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RESEARCH ARTICLE

Diet, Gut Dysbiosis and Liver Cirrhosis and their Influence upon Hepatic Encephalopathy

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ABSTRACT

Cirrhosis is the end stage of progressive liver fibrosis, resulted from chronic inflammation and liver injury. Early identification of risk factors and appropriate treatment for hepatic decompensation is paramount for positive health outcomes. In this review study, we revisited mechanisms associated with gut dysbiosis and intestinal hyperpermeability in advanced liver disease, and further discussed nutritional strategies for the management of dysbiosis in liver cirrhosis. In gut dysbiosis, proportionally lower concentrations of bacteria belonging to beneficial taxa such as Lachnospiraceae, Clostridiales, Ruminococcaceae and Veillonellaceae and others are observed, in relation to pathogenic taxa such as Enterobacteriaceae, Bacteroidaceae and others. Cirrhotic patients present decreased bowel motility, bacterial overgrowth and increased intestinal permeability. Dysbiosis may further exacerbate such conditions due to the ability of pathogenic bacteria to adhere to the epithelium, produce endotoxin, disrupt bile acid metabolism, activate the immune system and trigger inflammation, in a vicious cycle. The triad hepatic encephalopathy – cirrhosis – gut dysbiosis is an evident entity, and primary prevention as well as management strategies for those three conditions aim strongly at improving intestinal health by focusing on nutritional interventions. High-protein diets may be recommended for cirrhosis patients, and the protein source is a key factor to consider, and so are dietary fibre and carbohydrate compositions. Attention is given to reduce saturated fat intake. Supplementation with branched-chain amino acids, probiotics and prebiotics have also shown positive results.

Keywords: Diet; dysbiosis; microbiome; cirrhosis; hepatic encephalopathy

Introduction

Cirrhosis, the leading cause of mortality by liver diseases, is the end stage of progressive liver fibrosis resulted from various mechanisms of liver injury that lead to inflammation[1]. Recent data demonstrated that cirrhosis contributed to 2.4% of total deaths globally in 2017. In that same year, the prevalence of cirrhosis was 10.6 million decompensated cases, and 112 million compensated cases[1].

The chronic progression of cirrhosis leads to severe clinical manifestations including ascites, oesophageal variceal bleeding, hepatic encephalopathy (HE), and jaundice. Once decompensation occurs, the morbidity attributed to cirrhosis increases significantly, influencing quality of life, elevated disability-adjusted life years, and years of life lost. The 1-year case-fatality rate varies from 57 to 80%, depending on the cause of decompensation[2].

Considering the risk and relevance of decompensation on mortality rate of cirrhotic populations, the early identification of possible risk factors and appropriate treatment is of paramount importance for positive health outcomes. One of the several primary prevention strategies proposed focuses on improving intestinal health, which considers not only the gut-liver axis as pivotal for liver health but also the influence of gut microbiota (GM) on spontaneous bacterial peritonitis[3].

Cirrhotic patients present decreased bowel motility, bacterial overgrowth, and increased intestinal permeability[4]. Such manifestations increase the risk of microbial translocation to mesenteric lymph nodes, which predisposes patients to infection[5]. The GM is also a source of endotoxin and other bacterial products that affect vascular function[6]. The GM composition of patients suffering with advanced chronic liver disease (CLD) and HE appears to be different from the one found in healthy individuals[7].

A healthy GM provides a range of beneficial properties to its host, including the maintenance and integrity of the mucosal barrier, biotransformation and provision of key nutrients, and protection against pathogenic species[8]. In healthy individuals, bacteroid species are quantitatively amongst the most prevalent genera in the intestine. It has also been observed that the Enterobacteriaceae, Porphyromonadaceae and Alcaligeneacea genera are mostly pathogenic and, therefore, occur in lesser amounts in the healthy GM[9].

Gut dysbiosis is defined as an imbalance between the main phyla of bacteria residing in the intestine, characterized by an increase in pathogenic bacteria in relation to bifidogenic bacteria, followed with narrower taxonomic diversity. In gut dysbiosis, proportionally lower concentrations of bacteria belonging to beneficial taxa such as Lachnospiraceae, Clostridiales XIV, Ruminococcaceae and Veillonellaceae are observed, in relation to pathogenic taxa such as Enterobacteriaceae and Bacteroidaceae[10]. Dysbiosis may be particularly damaging to the host due to the ability of segmented filamentous bacteria to strongly adhere to the epithelium, activating the immune system and triggering inflammation[8].

In this study we have reviewed the mechanisms associated with gut dysbiosis and intestinal hyperpermeability in advanced liver disease and further discussed nutritional strategies for the management of dysbiosis in liver cirrhosis.

Western dietary patterns and their impact on obesity and global health

From the second half of the 20th century, advancements in food technology triggered changes in traditional dietary habits, making ultra-processed foods (UPFs) more easily available and affordable in westernised societies[11]. Due to its often-poor nutritional composition, UPFs are not recommended for prolonged consumption[12].

