinal tract, but it is most viscous in the distal colon.<sup>20</sup> Although the mucus layer prevents some organisms and large molecules such as food particles to directly access the epithelium, it can only slightly mitigate the flow of small molecules, ions, and water. The epithelial cell membrane is an efficient barrier against most hydrophilic solutes, but it would not be an appropriate barrier should the space between individual cells were not sealed by a series of intercellular junctions.<sup>20</sup>

## Cell types

Various cell types constitute the intestinal barrier's integrity. In order of frequency, we find absorptive cells or enterocytes, goblet cells, Paneth cells, enteroendocrine cells, and pluripotent stem cells (Figure 1).<sup>21</sup>

### Enterocytes or absorptive cells

These are characterized by a great abundance of microvilli on their apical surface, tightly distributed and parallel to each other,



**Figure 1** Components of the intestinal barrier. It is composed of intestinal microbiota, a mucin layer with IgA immunoglobulin (and other defense proteins) covering the mixed columnar epithelial cells (enterocytes, goblet cells, enterochromaffin cells and intestinal stem cells). Subepithelial region containing the lamina propria, the enteric nervous system, connective tissue and muscle layers. Intraepithelial lymphocytes are above, underlying basement membrane. There are many different immune cells, like macrophages, dendritic cells, plasma cells, lymphocytes and in some cases, neutrophils, and organized lymphoid tissue like lymphoid structures compound as the Peyer's patch (terminal ileum), containing M cells, dendritic cells and lymphocytes.

**Note:** Adapted with permission from Salvo-Romero E, Alonso Cotoner C, Pardo-Camacho C, et al. The intestinal barrier function and its involvement in digestive disease. *Rev Esp Enferm Dig.* 2015;107:686–696.<sup>21</sup>

**Abbreviations:** CNS, central nervous system; ENS, enteric nervous system; IEC, intestinal epithelial cell; IESC, intestinal epithelial stem cell; IgA, immunoglobulin A; EC, enterochromaffin cell; DC, dendritic cells; PC, plasma cell.

forming a structure known as a “brush border”. Each enterocyte has a mean of 3,000 microvilli, translating into a very large absorptive surface; enterocytes are key elements to the maintenance of the barrier's physical integrity. They also play a role in the development of immunologic activity since they express receptors implicated in the innate immune response,<sup>22</sup> and they act as nonprofessional antigen-presenting cells and release several cytokines and chemokines such as thymic stromal lymphopoietin, transforming growth factor- $\beta$ 1,<sup>23</sup> interleukin-25 (IL-25),<sup>24</sup> B cell proliferation stimulating factor (APRIL), and B-cell activating factor,<sup>25,26</sup> recruiting and activating leukocytes and regulating the local immune response.

## Intestinal microbiota

The term intestinal microbiota refers to the group of microorganisms residing in the human intestine that establish a symbiotic relationship with host. Most intestinal bacteria belong to ten phyla; five phyla represent the majority of bacteria that comprise the gut microbiota: 90% are Firmicutes (200 genera) and Bacteroidetes (25 genera). Other phyla are Actinobacteria, Proteobacteria, Verrucomicrobia, Fusobacteria, and Cyanobacteria.<sup>27</sup>

Microbiota functions can be grouped into three large areas: protective, trophic, and metabolic, and all play a role in the maintenance of epithelial cell integrity; they decrease colonization by pathogens by producing antimicrobial

substances, modify the pH, stimulate mucus secretion, and hence contribute to the baseline conservation of the host's defenses in a stationary state (Figure 1).<sup>27</sup>

## Intercellular junction proteins

Epithelial cells foster selective permeability in the following two ways: one is transcellular and the other is paracellular. The first (transcellular) involves nutrient absorption via

transporters or channels located on the apical or basolateral membrane, while the paracellular mechanism is associated with transport in the intercellular space between adjacent epithelial cells; it is regulated by apical union complexes and formed by tight junctions (TJ), anchoring junctions, and communicating junctions (GAP; Table 1).<sup>28–30</sup> Two types of intestinal permeability have been described: mediated by pores (characterized by its highly selective capacity in terms

**Table 1** Cellular junctions (intercellular bridge)

| Group                                  | Function   | Protein                             | Subtypes                                       | Specific function   | Location  |
|--|--|-------------------------------------|--|---|---|
| Tight Junctions (TJ)                   | Maintaining the barrier and epithelial polarity limits the diffusion of ions and translocation of luminal antigens from the apical region toward the basolateral membrane region | Occludin                            | Phosphorylated<br>Dephosphorylated             | Participates in the assembly and disassembly of TJ and control the passage of ions through the paracellular space   | TJ<br>Cytoplasm   |
|  |  | Claudins                            | 1, 3, 4, 5, 8, 9, 11 and 14<br>2, 7, 12 and 15 | Barrier   | Fibroblast  |
|  |  | Junctional adhesion molecules (JAM) | JAM-A<br>JAM-4                                 | Pore<br>Facilitate the assembly and the formation of functional and polarized TJ and regulate intestinal permeability and inflammation                            | Intercellular junctions<br>Intercellular junctions collocated with ZO-1 and MAGI-1<br>In the cell–cell contacts and collocated with ZO-1 in intestinal T84 cells                          |
|  |  | Tricellulin                         | –  | Stability and formation of the epithelial barrier, seal sheets, endothelial cells, without affecting ion permeability   | Intercellular contacts between three adjacent cells   |
|  |  | TJ adapter proteins                 | Zonula occludens (ZO)                          | ZO-1<br>ZO-2<br>ZO-3  | Regulation of cell permeability, adhesion, and stabilization of the TJ, transmission of signals from the junctions into cells for regulation of cellular processes such as cell migration |
| Anchoring junctions (AJ)               | Connect the cytoskeleton of each cell with the neighboring cell or to the extracellular matrix   | Adherens junctions                  | Cadherins<br>Catenins                          | Regulate adhesion between adjacent cells by transmembrane adhesion receptors and their regulatory proteins associated with actin                                  | Cytoskeleton  |
|  |  | Desmosome                           | Desmoglein<br>Desmocollin<br>Desmoplakin       | Transcellular network that confers mechanical strength to the tissues and allows cells to maintain their morphology   | Cytoskeleton  |
| Communicating junctions (GAP junction) | Allow communication between the cytoplasm of neighboring cells   | Connexin                            | –  | Regulates the mutual exchange of ions and small molecules of <1 kDa and it has a crucial role in the development, growth, and differentiation of epithelial cells | Cytoplasm   |

**Note:** Data from references.<sup>36–40</sup>

of particle size and charge) and by “leakage” (characterized by its low selective capacity).