The effects of westernised diets (WD) on weight gain and development of metabolic diseases are often attributed to the nutritional composition of UPFs, typically characterised by their high content of free or added sugars, sodium, saturated fats, trans fats and additives, and most importantly, high energy density[11].

Both obesity and WD patterns are known risk factors for non-alcoholic fatty liver disease (NAFLD). Obesity was associated with a modest increase in the risk of incident severe liver disease (adjusted HR 1.20, 95% CI 1.12-1.28)[13]. A recent meta-analysis found that WD patterns increased the risk of NAFLD by 56%[14]. Due to the marked connection between metabolic diseases and NAFLD, the latter condition has been recently re-named as Metabolic (dysfunction)-Associated Fatty Liver Disease[15].

The impact of dietary patterns on gut health

The GM metabolic activity is fundamental for nutrient digestion, biotransformation and absorption, with the resulting bioavailability of short-chain fatty acids (SCFAs), amines, phenols and indoles, sulphurous compounds, increased bioavailability of minerals, and metabolism of bile acids. Additionally, GM further aids in gut health by

displacement of pathogenic species, production of antimicrobial factors, regulation of enterocyte exchange rate, differentiation of epithelial cells, strengthening of the intestinal barrier, and maintaining the functioning of the intestinal mucosa immunity by inducing immunoglobulin A secretion[16]. The maintenance of a healthy microbial composition is critical for protection against pathogens and overall immune response[17].

Alongside food, environmental microorganisms gain access to the gastrointestinal tract (GIT) and compete with local microorganisms, posing a potential threat to gut integrity[8]. Considering the above, multiple factors dictate the composition of the microbiota and its activity, but one of the most significant roles is played by nutrition, which includes diet composition, dietary patterns, and long-term dietary habits[17].

Among macronutrients, carbohydrates (CHOs) play a pivotal role in GM remodelling. Simple CHOs can rapidly deteriorate the GM, whilst complex CHOs confer a more protective role upon GM composition[18]. Complex carbohydrates are found abundantly in vegetables but as the human GIT cannot digest plant cell wall polysaccharides and resistant starch, such nutrients become available for microbial fermentation, resulting in SCFAs production- acetate, propionate and butyrate. Butyrate is involved in the maintenance of the intestinal barrier and, along with propionate, promotes intestinal gluconeogenesis. In hepatocytes and adipocytes, SCFAs not only modulate the activity of nuclear transcription factors, including the peroxisome proliferator-activated gamma receptor, and G-protein-coupled receptors but have also been implicated in the regulation of free fatty acid metabolism, inflammation mediation and cancer risk.[17].

Protein products metabolised by a diverse GM normally include indoles, amines, phenols, thiols, hydrogen sulphide (H_2S), carbon dioxide (CO_2), methane (CH_4) and Hydrogen gas (H_2). However, elevated production of acetic acid, CH_4 and CO_2 suggest overgrowth of anaerobic bacteria[19]. The effects of dietary protein on GM have been associated with increased Proteobacteria, Bifidobacterium and Lactobacillus and decreased *Bacteroides fragilis* and *Clostridium perfringens*. Protein consumption is also positively correlated with microbial diversity, and significant differences in bacterial enterotypes have been observed between diets with animal-derived protein versus plant-derived protein[20].

The amount and composition of dietary fats seem to influence GM composition. A monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA)-rich diet increases the Bacteroidetes / Firmicutes ratio, as well as lactic acid-related species such as Bifidobacteria and Akkermansia muciniphila. On the other hand, a SFA-rich diet facilitates the growth of Bilophila and Faecalibacterium prausnitzii, and at the same time reduces the counts of Bifidobacterium, Bacteroidetes, Bacteroides, Prevotella, and Lactobacillus ssp[21-22].

The effects of abundant SFA on GM also appear to influence bile acid metabolism. The SFA stimulus for increased bile acid secretion appear to involve the overproduction of hydrophobic secondary bile acids, including deoxycholic acid, leading to changes in microbial composition and function[9,23-24]. Furthermore, SFA, as a component of the lipid portion of lipopolysaccharide (LPS) of pathogenic species, have been shown to activate inflammatory cascades via toll-like receptor 4 (TLR4)[17] (Figure 1).

Westernised diets, dysbiosis and gut hyperpermeability

Chronic exposure to WD is associated with negative outcomes upon GM diversity and function[25]. WD is known to decrease not only the count of total species but also the count of commensal Bifidobacterium and Eubacterium species[26]. It has been suggested that chronic WD consumption may be associated with irreversibly reduced microbial diversity and depletion of specific bacterial species due to its low content of microbiota-accessible CHOs[27]. Several studies emphasize that WD influences GM and potentially trigger chronic pro-inflammatory diseases[28,29].