The paracellular pathway is associated with transport through the intercellular space between adjacent epithelial cells; it is regulated by apical junctional complexes and conformed by TJ and adhesion junctions. The latter, in along with desmosomes, create strong links between epithelial cells and foster intercellular communication but do not determine paracellular permeability.<sup>31</sup> TJ not only determine paracellular selective permeability to solutes and create the barrier to noxious molecules but also form permeable pores to ions, solutes, and water. TJ are complexes formed by multiple proteins such as claudins, occludin, and zonula occludens 1 and a broad spectrum of cytosolic proteins. Modifications to the TJ barrier and paracellular permeability are dynamically regulated by several extracellular stimuli, closely linked to our health or disease susceptibility (Table 1).<sup>32</sup>

Injury to the TJ-mediated barrier and the consequent increase in paracellular permeability and luminal passage of proinflammatory proteins may lead to activation of the immune system associated with mucosa, a sustained inflammatory process and perpetuating tissue injury. Experimentally, the TJ-mediated barrier is evaluated by measuring the transepithelial electrical resistance and the paracellular passage of small molecules such as mannitol, dextran, and inulin.<sup>32–37</sup>

### TJ regulation

TJ regulation has been studied in physiological conditions, in cell culture, and in animal models. TJ barrier dysfunction mediated by cytokines leads to immune activation and tissue inflammation, which may be an important factor triggering and/or in the development of several intestinal and systemic diseases.<sup>30</sup>

1. Interferon gamma: this cytokine is mainly secreted by T-cells and natural killer cells. It is a proinflammatory cytokine associated with increased paracellular intestinal permeability due to TJ protein redistribution; induces TJ internalization and a subsequent increase in intestinal permeability.
2. TNF- $\alpha$ : this is a proinflammatory protein preferentially produced by T-cells and macrophages. This factor is known to induce apoptosis and inflammation of intestinal epithelial cells while also interferes with the intestinal TJ barrier through different mechanisms. TNF- $\alpha$  alters the TJ intestinal barrier by rearranging the cytoskeleton and TJ expression.<sup>30</sup> In enterocytes decreases occludin mRNA expression but increases that of claudin 2 and 8.<sup>38</sup>

3. IL-4: it is mainly secreted by T-cells, basophils, and mastocytes, and plays a significant role in the humoral and adaptive immune responses. The mechanism through which IL-4 alters TJ remains unclear, but it has been shown to decrease the epithelial electric resistance and increase the flow of dextran.<sup>30</sup>
4. IL-6: this is a cytokine with pleiotropic activities, whose expression is important in host defense against a great number of pathogens. IL-6 selectively increases intestinal permeability to cations but not to macromolecules, by increasing the pores formed by claudin 2.
5. IL-10: this protein is primarily produced by regulatory T-cells, monocytes, macrophages, and dendritic cells. It appears that IL-10 has a protective effect on the TJ barrier since it is an antagonist to some of the effects of TNF- $\alpha$ . In intestinal cells, IL-10 prevents the effects of interferon gamma-mediated increase in mannitol flow. Injury from parenteral nutrition in animal models is associated with decreased mucosal IL-10 levels.<sup>30</sup>

### Intestinal immunity

The intestinal barrier does not totally impede the passage of antigens and their penetration is restricted by local immune mechanisms. In particular, the subepithelial lamina propria contains a large number of antigen-presenting dendritic cells that sample the environment and process commensal and pathogenic bacteria; they subsequently migrate to mesenteric lymph nodes or Peyer patches and prime virgin T-lymphocytes. Live enteric bacteria have been found in the liver although contained by Kupffer cells, suggesting that the liver is a second barrier limiting and depurating commensal or pathogenic bacteria if the first line of defense mechanisms have been overwhelmed.<sup>39</sup>

Another fundamental component of the intestinal barrier is constituted by intestinal macrophages (CX3CR1) that through TLRs can detect and subsequently activate innate lymphoid tissue secreting IL-22 that in turn promotes the repair and maintenance of the epithelium's integrity (Figure 1).<sup>40</sup>

### Effects of liver cirrhosis on the intestinal barrier

#### Inflammation and TJ in patients with liver cirrhosis

Recent studies have shown that patients with high Child–Pugh–Turcotte scores have increased serum TNF- $\alpha$  levels.<sup>15,41</sup> Other cytokines and the presence of necrosis may lead to short- and long-term liver decompensation. In cirrhosis, one of the main contributors to TJ abnormalities is the



increased production of TNF- $\alpha$  by monocytes in mesenteric lymph nodes,<sup>42</sup> favoring the decreased expression of proteins such as occludin but an increase in claudin 2 and 8.

A recent study by Assimakopoulos et al<sup>43</sup> revealed altered intestinal TJ protein expression in patients with liver cirrhosis as a pathogenic mechanism of increased intestinal permeability; they demonstrated that occludin and claudin 1 expression is decreased in the intestinal epithelium of these patients. According to their immunohistochemistry results, this downregulation was more significant in decompensated cirrhosis (CHILD B and C). A specific intestinal pattern was observed in the case of occludin, with a gradual decrease in the expression of crypts in villi.

The presence of activated CD33<sup>+</sup>CD14<sup>+</sup> intestinal macrophages (nitric oxide for producing cells: TREM-1 iNOS), which in other diseases such as necrotizing enterocolitis are associated with intestinal mucosal repair,<sup>44</sup> has been recently found in duodenal epithelium in patients with decompensated cirrhosis; they were associated with increased serum levels of lipopolysaccharide, nitric oxide, IL-8, IL-6, and claudin levels, decreased duodenal resistance and increased intestinal permeability.<sup>45</sup>

The chemoattractant cytokine CX3CR1, plays an important role in modulating inflammatory responses, including monocyte homeostasis and macrophage phenotype and function.<sup>46</sup> In a nonalcoholic steatohepatitis mouse model, CX3CR1 was shown to limit the progression of liver inflammation and early glucose intolerance by maintaining the intestinal barrier's integrity; this effect hinged on the intestinal microbiota's composition and the receptor acts as a gatekeeper in diet-induced steatohepatitis, maintaining intestinal permeability.<sup>40</sup>

## Intestinal mucus in the patient with liver cirrhosis

Mucin secretion is affected by transcription factors, such as nuclear factor- $\kappa$ B, growth factors, lipopolysaccharides, the presence of microorganisms, and proinflammatory cytokines.

Increased mucins MUC2 and MUC3 mRNA expression has been reported in the ileum of rats with cirrhosis compared with rats without cirrhosis.<sup>47</sup> Intestinal mucus modulates bacterial adhesion to the intestinal surface.

## Intestinal microbiota in the patient with liver cirrhosis

Two pathological processes of intestinal microbiota have been described in patients with liver cirrhosis, fostering an intestinal and systemic inflammatory state: small intestinal

bacterial overgrowth (SIBO) and bacterial dysbiosis, two phenomena that are not mutually exclusive.<sup>48</sup>

### Small intestinal bacterial overgrowth

SIBO is defined as excessive bacterial growth in the small intestine and the constellation of symptoms to which it leads, but there is no clear consensus on this entity's definition. Tests such as proximal jejunal aspirate culture have historically defined SIBO as bacterial growing at  $\geq 10^5$  colony forming units, and noninvasive tests such as breath tests with different carbohydrates have also been used for diagnosis.<sup>49</sup> Factors fostering SIBO development in the liver cirrhosis population include decreased gastric acid production, alterations in gastrointestinal motility (increased intestinal transit time), decreased intestinal biliary acid availability as well as decreased peptides with antimicrobial activity.<sup>50</sup>

SIBO appears to play an important role in the pathogenesis of bacterial translocation; it has been frequently associated with the severity of liver disease, particularly in patients with a history of SBP and/or HE.<sup>51</sup> Clinical and experimental research has shown that inhibition of intestinal Gram-negative aerobic flora growth decreases the incidence of SBP in patients with cirrhosis.<sup>10</sup>

### Intestinal dysbiosis in the patient with liver cirrhosis

Dysbiosis refers to the altered diversity in bacterial families in the gastrointestinal tract, resulting from a decrease in commensal microbiota; dysbiosis has been described in the study of the stool, saliva, and colonic mucosa of patients with liver cirrhosis and it appears to correlate with disease severity, systemic inflammation, and the number of hospitalizations.<sup>52-54</sup>

Changes in diet and intestinal inflammation may promote intestinal dysbiosis; for example, a high-fat diet changes the ratios from *Firmicutes* and *Proteobacterias* to *Proteobacterias* and *Bacteroides*, thus activating the inflammasome and leading to the development of steatohepatitis in a mouse model.<sup>55</sup>

Abnormalities in intestinal microbiota have also been shown in patients with HE when compared with patients with hepatic cirrhosis and no HE and healthy individuals; certain bacterial families appear to be associated with HE (*Alcaligenaceae*, *Porphyromonadaceae*, and *Enterobacteriaceae*), cognitive dysfunction, and inflammation in these patients.<sup>56</sup>

### Intestinal transit

Another important factor in bacterial translocation and bacterial overgrowth is the delay in intestinal transit time observed

in the cirrhotic patient and in experimental cirrhosis models. Patients with decompensated cirrhosis have a slower transit time compared with patients with compensated cirrhosis and healthy individuals.<sup>50</sup> Moreover, some prokinetic drugs such as cisapride have been shown to decrease bacterial translocation and overgrowth in cirrhotic patients.<sup>11</sup> This effect has also been described with the use of nonselective beta-blockers (propranolol) in cirrhosis animal models, and they also accelerated their intestinal transit time and decreased bacterial translocation.<sup>12</sup>

### Intestinal wall structural changes

Portal hypertension-induced vascular stasis and that may lead to an increase in intestinal permeability. Structural changes such as mucosal congestion and edema have been observed in cirrhotic patients in whom broadening of intercellular spaces has also been described.<sup>13</sup>

There is also recent evidence of probable susceptibility in this population to increased intestinal permeability, since patients with the NOD2 and TLR2 polymorphisms are at greater risk of developing SBP and have greater mortality than patients with minor alleles, associated with increased intestinal permeability and a greater presence of markers derived from bacterial products.<sup>14,15</sup>

### Intestinal permeability in the patient with liver cirrhosis

Alteration in intestinal permeability (increase) is a common finding in patients with liver injury, particularly in patients with liver cirrhosis although it has also been described in different entities associated with chronic liver disease such as alcohol-induced injury, nonalcohol fatty liver disease, and HCV-mediated injury.<sup>57</sup> In patients with liver cirrhosis, alterations in intestinal permeability correlate with the degree of liver injury whereby Child–Pugh C patients and those with ascites or a positive history of SBP have increased intestinal permeability to the passage of molecules<sup>58</sup> although patients with compensated cirrhosis have been shown to have increased colonic permeability but not gastroduodenal or intestinal.<sup>59</sup> However, other authors have been unable to reproduce these results.<sup>60</sup>

### Consequences of increased intestinal permeability

Increased intestinal permeability may precede and promote translocation of bacteria,<sup>6</sup> endotoxins (such as lipopolysaccharides), and pathogen-associated molecules into the portal venous system and extraintestinal sites.

### Bacterial translocation

There are at least four mechanisms underlying bacterial translocation: intestinal bacterial overgrowth, bacterial dysbiosis, immune system abnormalities, and increased intestinal permeability. The gastrointestinal tract is an actively immune organ that essentially includes all leukocyte types involved in the immune response. Changes in the local and systemic immune system are clinically relevant since they foster bacterial translocation in cirrhosis. Patients with cirrhosis have immunologic abnormalities that facilitate the development of infections and bacterial translocation. In summary, these four previously mentioned factors play a pivotal role in the pathogenesis of bacterial translocation in cirrhosis and also explain part of the high prevalence in cirrhosis compared with other clinical scenarios in which only some of these factors are actively involved.

Experimental studies have also suggested that bacterial translocation is associated with deterioration in the hemodynamics of patients with cirrhosis.<sup>61–63</sup>

### Liver injury

Intestinal inflammation and bacterial translocation impact directly the progression to liver fibrosis and decompensation of preexisting chronic liver damage.

The products of bacterial metabolism may be hepatotoxic substances (phenols, ethanol, acetaldehyde, ammonia, and benzodiazepines) that are metabolized in the liver via the portal circulation.<sup>48</sup> Moreover, the lipopolysaccharides released by Gram-negative bacteria attach to the lipopolysaccharide-binding protein that activates Kupffer cells by binding to CD14. The association between CD14 and TLR4 on the cell surface triggers the inflammation cascade, suggesting that microbiota may directly mediate inflammatory processes in the liver.

The persistent liver inflammation generated by an aggressive stimulus (ie, alcohol, virus, fat deposits, etc) plus the inflammation resulting from the translocation of bacteria or their products play an important role in the development of liver fibrosis through the activation of TLR2, a receptor for Gram-positive bacterial products that promotes an inflammatory cascade mediated by the monocytes in the intestinal lamina propria after its activation via the tumor necrosis factor receptor type I (TNFR1). A study of knock-out mice for the TLR2 gene (TLR2<sup>-/-</sup>) with cholestatic liver injury revealed decreased bacterial translocation into the mesenteric lymph nodes and less endotoxemia compared with wild mice; furthermore, TNFR1<sup>-/-</sup> mice are protected against the development of liver fibrosis due to decreased extracellular matrix deposits.<sup>64</sup> Decreasing the levels of serum

lipopolysaccharides with nonabsorbable antibiotics (neomycin) attenuated liver fibrosis development in a nonalcoholic steatohepatitis animal model, by decreasing intestinal permeability as a result of increased TJ expression and decreased TLR4 activity and hepatic stellate cell activation.<sup>65</sup>