Caesar and colleagues analysed the GM of mice fed high fat diets enriched with lard or fish oil, and found higher counts of the commensal species Akkermansia muciniphila, Lactobacillus, and Bifidobacterium in the mice fed the fish oil diet. In the opposite direction, the researchers found increased TRL4 activation and inflammation in the white adipose tissue of lard-diet fed mice[30]. Interestingly, a systematic review appraising interventional studies in humans found that high consumption of fat and SFA might unfavourably affect microbiota diversity, and also that MUFA-rich diets might decrease total bacterial abundance, whereas PUFA-rich diets may not significantly modulate GM diversity[31].

An experimental study investigated the effects of various types of dietary protein upon GM composition of male rats[20]. The white meat-fed group showed higher Lactobacillus count when compared to the red meat and non-protein-fed groups, and higher Firmicutes count followed with

lower Bacteroidetes count when compared to casein-fed and soy-fed groups. The researchers also found that the soy-fed group showed higher Bacteroidetes count, the beef-fed group showed higher Proteobacteria count, and the chicken-fed group showed higher Actinobacteria count[20].

A small clinical trial investigated the effects of two distinct diets, the first a plant-based diet and the second an animal-based diet rich in meats, eggs, and cheeses, upon GM composition. Nine subjects participated in both diet arms of the study, separated by 1 month period. The results showed that the animal-based diet increased the abundance of bile-tolerant microorganisms (Alistipes, Bilophila, and Bacteroides) and decreased the levels of Firmicutes that metabolise dietary plant polysaccharides (Roseburia, Eubacterium rectale, and Ruminococcus bromii)[26].

Manifestations associated with dysbiosis induced by WD chronic consumption include increased barrier permeability, which triggers low-grade inflammation and subsequent metabolic disorders[32]. Additionally, exaggerated penetrability and reduced growth rate of the inner mucus layer facilitate the susceptibility to infections[33].

Dysbiosis has the potential to negatively influence the activity of tight junction proteins, including zonula occludens-1 (ZO-1), claudin and occluding[34]. Additionally, upregulated zonulin facilitates intestinal hyperpermeability[35]. In combination, such disturbances increase intestinal permeability and the transfer of intraluminal molecules, including dietary antigens and LPS, into the bloodstream, further contributing to low-grade systemic inflammation. Reduced intestinal mucous layer thickness is observed in mild chronic inflammation, further feeding into a vicious cycle of increased intestinal permeability and inflammation. In the lumen and across the intestinal barrier, LPS and other bacterial-derived compounds such as lipoteic acid, peptidoglycan, flagellin and bacterial DNA, can further stimulate the immune system and induce inflammation via TL4R activation[18].

Low quantities of microbiota-accessible CHOs facilitate the erosion of the colonic mucus barrier as a consequence of switched GM composition towards species that utilize secreted mucins as nutrient sources. A study conducted in experimental models showed that dietary fibre deprivation, and subsequent erosion of the colonic mucus barrier, promoted greater epithelial access, susceptibility to pathogens and enhanced expression of pro-inflammatory markers[36].

A high consumption of sugary foods and beverages has been identified as contributor to the excessive intake of high fructose corn syrup (HFCS) and sucrose. Chronic intake of HFCS induces insulin resistance with a greater risk for metabolic syndrome and liver diseases such as steatosis and non-alcoholic steatohepatitis (NASH)[37-39].

Furthermore, chronic intake of high-sucrose and HFCS foods has been associated with reduced diversity of bacteroids and Prevotella, alongside elevated concentration of toxic molecules derived from the fermentation of tryptophan and tyrosine, such as p-cresol and indoxyl sulphate[40]. Excess fructose also increases intestinal translocation and plasma endotoxin levels, which associated with the production of toxic metabolites in the intestine, favours inflammation and the development of NAFLD[41].

Dysbiosis and unfavourable clinical outcome for patients with advanced Chronic Liver Disease

The degree of dysbiosis in the patient with severe liver disease worsens as the disease progresses, and often accelerates its evolution, causing a vicious cycle[42-43]. Cirrhosis patients in the more advanced stages often show clinical decompensation, such as upper digestive haemorrhage, obstructive jaundice, HE, ascites, and spontaneous bacterial peritonitis[42]. Some cirrhosis complications are related to imbalanced GM and intestinal hyperpermeability, usually caused by the presence of dysbiosis, intestinal dysmotility, portal hypertensive vasculopathy, and overconsumption of alcoholic beverages[44]. In addition to the aforementioned factors, alteration in the flow of bile acids, with a consequent impairment of the bacteriostatic activity of bile salts[45], also favours endotoxemia and increased risk of liver disease complications[44]. Intestinal dysfunction in CLD patients, characterized by changes in intestinal motility and permeability, bacterial overgrowth and bacterial translocation, can further increase the risk of cirrhosis complications such as bacterial peritonitis and HE[45,46].

Gastrointestinal motility and gastric emptying are reduced in cirrhosis when compared to healthy individuals, and is further delayed as the liver disease progresses[48,49]. A clinical study assessing cirrhosis patients found delayed gastric emptying time in 24% of the patients, and delayed intestinal transit in approximately 38%. The alterations in gastric emptying and delayed intestinal transit were also positively associated with feelings of postprandial fullness, early satiety, nausea, diarrhoea, and abdominal pain[49].

The dysbiosis commonly observed in cirrhosis patients is often characterised by quantitative

changes in the Bacteroides Firmicutes ratio, predominance of potentially pathogenic families such as Enterobacteriaceae and Alcaligenaceae, and reduced population of commensal species[7]. Elevated counts of the pathogenic family of Enterobacteriaceae observed in cirrhosis patients favours increased LPS production with a consequent exacerbation of inflammation[3].

LPS derived from species belonging to the Enterobacteriaceae family shows four to fifty-times greater potency to activate inflammation and increase TNF-α as compared to bacterial fragments derived from the genus Bacteroidetes[9]. Activation of TLR2, TLR4 and TLR9 receptors in hepatocytes contributes to the pathogenesis of metabolic liver diseases by triggering pro-inflammatory events, differentiation of pro-inflammatory Th1 cells, as well as increased synthesis of TNF- α, interleukin (IL) 6 and IL-1B[3,9].

A prospective case-control study found in cirrhotic patients a worsened clinical evolution in those presenting higher pathogenic bacterial count in their GM[50], which reinforces the hypothesis that cirrhosis patients show progressive changes in their GM, and such changes become more dramatic when the disease is decompensated by infection or HE. Further studies have shown in hospitalized patients that the presence of dysbiosis hinders recovery of severe HE and compromises the resolution of acute liver failure, in addition to increasing the length of ICU stay, risk of organ failure and higher mortality rate[42,51].

Clinical studies have attempted to correlate microbiota patterns with the prognosis of cirrhosis, and in general, cirrhosis patients show higher counts of the phylum Proteobacteria and lower counts of the phylum Firmicutes[42]. The Cirrhosis Dysbiosis Ratio (CDR) and the Gut Dysbiosis Index (GDI) correlate the phyla firmicutes, bacteroids and protobacteria to determine the degree of dysbiosis and to predict the onset of organ dysfunction within 30 days and mortality rate[44-45]. The CDR assesses the ratio between firmicutes / bacteroids and protobacteria and, therefore, the lower the score found the worse the prognosis. On the other hand, in GDI the proportion is inverted and high scores indicate worse deregulation of the microbiota and greater severity of liver disease[45].

Liver-gut axis, dysbiosis and hepatic encephalopathy

An association between dysbiosis and disturbances in the liver-gut axis, and how that association influences the aetiology of HE, have been identified. A meta-analysis showed that the prevalence of small intestinal bacterial overgrowth (SIBO) in CLD patients was significantly higher as compared to healthy controls- 68.31% vs 7,94%[52]. Although most studies appraised in that meta-analysis were conducted in CLD patients in more advanced stages, SIBO was also prevalent in patients with NAFLD without fibrosis[52]. The occurrence of SIBO appears to be related to changes in intestinal microvilli, being related also with endotoxemia and greater severity of cirrhosis[53]. It should be noted that cirrhosis patients often present hypochlorhydria, regardless of the use of acid secretion-inhibiting medication, favouring SIBO. Furthermore, SIBO is worsened by proton pump inhibitors, and a recent clinical study has shown that such class of medication increases the severity of HE in cirrhosis patients[54].

HE is possibly the most dramatic clinical manifestation attributed to dysregulation of the intestine and liver-brain axis, leading to systemic inflammation, neuroinflammation and hyperammonaemia[42]. HE is defined as a neurological dysfunction caused by liver failure and can be manifested in a wide spectrum of neurological and or psychiatric manifestations. Minimal HE (MHE) and Grade I HE are classed together as covert encephalopathy, whilst HE grades II through to V are classed as HE overt or evident[55].

MHE is defined as a brain disorder characterised by the presence of small cognitive changes but without evident clinical signs, such as disorientation and asterixis[55-56]. Cirrhosis patients suffering with MHE show increased Streptococcus salivarius counts in their GM, alongside hyperammonaemia[43].

Some studies have shown that specific bacterial taxa occur differentially in the gut microbiome of MHE patients in relation to overt encephalopathy. In the study of Bajaj and colleagues[57], a greater abundance of the beneficial genus Roseburia was found in patients without HE, while a greater abundance of the genera Enterococcus, Veillonella, Megasphaera, Bifidobacterium and Burkholderia was found in the mucosa, but not in the faeces, of HE patients[57]. Interestingly, a similar study carried out by the same research group found significant differences in the microbiota composition in the colon mucosa of overt HE patients when compared to healthy individuals, but not when compared to MHE[58].