Acute-on-chronic liver failure refers to a condition that develops in patients with chronic liver disease leading to organ failure and associated with high short-term mortality rates; it is a distinct entity from liver decompensation or acute liver failure.<sup>66</sup> The most frequent precipitating factor is infection, particularly SBP, pneumonia, and urinary tract infection.<sup>67</sup>

## Variceal hemorrhage

Patients with cirrhosis and upper gastrointestinal bleeding are at increased risk of acquiring bacterial infections (25%–65%), particularly SBP during the first 7 days after bleeding; furthermore, bacterial infections increase the risk of early rebleeding.<sup>68</sup> Patients with liver cirrhosis and increased intestinal permeability, increased lipopolysaccharide binding protein, and elevated IL-6 levels represent a population highly prone to develop variceal hemorrhage.<sup>15</sup>

Infectious processes have been described to increase portal pressure and changes in homeostasis, and the administration of prophylactic antibiotic therapy appears to have a beneficial role in terms of controlling hemorrhage and preventing rebleeding. Current international guidelines actually recommend the use of prophylactic antibiotics in patients with cirrhosis and gastrointestinal bleeding, regardless of the presence or lack of ascites.<sup>37,69</sup> A recent meta-analysis of 12 studies comparing a population of 1,241 patients with cirrhosis and gastrointestinal bleeding determined that the use of prophylactic antibiotics significantly decreased the incidence of bacterial infections, the incidence of rebleeding, the duration of hospitalization and mortality.<sup>70</sup>

## Infections, SBP

Bacteremia and SBP are the main consequences of bacterial translocation in patients with cirrhosis.<sup>71</sup> Patients with decompensated cirrhosis and high-risk alleles for the NOD2 associated with functional alterations of the intestinal wall are at increased risk of developing SBP and bacteriascites.<sup>72,73</sup>

Almost half of cirrhotic patients are infected at hospital admission or develop infections during their hospitalization.<sup>71</sup> Inhospital mortality is greater in patients who develop bacterial infections than in those who do not.<sup>74</sup> The prognosis of

bacterial peritonitis has improved in the past few decades due to early diagnosis, better established criteria, and the timely use of antibiotic therapy.<sup>10</sup>

Bacterial DNA translocation in patients with ascites and portal hypertension worsens systemic circulation, leading to subsequent exacerbation of peripheral vasodilation; this has been related to an increase in the inflammatory status, characterized by elevated TNF- $\alpha$  levels.<sup>62</sup> Worsening of hyperdynamic circulation in this inflammatory state due to bacterial translocation has been suggested to play a role in the development of portal hypertension complications, particularly the hepatorenal syndrome. Recent experimental studies have shown that in the cirrhotic population, the expression of TLR4 and proinflammatory cytokines (TNF- $\alpha$ ) are increased in the kidney and more prone to inflammatory insult in the presence of bacterial translocation.<sup>75</sup>

The presence of bacterial DNA in refractory ascites showed that translocation was associated with abnormalities in cardiovascular and kidney function, as well as a greater risk of hepatorenal syndrome and death; the use of antibiotics like rifaximin improves systemic hemodynamics alterations and renal function.<sup>76,77</sup>

One-third of patients with SBP with antibiotics develop kidney failure that may be transient, permanent, or rapidly progressive in 25%, 33%, and 42% of cases, respectively.<sup>78,79</sup>

International associations recommend that primary prophylaxis of SBP be administered to cirrhotics with a low protein concentration in ascitic fluid (<1.5 g/dL), as well as to patients with renal dysfunction (serum creatinine  $\geq$ 1.2 mg/dL, BUN  $\geq$ 25 mg/dL, or serum sodium  $\leq$ 130 mEq/L) or severe liver dysfunction (Child–Pugh–Turcotte  $\geq$ 9 and serum bilirubin  $\geq$ 3 mg/dL), and those with a previous episode of SBP. The antibiotics of choice are norfloxacin, ciprofloxacin, and trimethoprim–sulfamethoxazole.<sup>10</sup>

## Hepatic encephalopathy

In the last decade, several studies have suggested a synergistic effect of inflammation and infection on the pathogenesis of HE.<sup>80,81</sup>

HE is usually associated with signs of the systemic inflammatory response syndrome.<sup>82</sup> A prospective study proved the association between systemic inflammatory response syndrome or infection and the development of severe HE, independently of ammonia levels.<sup>83</sup> Systemic inflammation associated with bacterial translocation may play a role in the pathogenesis of HE. SIBO has also been associated with bacterial translocation and an increased prevalence of HE in liver cirrhosis.<sup>84</sup>



Bajaj et al<sup>52</sup> demonstrated an association between certain intestinal bacterial families, altered cognition, and IL-17/IL-23-mediated inflammation in patients with HE.

## Analytic methods to determine intestinal permeability

Intestinal permeability refers to the property that allows the exchange of solutes and fluid between the intestinal lumen and tissues.

Several tools have been developed to measure it, both *in vivo* and *ex vivo*. Although there are accessible and applicable methods to measure permeability in humans, result interpretation may be complex. Various authors still debate the clinical significance of increased intestinal permeability and even the normal values of intestinal permeability settings (Table 2). These differences might be explained by several factors such as intestinal transit time, surface area, kidney function, different liver disease entities, time evolution of the disease, comorbidities, the type of test used to measure intestinal permeability (testing with isotopes is considered the gold standard test), and the considered cutoff limits (Table 2).

Studies using sugar as a differential marker in absorption tests, combining an oligosaccharide and a monosaccharide, such as in lactulose/mannitol (L/M) and saccharose/mannitol, revealed increased intestinal permeability in patients with liver cirrhosis.<sup>6,15,57–59,85–96</sup>

Intestinal permeability can be measured with the administration of nondigestible markers that cross the mucosal barrier by passive diffusion.<sup>97</sup> These markers have been observed to pass through the intestinal lumen to the extraintestinal space and can be detected in blood, urine, tissues, and other organs. The most commonly used markers are some saccharides, radioisotopes such as Cr-EDTA<sup>58</sup> and polyethylene glycols.<sup>96,98</sup>

In the 1970s, oligosaccharides were introduced for intestinal permeability evaluation.<sup>99</sup> Throughout the world, saccharides with different molecular weights, absorption routes, and levels of urinary excretion have been used as parameters to determine intestinal permeability.<sup>100–104</sup>

Several saccharide quantification methods have been developed to determine changes in intestinal and gastroduodenal permeability, since sugars, such as lactulose, saccharose, mannitol, xylose, and rhamnose, among others, are known to provide this information.<sup>98,101,105–110</sup>

Mannitol is one of the most studied monosaccharides. It is absorbed in healthy small intestinal mucosa, travels via the transcellular aqueous pores, the hydrophilic region of the

cell membrane, ultimately reflecting the absorption of small molecules.<sup>106</sup> Lactulose and saccharose, which are absorbed through the cell wall union complexes, that is, the TJ and extrusion zones of intervillous spaces, reflecting permeability to large molecules.<sup>104,107</sup>

Mannitol is currently considered a marker of the mucosal absorption zone, while lactulose absorption is a marker of TJ integrity in the intestinal mucosa.<sup>11,12</sup> The L/M ratio is an index evaluating intestinal permeability; when it increases, one can conclude that intestinal permeability is also increased. However, lactulose and mannitol are degraded by colonic bacteria and are of no use in evaluating colonic permeability. Lactulose is large in size and can only cross the barrier if there is intestinal epithelial injury, so it is considered a marker of epithelial integrity. Furthermore, mannitol is one-third the size of lactulose; it crosses the intestinal epithelium through pores and it is used to determine the surface area. In patients with liver cirrhosis and in other diseases compromising the intestinal wall, intestinal permeability is increased.<sup>13,111</sup>

The saccharose/mannitol ratio is used as an index of gastroduodenal permeability.<sup>112</sup> Saccharose is an ideal marker to evaluate gastric permeability and it has been evaluated in endoscopic studies analyzing mucosal injury after the ingestion of acetylsalicylic acid,<sup>113</sup> alcohol use,<sup>114</sup> and other diseases.<sup>115</sup> In the liver cirrhosis population, gastric permeability to saccharose has been demonstrated in patients with portal hypertension and it correlates with the severity of gastric lesions (urinary retrieval  $\geq 0.15\%$ ); gastric permeability to saccharose returned to normal after the administration of nonselective beta-blockers.<sup>116,117</sup>

The quantitative analysis of sugars is difficult since they are highly polar compounds, as well as hydrophilic, thermosensitive, their structure lacks chromophores, their  $pK_a$ 's are  $>10$ , and they are not easily ionized due to their low acidity and volatility.

However, saccharides have been identified and quantified with several extraction techniques and different detection instruments. High-pressure liquid chromatography is the most often used instrument, coupled to various types of detectors such as ultraviolet or visible UV light,<sup>118</sup> fluorescence, electrochemical, evaporative light scattering detector,<sup>119</sup> refraction index detector,<sup>120</sup> charged aerosol detector,<sup>121</sup> and masses.<sup>104,108–110</sup>

Additionally, gas chromatography–mass spectrometry<sup>122,123</sup> in which some mono- and disaccharides are derivatized for quantification is used; other methods include high-performance anion-exchange chromatography with pulsed

**Table 2** Intestinal permeability markers, analytic techniques, and ratios in urinary samples of patients with cirrhosis

| Marker             | N   | Aim of study   | Analytic technique and dosage   | Results  | Study                             |
|--------------------|---|--|---|--|-----------------------------------|
| L/M                | 46 patients with LC<br>16 healthy controls  | Analyze the impact of increased IP on mortality and development of infections in patients with cirrhosis   | LC-MS/MS<br>Osmotic solution (100 mL) prepared with 2 g of M, 5 g of L, and 40 g of G; urine collected for 5 hours  | Thirty nine (85%) patients with LC have a pathologically increased IP ratio (L/M >0.07) compared with four (25%) healthy controls (P<0.0001).<br>Comparing controls with LC patients, L recovery was higher in LC patients (median 2.0% vs 0.6%, P=0.005), while M recovery was decreased (11.2% vs 16.1%, P=0.03). Pathologically increased IP was defined by an IP ratio >0.07. IP index did not predict time to infection, infection-free survival, or overall survival. IP was significantly greater in LC patients than in healthy controls.  | Vogt et al <sup>85</sup> 2016     |
| L/M                | 18 patients with LC<br>15 healthy controls  | Determine if there is an association between nutritional status through subjective overall evaluation, anthropometry, dynamometry, phase angle, and IP in LC patients who are candidates for liver transplantation | Ion exclusion chromatography<br>Isosmolar solution, 120 mL with 6.25 g of L and 3.0 g of M<br>Urine collection for 5 hours in LC patients and for 6 hours in controls | In LC patients, the percentage of L (n=14) was 0.14 (0.01–2.74), while that of M was 9.09 (2.44–24.34) and the L/M ratio was 0.0209 (0.0012–0.1984).<br>In controls, the percentage of L (n=15) was 0.07 (0.05–0.28), while that of M was 21 (18.30–28) and the L/M ratio was 0.003 (0.0020–0.0013, P=0.01).<br>However, IP was not affected by the nutritional status of LC patients. There was no significant difference in IP in malnourished patients (median, 0.032; range, 0.002–0.079) vs well-nourished LC patients (median, 0.010; range, 0.001–0.198), according to overall subjective evaluation. | Liboredo et al <sup>86</sup> 2015 |
| L/M                | Eleven subjects with cystic fibrosis (CF) and LC (CFLC)<br>19 with CF and no liver disease (CFnoLD) | Determine the frequency of macroscopic intestinal lesions, intestinal inflammation, IP, and characterized fecal microbiome in subjects with CF or CFnoLD   | Cation-exchange chromatography<br>5 g of L and 2 g of M dissolved in 100 mL water<br>Urine was collected for 5 hours  | IP measured by L/M ratio was abnormal (>0.03) in 27/28 of subjects. However, L/M was slightly higher in patients with CFnoLD than in subjects with CF (0.11±0.05 vs 0.08±0.02, P=0.04). Neither the absorption percentage of L nor M was different between groups.   | Flass et al <sup>87</sup> 2015    |
| L/M                | 39 patients<br>21 controls  | Determine the prevalence of altered IP in children with NASH and its possible association with the stage of liver disease  | LC-MS/MS<br>5 g of L and 1 g of M dissolved in 120 mL water<br>Urine was collected for 6 hours  | L/M was abnormal in 12/39 patients (31%) and none of the controls. IP was highest in children with NASH, with an L/M ratio of 0.038±0.037 vs 0.008±0.007 (P<0.05). In the group of children with NASH, IP increased in those with steatohepatitis compared with those with only steatosis (0.05±0.04 vs 0.03 vs 0.03, P<0.05).   | Giorgio et al <sup>88</sup> 2014  |
| SS/R<br>L/R<br>S/E | 26 patients with compensated LC (CLC)<br>27 controls  | Evaluate IP in patients with stable CLC and controls by determining protein  | High-pressure ion-exchange chromatography<br>1 g of SS, 1 g of L, 0.5 g   | Gastrointestinal permeability was reflected by SS/R urinary excretion between 0 hour and 5 hours; there was no difference between CIR patients and controls  | Pijls et al <sup>89</sup> 2014    |