Changes in GM composition have been associated with higher occurrence of HE. A study showed that increased counts of the families Alcaligenaceae, Porphyromonadaceae, Veillonellaceae, genus Megasphaera and Burkholderia, in addition to the

species Enterococcus, were related to hyperammonaemia, systemic inflammation and worsening of HE[45].

Both covert and overt HE occur more frequently in patients suffering with dysbiosis, malnutrition, sarcopenia, and in those with hyperammonaemia[42,49]. Low muscle reserve is an independent risk factor for HE in patients undergoing insertion of transjugular intrahepatic portosystemic shunt (TIPS), since muscle mass is essential for extra-hepatic ammonia metabolism[49,57].

Although significant amounts of circulating ammonia are derived from hepatic, renal and muscle metabolism, it has been recently reported that the GIT is a major site for the synthesis of ammonia. Pathogenic microbiota, through metabolization of food residues mainly containing protein, contribute to elevated ammonia concentration in the portal vein[59]. Thus, elevated release of ammonia into the intestine occurs through the actions of urease-producing bacteria that hydrolyse urea, and well as through bacterial protein deamination and glutamine metabolism in the intestinal mucosa.

Additionally, Helicobacter pylori metabolism in the stomach associated with intestinal dysbiosis also show great relevance for endogenous ammonia synthesis[55,59]. Along those lines, patients with cirrhosis and liver failure experience increased bacterial synthesis of ammonia associated with reduced urea cycle reactions by the liver, resulting in significantly increased ammonia levels in the plasma, which in turn crosses the blood-brain barrier and triggers HE[45,55]. As dysbiosis predicts decompensation of CLD, nutritional strategies for the management of complications associated with advanced liver disease should prioritize GM modulation[45,59].

Recently, a randomized, placebo-controlled, single-blind clinical trial evaluated the safety, tolerability, and effects of capsular faecal microbiota transplantation (FMT) on the microbiota and brain function of patients with cirrhosis and recurrent HE. Preliminary but promising results showed that FMT improved dysbiosis and duodenal mucosa microbial diversity in patients with cirrhosis and Model for End-Stage Liver Disease scores (MELD) < 17 on rifaximin and lactulose therapy. Six patients in the placebo group required hospitalizations, compared to one in the FMT group, which was supposed by the researchers as unrelated to the therapy[60]. In addition, an improvement in neurocognitive performance was observed in the FMT-treated patients[61]. However, further studies are needed to assess the effectiveness of this therapy.

Dysbiosis and the risk of Hepatocellular Carcinoma

Studies have shown that dysbiosis may be related to a higher risk for the evolution of both liver cirrhosis of viral origin and NASH to Primary Hepatocellular Carcinoma (PHCC)[62,63]. A clinical study with 68 patients with PHCC were compared against 18 matched healthy individuals who had not been treated with antibiotics or probiotics in the 2 months prior to the study, and the results showed that PHCC patients presented significantly increased pro-inflammatory bacteria in their faecal microbiota[62].

A recent clinical trial detected differences amongst the GM of control volunteers, HCC patients with associated chronic hepatitis B (CHB), and HCC patients with an aetiology not associated with viral hepatitis[64]. The HCC-CHB group showed a wider variety of species in the faecal microbiota, compared to both the control and non-viral HCC groups. The non-viral HCC group, in turn, showed greater relative diversity of the phylum Proteobacteria, mainly from the genera Salmonella, Proteus, Shigella and Yersinia, in relation to the control and HCC-CHB groups. Such results are likely to be attributed to dietary differences across the groups, which is further corroborated by the finding of relatively high alcohol consumption by 73% of patients in the non-viral HCC group[64].

Thirty-two NAFLD-related HCC patients, 28 NAFLD-related cirrhosis, and 30 non-NAFLD control subjects took part in a study that assessed the microbiome profile in cirrhotic patients with and without HCC. Metagenomic sequencing results showed dysbiosis in NAFLD-HCC and NAFLD-cirrhosis compared to the controls. NAFLD-HCC subjects showed elevated Proteobacteria phylum compared to non-NAFLD controls. Furthermore, NAFLD-HCC showed higher proportion of the Enterobacteriaceae family when compared to NAFLD-cirrhosis and non-NAFLD controls. In addition, the researchers found increased SCFA serum levels in patients with NAFLD-HCC. Such findings suggest that a dysbiotic gut composition in NAFLD-HCC subjects could potentially modulate the peripheral immune response[65].

Acute-on-Chronic Liver Failure

Although Acute-on-Chronic Liver Failure (ACLF) is the most severe clinical stage of cirrhosis, currently little is known about its relationship with GM. A study evaluated patients with advanced CLD, with and without ACLF. Microbial genomic profile was

investigated in stool samples and grouped into clusters, defined as metagenomic species. Results show that progression of cirrhosis was associated with significant reduction of microbial genes and metagenomic species, particularly in ACLF[66]. In that study, the GM profile was associated with functional changes and could predict patient survival. Additionally, ACLF was associated with significantly increased Enterococcus and Peptostreptococcus sp, and reduced autochthonous bacteria. Of the 171 patients included in the analysis, 34 died during the 3-month follow-up period, 8% of those in the decompensated cirrhosis group and 42% in the ACLF group.

Whilst some species were strongly associated with better prognosis, particularly Paraprevotella clara, Bacteroides salyersiae, Clostridium sp, and Roseburia hominis, other species were associated with worsened prognosis and poorer short-term survival, including E faecium, Streptococcus thermophilus, and Ruminococcus lactaris. Patients who died during the study had a significant loss of gene richness, and presented more abundant Enterococcus species, as compared to survivors. The study also found that GM alterations in end-stage liver disease, Child-Pugh scores and organ failure were associated with more prevalent HE and infections[66].

Nutritional strategies in the management of dysbiosis in liver cirrhosis

The European Society for Clinical Nutrition and Metabolism (ESPEN-2020)[67] and the European Association for the Study of the Liver (EASL-2018)[57] recommend the prescription of a high-protein diet for cirrhosis patients, including those with covert and overt HE. For patients with adequate nutritional status, the daily protein should be 1.2 g / Kg BW, while for malnourished or sarcopenic patients, the recommendation is 1.5 g of protein / Kg BW. Plant and dairy-derived protein are recommended for patients with intolerance to animal protein. Supplementation with branched-chain amino acids (BCAA) can be implemented as prophylactic or therapeutic measure for HE at the daily dosage of 0.25 g / kg BW. Guidelines also recommend that cirrhosis patients refrain from fasting, avoiding periods longer than 3 hours without food intake[57,67]. A meta-analysis showed that the introduction of a late-night snack reduced the post-absorptive phase, had the potential to reverse anabolic resistance, and improved sarcopenia and quality of life in cirrhosis patients. The results suggest that the evening snack reduced lipid peroxidation and improved nitrogen balance[68]. Although the composition of an ideal evening snack has not yet been fully defined, ideally a serving of approximately 400 calories containing 50 g of complex carbohydrates and 20 g of protein and supplemented with BCAA is recommended[68].

A clinical study investigated associations among diet, GM and their impact on cognition and clinical evolution of patients with compensated or decompensated cirrhosis. The results showed that all decompensated patients followed up for 90 days had lower intake of fat and protein of both animal and vegetable origin. Those patients also showed reduced GM diversity and the relative consumption of vegetables, animal fats and eggs were lower in patients with decompensated cirrhosis. The latter also showed lower Prevotellaceae counts, particularly in those patients with lower consumption of animal fat and protein. Multi-varied regression analysis showed that higher concentrations of species from the Prevotellaceae, Ruminococcaceae and Lachnospiraceae families reduced the risk of hospitalization independently of the patients' MELD scores and ascites[69].

Another cohort study compared patients with compensated or decompensated cirrhosis. The consumption of vegetables, cereals and foodstuffs with high content of polyphenolic compounds (PP) such as dark chocolate, coffee and tea, and fermented milk drinks showed positive effects on microbial diversity, which in turn was a factor associated with lower risk of hospitalization[70]. Previous studies have shown PP beneficial effects upon the GM by increasing the abundance of Bifidobacterium and Lactobacillus and increasing SCFA production, and that a reduction in the pathogenic species Clostridium perfringens and Clostridium histolyticum was detected in response to PP intake[71].

PP beneficial effects have been observed in cirrhosis patients. Coffee consumption has been associated with lower cirrhosis mortality and, in healthy patients, an association with higher counts of Bifidobacteria was found[72-73]. A randomized study carried out with cirrhosis patients showed that a liquid meal containing dark chocolate with 85% cocoa (0.55 g / kg of body weight) significantly attenuated the postprandial increase in the hepatic venous pressure gradient and portal hypertension was observed when compared to white chocolate consumption[74].