|             |  |   |  |                                       |
|-------------|--|---|--|---------------------------------------|
|             | expression in T] in duodenal and sigmoid mucosa biopsies   | of R, 1 g of E, and 1 g of S dissolved in 150 mL drinking water<br>Urine was collected for 24 hours in two separate fractions: 0–5 hours for SS/R and L/R, and 5–24 hours for S/E | [5.979 (0.547–24.880) vs 6.265 (2.226–30.469) $\mu\text{mol}$ , $P=0.881$ ].<br>The 0–5-hour urinary L/R ratio reflecting increased small IP was also similar in both groups [0.023 (0.010–0.115) vs 0.023 (from 0.006 to 0.069), $P=0.455$ ].<br>However, colon IP was significantly increased in patients with LC compared with controls, reflected in an elevated S/E ratio in 5–24-hour urine [0.019 (0.008–0.051) vs 0.015 (0.008–0.034), $P=0.006$ ].<br>The urinary S/E 0–24 hours ratio, reflecting IP of the entire intestine, was also significantly higher in patients with LC compared with controls [0.020 (0.010–0.037) vs 0.016 (from 0.008 to 0.028), $P=0.003$ ].<br>Gastroduodenal and IP were abnormal in 72% and 59% of patients, respectively.<br>Urinary saccharose was $76.2\pm 56$ mg/dL, $P=0.049$ ( $n=50$ ).<br>S/M ratio was $0.030\pm 0.025$ , $P=0.007$ ( $n=50$ ).<br>IP ratio was $0.038\pm 0.030$ , $P=0.030$ and $P=0.002$ .<br>Abnormal gastroduodenal permeability was defined as an SS urinary concentration $>43$ mg and/or an SS/M $>0.0142$ .<br>IP index was calculated on the basis of L and M excretion in every urine collection for 5 hours.<br>The normal reference permeability is defined as $<0.030$ of the L and M percentages.<br>The use of nonselective beta-blockers improved gastroduodenal/intestinal permeability and decreased bacterial translocation independently from hemodynamic effects. | Reiberger et al <sup>15</sup><br>2013 |
| SS/M<br>L/M | Evaluate gastrointestinal permeability and bacterial translocation in patients with PHT and LC, before and after treatment with nonselective beta-blockers | HPLC<br>100 mL water with 20 g of SS, 10 g of L, and 5 g of M<br>Urine was collected for 5 hours  |  |                                       |
| SS/M<br>L/M | 50 patients with LC (72% males, 18% with ascites, 60% alcohol-induced)   | HPAEC-PAD<br>Solution of 10 g of L, 5 g of M, and 20 g of SS dissolved in 100 mL water.<br>Administer six pills with 5 g of S each<br>Urine was collected for 5 hours             | Norman et al <sup>89</sup><br>2012   |                                       |
| SS/M<br>L/M | Determine if malnutrition is associated with IP, measured by urinary excretion of nonmetabolized sugars, in patients with LC                               | 64 patients (21 well nourished, 23 moderately nourished, and 20 severely malnourished LC patients) 63 healthy controls  |  |                                       |
| PEG         | Compare ciprofloxacin PO vs ceftriaxone  | Mixture of PEG of different molecular weights   | Kim et al <sup>90</sup> 2011   |                                       |

(Continued)

Table 2 (Continued)

| Marker                | N   | Aim of study   | Analytic technique and dosage  | Results   | Study                                |
|-----------------------|---|--|--|---|--------------------------------------|
| L/M                   | 217 subjects (134 controls and 83 patients with CLI)                | intravenous as prophylaxis against bacterial infections in patients with decompensated LC and gastrointestinal bleeding<br>Explore the relationship between IP, the type and degree of plasma levels of proinflammatory cytokines and S-nitrosothiols and nitrites in patients with different types and degrees of CLI | Urine was collected 24 hours after mixture administration<br><br>HPAEC-PAD<br>Oral bolus of 5 g of L and 2 g of M dissolved in 150 mL drinking water<br>Urine was collected for 5 hours  | The permeability index may be a tool to predict the development of infections in this population.<br><br>Significant differences ( $P < 0.01$ ) in IP values between controls and CLI patients (mean $\pm$ SD was $0.016 \pm 0.014$ in controls, $0.037 \pm 0.04$ in CLI patients, and $0.056 \pm 0.08$ in LC).<br>IP values were considered normal when the ratio of the two sugars (L/M) was $< 0.030$ ; this cutoff limit equalled the calculated mean + 2SD value in a large group of healthy subjects. | Cariello et al <sup>17</sup> 2010    |
| L/M                   | Seven patients with LR (Child–Pugh A and B)                         | Detect bacterial DNA in CIR patients with or without ascites and correlate with intestinal transit time and IP   | Urine analyzed by HPLC<br>L/M test<br>Urine collected for 5 hours  | IP expressed as the ratio between the % excreted L and the % excreted M ( $R = \%L/\%M$ ), healthy controls to determine normal value.<br>IP was increased in all patients with CIR compared with healthy subjects ( $0.05 \pm 0.01$ vs $0.02 \pm 0.005$ ).<br>Six patients had an abnormal intestinal transit time, only one patient had bacterial DNA in blood and ascitic fluid.   | Thalheimer et al <sup>6</sup> 2010   |
| <sup>51</sup> Cr-EDTA | 52 patients with LC<br>48 healthy controls                          | Compare IP in LC patients with or without SBP  | Urine analyzed with $\gamma$ -counter<br>PO administration of 96 MBq <sup>51</sup> Cr-EDTA in 10 mL water<br>Urine collected for 24 hours<br><sup>51</sup> Cr-EDTA clearance was determined with the formula: $([\text{mean urinary counts} \times \text{urinary volume}] \times [\text{standard count} \times 50]) - 1$ | Altered IP = Cr-EDTA $\geq 3\%$ .<br>IP was abnormal in 45% of CIR patients vs 4% controls. Altered IP was associated with the degree of liver injury (determined by Child–Pugh score):<br>– 75% in Child C vs 39% Child B vs 22% Child A.<br>– Patients with ascites 60% vs no ascites 31%.<br>– 100% in patients with a previous history of SBP vs 50% in those with no previous SBP.<br>– Cr-EDTA was found in ascitic fluid, suggesting altered permeability of splanchnic vessels and peritoneum.      | Scarpellini et al <sup>18</sup> 2010 |
| L/M                   | 86 patients with PBC<br>69 with hepatitis C<br>155 healthy controls | Compare the measured gastrointestinal permeability in patients with PBC to that in patients with liver disease (hepatitis C) and healthy controls  | Thin-layer chromatography<br>100 g of SS, 5 g of L, and 2 g of M dissolved in water<br>Urine was collected for 8 hours   | S urinary excretion in PBC patients ( $133.89 \pm 72.56$ mg) was significantly higher than in patients with hepatitis C ( $101.07 \pm 63.35$ mg) and healthy controls ( $89.46 \pm 41.76$ mg) ( $P = 0.0001$ ), suggesting abnormal gastric permeability or abnormal IP of the proximal small intestine. Values in the hepatitis C cohort were not significantly different to those of healthy controls.  | Feld et al <sup>11</sup> 2006        |

The L/M excretion ratio reflects small IP, which was also increased in the PBC group (0.017±0.012) compared with healthy controls (0.012±0.007;  $P=0.0001$ ) but the same as in patients with hepatitis C (0.016±0.011). Both PBC and hepatitis C patients had significantly higher L/M ratios than healthy controls (0.012±0.007, median =0.011) ( $P=0.0001$ ). In PBC group, 21 (25.0%) had "abnormal" permeability, defined as > 2SD above the mean values in healthy controls.