Higher protein intake may be associated with greater GM variety and uniformity in cirrhosis patients when compared to cirrhosis patients with protein intake below recommendations. A study found that Prevotella-9 genera and Agathobacter

counts were higher in cirrhosis patients with higher protein intake[75]. In addition to BCAA as well as ornithine and arginine, plant proteins provide soluble fibre, which are precursors of SCFAs, promoting a reduction in intestinal pH and better GM composition. On the other hand, animal protein has been associated with higher levels of ammonia and trimethylamine N-oxide (TMAO). TMAO is an amine oxide synthesized by GM through choline, betaine and carnitine metabolism. Pathogenic bacteria and products of their metabolism play an important role in systemic inflammation and are associated with HE[76].

lebba and colleagues[77] sequenced the 16S rRNA and analysed functional metagenomic networks of GM, gut biopsy, peripheral blood and portal blood of cirrhotic patients. The analyses of faecal material and caecum biopsies showed marked dysbiosis, with higher proportions of Enterobacteriaceae. The faecal bacterial metabolism results showed imbalanced synthesis of SCFAs, trimethylamine and metabolites containing carbon and methane, which were positively correlated with plasma levels of pro-inflammatory cytokines. In the opposite direction, the researchers found that higher proportions of the family Ruminococcaceae and increased SCFAs had a protective effect by reducing markers of systemic inflammation[77]. Bacterial metabolites such as methanol, threonine and TMAO have been linked to clinical markers of systemic inflammation and HE[76,77].

The effects of BCAA upon the GM of cirrhotic patients is an area yet to be fully elucidated, but it is already known that specific GM species have the potential to modulate BCAA biosynthesis, transport and metabolism. It has been observed in Type 2 diabetesT2D that higher counts of the Prevotella capri species was associated with higher circulating levels of BCAA and worsened insulin resistance[78]. BCAA supplementation is recommended for patients intolerant to animal protein and in the treatment of HE but which must be followed with close monitoring of glycaemic parameters[79].

Previous studies have investigated the usefulness of probiotic supplementation in the primary prophylaxis of HE in cirrhosis patients. For example, a study adopting a three-month probiotic cycle model found that probiotic supplementation improved psychometric tests, reduced hyperammonaemia and decreased the risk of HE development in cirrhosis patients as compared to the placebo-receiving group[80]. For the secondary prophylaxis of HE in cirrhosis patients, a clinical trial showed that probiotic supplementation was as effective as lactulose in preventing new HE episodes[81]. It has also been shown that probiotic supplementation improved MHE, reduced hospitalization rates and the evolution to overt HE, when compared to placebo or standard treatment with lactulose, but however interesting, no significantly beneficial results were found regarding prognosis or mortality rate[82].

Lactulose, lactitol, fructooligosaccharides and galactooligosaccharides are prebiotics commonly used to prevent and manage complications associated with CLD. Lactulose reaches the colon almost intact, where it is partially metabolised by saccharolytic bacteria, producing lactic acid and small amounts of acetic acid and formic acid. Intestinal acidification reduces ammonia synthesis by urea-producing bacteria, which results in greater conversion of NH3 into ammonium ions, which for not being absorbable are excreted through faeces[83]. Non-absorbable disaccharides can inhibit glutaminase activity, thereby reducing intestinal ammonia production and favouring the proliferation of beneficial saccharolytic bacteria such as Bifidobacterium and Lactobacillus, which in turn antagonise the growth of ammonia-producing species[53]. A clinical trial showed that the offer of yogurt containing the species *Bacillus bifidus, Lactobacillus acidophilus, Lactobacillus bulgaricus,* and *Streptococcus thermophilus* for cirrhosis patients was associated with reduced E. coli counts, improved appetite and reduced abdominal distention symptoms[84].

A systematic review concluded that probiotic supplementation could improve outcomes in overt HE, enhance quality of life and reduce hyperammonaemia, but with little effect on mortality rate[82]. Interestingly, most clinical trials appraised in that review did not show high quality, and therefore the evidence is not yet totally conclusive. A meta-analysis published one year later showed that probiotic supplementation was associated with reduced circulating ammonia and endotoxin levels, improved MHE and lowered the occurrence of overt HE in cirrhosis patients[85]. That same study however showed that lactulose appeared to be more effective in improving the number-connection test results compared to probiotics. Table 01 summarizes the main nutritional strategies for GM modulation in cirrhosis patients.

Conclusions

Dysbiosis is positively associated with the development of liver decompensating complications including HE, systemic infections, ACLF and overall worsened clinical evolution in cirrhotic patients. If untreated, cirrhotic patients present progressive

changes in their GM, which become more significant when the disease is decompensated by infection or HE. Therefore, the early identification of dysbiosis in cirrhotic patients is a useful indicator for risks of complication that accompany the evolution of cirrhosis. Nutritional interventions that rely not only on improved macronutrient composition but also on probiotic and prebiotic supplementation are efficient strategies, shown to improve liver health in liver diseases.