Zuckerman et al<sup>92</sup>  
2004

Comparing controls with LC patients, the L/R ratio in LC and ascites was significantly higher (0.058 vs 0.037,  $P<0.001$ ), but not in LC without ascites (0.041 vs 0.037). Patients with ascites had a significantly lower mean retrieval of 3-O-methyl-D-glucose (3mGlc) (23.0% vs 49.1%;  $P<0.001$ ), × (18.8% vs 34.5%;  $P<0.001$ ), R (4.0% vs 9.1%;  $P<0.001$ ), and L (0.202% vs 0.337%;  $P<0.001$ ) than normal subjects.

However, the mean R/3mGlc ratio was the same in LC with ascites and in normal subjects (0.189 vs 0.189), suggesting that decreased sugar retrieval is due to nonmucosal factors.

Comparison of controls and patients with LC and ascites showed that LC and ascites patients had abnormal IP, measured by the L/R urinary excretion and normal absorption in the small intestine, determined by the R/3m Glc urinary ratio. In LC patients, low sugar retrieval was observed apparently, as a result of nonintestinal factors compromising clearance and not due to decreased intestinal absorption.

Pascual et al<sup>93</sup> 2003

The L/M ratio was significantly higher in LC than in controls, particularly in the final stages of LC. Patients with ascites or encephalopathy had a statistically significant increase in the L/M ratio compared with patients without these complications.

Xu et al<sup>94</sup> 2002

L and M excretion rates (L%) and (M%) were determined and the L/M ratio was calculated. Treatment with TIPS and modified Sugiura decreased portal pressure and directly improved IP

Thin-layer chromatography  
Isosmolar solution (300 mOsm/kg) prepared with 0.2 g of 3mGlc, 0.5 g of ×, 1.0 g of R, and 5.0 g of L, diluted to 100 mL with drinking water  
Urine was collected for 5 hours

Determine if patients with LC and ascites have altered intestinal function; patient IP and absorption were compared between patients with liver disease and normal subjects

66 patients with LC (48 with ascites, 18 without ascites)  
74 healthy controls

L/R  
R/3mGlc

HPLC  
10 g of L and 5 g of M  
Urinary volume measured for 5 hours

Determine if abnormalities in the intestinal barrier in LC correlate with the degree of liver failure and are associated with other clinical complications  
Evaluate the effect of portal hypertension on IP in LC population

79 patients with LC  
25 controls

L/M

Urinary L/M concentrations were determined by HPLC  
10 g of L and 5 g of M dissolved in 50 mL water

20 patients with LC + portal hypertension (Child-Pugh A, B, and C) treated with TIPS + modified Sugiura

L/M

(Continued)



Table 2 (Continued)

| Marker | N  | Aim of study   | Analytic technique and dosage  | Results   | Study                             |
|--------|--|--|--|---|-----------------------------------|
| L/R    | Controls were healthy subjects<br>35 patients with LC<br>Six controls                    | Evaluate IP in LC patients complicated by portal colopathy                                     | Urine was collected for 6 hours<br>Gas chromatography<br>27 g of L and 1 g of R dissolved in 100 mL water<br>Urine was collected for 8 hours | (IP: 0.128±0.072 pre-TIPS vs 0.050±0.029 post-TIPS vs 0.036 post-Sugiura).<br>IP increased in LC patients complicated by portal colopathy.<br>L urinary excretion rate was significantly higher (P=0.05) in CIR patients (0.561%±0.078%) than in controls (0.15%±0.043%).<br>R urinary excretion rate was 4.390%±0.411% and 3.493%±0.961% in LC patients and controls, respectively. The difference was not significant.<br>L/R urinary excretion ratio (determined by the % of oral dose/8 hours) per group was significantly higher (P<0.05) in LC patients (0.124±0.012) than in controls (0.049±0.008). | Fujii et al <sup>15</sup> 2001    |
| PEG    | 54 patients with alcohol-induced liver injury, 19 with alcohol-induced LC<br>30 controls | Evaluate IP to macromolecules in patients with several degrees of alcohol-induced liver injury | Exclusion by size or reverse-HPLC<br>Different PEG molecular weights (400–10,000) diluted in water<br>Urine collected for 24 hours           | There was increased IP to large molecules in the population with alcohol-induced liver disease (no difference in terms of liver injury severity) compared with healthy subjects; likewise, serum endotoxin levels were also increased.  | Parlesak et al <sup>16</sup> 2000 |

**Abbreviations:** CF, cystic fibrosis; CIR, cirrhosis; CLC, compensated liver cirrhosis; CLI, chronic liver injury; E, erythritol; G, glucose; HPAEC-PAD, high-performance anion-exchange chromatography coupled with pulsed amperometric detection; HPLC, high-performance liquid chromatography; IP, intestinal permeability; L, lactulose; LC, liver cirrhosis; LC-MS/MS, liquid chromatography coupled to a mass spectrometer; M, mannitol; 3mGlc, 3-O-methyl-D-glucose; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; PEG, polyethylene glycol; PHT, portal hypertension; R, rhamnose; S, sucralose; S, sucrose; SBP, spontaneous bacterial peritonitis; SD, standard deviation; SS, sucrose; TIPS, Transjugular Intrahepatic Portosystemic Shunt; TJ, tight junctions; X, D-xylose.

amperometric detection,<sup>124,125</sup> capillary electrophoresis, among others.

Due to the complexity of sugar quantification, derivatization has been used as an alternative for their measurement. Some examples of compounds used for derivatization are 1-phenyl-3-methyl-5-pyrazolone (1-(2-naphthyl)-3-methyl-5-pyrazolone)<sup>126</sup> and *p*-aminobenzoic ethyl ester that react by reducing carbohydrates<sup>127</sup> for ultraviolet detection; in gas chromatography, trimethylsilyl or acetate derivatives<sup>123,128</sup> are used before analysis since saccharides need to become volatile and stable derivatives. Quantification by fluorescence, benzamidine has been used as a reducing agent<sup>129</sup> and 8-aminopyrenesulfonic acid for derivatization.<sup>130</sup>

Additionally, in general, sugar extraction from the biological matrix (urine) is through direct precipitation and filtration in order to clean the sample<sup>104,108–110</sup> although solid-phase extraction has also been used; its disadvantage is its high cost compared with precipitation,<sup>131</sup> so numerous assays have been performed to obtain the optimal quantification conditions.

Most techniques developed for sugar determination have been applied to the study of food products and drinks, but they are also of use in studies of intestinal permeability in patients with diseases such as cystic fibrosis, Crohn's disease, malnutrition, pancreatitis, colon cancer, and cirrhosis.<sup>89,104,108–110,132–134</sup>

Several studies on intestinal permeability in cirrhotic patients have been conducted by many authors; Table 2 shows some of these and the different quantification methods that have been applied as well as results on the sugar ratios reflecting permeability.

There are multiple results on the L/M quantification ratio and they are generally expressed as the percentage of excreted lactulose over the percentage of excreted mannitol. The normal proposed value is  $\leq 0.03$ ,<sup>57</sup> and a ratio of  $>0.03$  reflects increased small intestine permeability, and higher ratios are associated with even greater permeability.

## Measures to diminish increased intestinal permeability in the liver cirrhosis population

### Intestinal decontamination

Selective intestinal decontamination refers to a use of antibiotics to modify and select intestinal microbiota; it is common practice in patients with cirrhosis in the management of

certain complications (HE, variceal hemorrhage, prophylaxis of bacterial peritonitis, etc) and it is recommended in the international guidelines on the management of each of these complications.<sup>10,69,135</sup>

Rifaximin is an antibiotic with luminal activity and minimal systemic absorption; it is a broad-spectrum antibiotic against Gram-positive bacteria and its risk of fostering resistance has been reported to be low. Rifaximin in conjunction with lactulose is effective in the prevention of overt HE recurrence.<sup>135</sup> A study published by Vlachongiannakos et al<sup>136</sup> reported that the use of rifaximin was associated with a significant decrease endotoxemia and simultaneously, portal venous gradient in a population of decompensated alcoholic-related cirrhosis. Rifaximin has also been associated with improvement in cognitive function as well as in endotoxemia in patients with minimal HE; Bajaj et al<sup>137</sup> proved that rifaximin modified bacterial networks and their metabolites, fostering the generation of beneficial metabolites (saturated and unsaturated fatty acids) that ameliorate cognitive function.

### Probiotics

The use of probiotics has been associated with decreased intestinal permeability and decreased bacterial translocation and endotoxemia in animal models and clinical studies.<sup>138</sup> Patients eligible for liver transplants presented with increased intestinal permeability compared with healthy controls, a 30-day treatment with *S. boulardii* did not improve this intestinal permeability or the severity scores.<sup>139</sup>

Better trials are needed to establish that probiotics effectively improve intestinal permeability and their clinical effects; a recent meta-analysis was unable to confirm that probiotics were effective in the management of HE.<sup>140</sup>

### Increased gastrointestinal motility

The use of prokinetics has been evaluated as a measure to decrease bacterial translocation. A study conducted in humans and rats showed that cisapride (5-HT<sub>4</sub> agonist) decreased SIBO and bacterial translocation.<sup>11</sup> Moreover, a study of rats with liver cirrhosis analyzed bacterial translocation, SIBO, and intestinal transit time before and after cisapride or placebo administration: rats on cisapride had lower rates of bacterial translocation, endotoxin translocation, and intestinal bacterial overgrowth that appeared to depend on an increase in intestinal transit time and improved intestinal permeability.<sup>141</sup> The use of combined norfloxacin and cisapride versus cisapride alone decreases the incidence of SBP in patients with liver cirrhosis and ascites at high risk

of developing SBP (alcohol-induced cirrhosis, low albumin levels in ascitic fluid, gastrointestinal hemorrhage, and low serum albumin).<sup>142</sup>

In an animal model,<sup>12</sup> the administration of propranolol to rats with liver cirrhosis was evaluated and compared with placebo. The rats on propranolol had a lower portal pressure (20.9±4 mmHg vs 17.2±4 mmHg), a lower intestinal transit time (0.23±0.1 vs 0.44±0.1), lower rates of SIBO (67% vs 15%), and bacterial translocation (58% vs 15%).

## Other strategies

Studies in rats have shown that the duodenal administration of a 15% alcohol solution or the application of red wine to the duodenal intraluminal surface increases intestinal permeability, while the application of melatonin can prevent this effect of alcohol.<sup>143</sup>

Diets that improve intestinal saturated fatty acid levels may decrease alcohol-induced liver injury by stabilizing the intestinal barrier and preserving intestinal eubiosis; this is suggested in a study conducted in humans and mice by Chen et al.<sup>144</sup>

The use of propranolol in patients with liver cirrhosis not only decreased the portal venous pressure gradient but also intestinal permeability when tested with the three-sugar test; it also decreased bacterial translocation and serum IL-6 levels, an effect that was not limited to hemodynamically responding patients.<sup>15,117</sup>

Úbeda et al<sup>145</sup> showed that administration of obeticholic acid (a potent farnesoid X receptor agonist) for 2 weeks to cirrhotic rats with ascites decreased bacterial translocation, increased the expression of antimicrobial peptides in the ileum, and also increased TJ protein expression (zonulin 1 and occludin); it also decreased the degree of liver fibrosis and normalized the expression of inflammatory cytokines and TLR4. In an experimental model of cholestatic liver injury, obeticholic acid improved intestinal barrier function at the ileal level and increased the expression of claudin 1 and occludin; it also led to a significant decrease in bacterial translocation by attenuating the degree of intestinal permeability.<sup>146</sup>

A new potential therapeutic target may be the inhibition of microRNA-155 (miRNA-155) expression. Mice that are deficient in miRNA-155 were protected from alcohol-induced intestinal inflammation and they showed no elevation in endotoxin levels, suggesting that miRNA-155 may play a role in the maintenance of epithelial integrity after alcohol ingestion.<sup>147</sup>

## Conclusion

Patients with liver cirrhosis deteriorate as a result of several complications secondary to the presence of bacterial

translocation and their products from the intestinal lumen into the systemic circulation. Identification of the mechanisms implicated in this increase in intestinal permeability will promote the development of therapeutic targets that may prevent decompensation or death in this population. The usefulness and limitations of selective decontamination should be more clearly defined. Further research including innate immune responses against structural components of microbes may open new possibilities to manage this alteration. It remains to define the true impact of short- and long-term use of beta-blockers in the management of intestinal permeability, with a cautious use in people with advanced liver disease and refractory ascites.

There are other promising therapeutic strategies such as the use of obeticholic acid and miRNAs, which are still in evaluation. Studies attempting to standardize the available methods to evaluate intestinal permeability in the liver cirrhosis population are lacking; the different results might be explained by several factors such as intestinal transit time, surface area, kidney function, different liver disease entities, time evolution of the disease, comorbidities, the type of test used to measure intestinal permeability, and the considered cutoff limits.

## Disclosure

The authors report no conflicts of interest in this work.

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