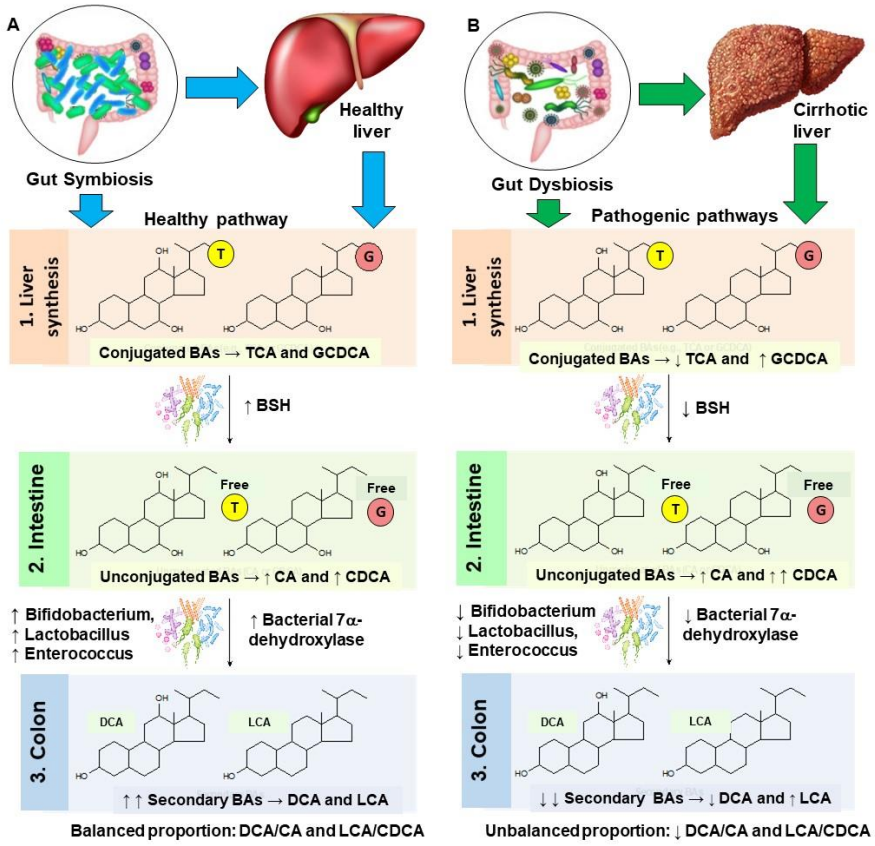
Footnotes

Conflict of interest statement: Authors declare that there are no conflict of interests to disclose.

Table 1: Nutritional strategies for GM modulation in cirrhotic patients

Nutrient / Supplement	Nutritional recommendations
Protein	Plant protein is preferable, particularly when animal protein not tolerated. 1.2 to 1.5 g / Kg / day
Plant protein	Its high fibre content accelerates intestinal transit and reduces circulating mercaptans and indoles, facilitating ammonia detoxification.
Branched chain amino acids	0.25 g / Kg / day
Carbohydrates	Preference for complex carbohydrates. Avoid processed and ultra-processed carbohydrate-containing foods
Fat	Reduce saturated fat and eliminate trans fat.
Prebiotic fibre	Fructooligosaccharides and galactooligosaccharides
Drugs to lower pH	Lactulose, lactitol
Probiotics (main strains administered)	*Lactobacillus acidophilus, L. casei strain Shirota, L. bulgaricus, L. plantarum, Bifidobacterium lactis, Streptococcus thermophiles, Bifidobacterium breve, Bacillus subtilis, Enterococcus faecium*

Figure 1. Bile Acid metabolism can be differently modulated following either physiological or pathogenic pathways in dysbiosis and cirrhosis.



A: The secretion, composition and metabolism of biliary acids (BA) are highly influenced by the type and diversity of the gut microbiota (GM), occurring differently in the conditions of eubiosis or dysbiosis. 1:Primary bile acids (BAs) are synthesized in the liver: taurine-conjugated cholic acid (TCA) and glycine-conjugated chenodeoxycholic acid (GCDCA). 2:Bile salt hydrolase (BSH) catalyses the bioconversion of conjugated BA into the free BAs cholic acid (CA) and chenodeoxycholic acid (CDCA). 3:CA and CDCA are metabolised in the intestine by 7α-dehydroxylase-expressing microbiota (e.g., Bifidobacterium, Lactobacillus, Enterococcus) forming mainly the highly hydrophobic secondary BAs deoxycholic (DCA) and lithocholic (LCA) acids, as well as ursodeoxycholic (UDCA) and chenodeoxycholic acids. Around 20 other types of secondary BAs can be formed depending on GM type and diversity.

B: Cirrhotic patients show reduction in BA production due to the suppression of the healthy pathway by pro-inflammatory cytokines. In those conditions, the acid pathway for BA synthesis becomes more activated, elevating the synthesis of CDCA in detriment of CA. Lowered concentrations of primary BA in the intestine reduce the 7α-dehydroxylating bacterial populations, with consequent imbalance in the ratios of primary and secondary BAs (DCA/CA and LCA/CDCA). Reduced and disproportionate composition of BA pool negatively influences GM composition and diversity, which further affects BA metabolism and induce greater hepatocellular dysfunction[9,23,24].

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