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Review article: the gut microbiome as a therapeutic target in the pathogenesis and treatment of chronic liver disease

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Summary

Background: Mortality from chronic liver disease is rising exponentially. The liver is intimately linked to the gut via the portal vein, and exposure to gut microbiota and their metabolites translocating across the gut lumen may impact upon both the healthy and diseased liver. Modulation of gut microbiota could prove to be a potential therapeutic target.

Aim: To characterise the changes in the gut microbiome that occur in chronic liver disease and to assess the impact of manipulation of the microbiome on the liver.

Methods: We conducted a PubMed search using search terms including 'microbiome', 'liver' and 'cirrhosis' as well as 'non-alcoholic fatty liver disease', 'steatohepatitis', 'alcohol' and 'primary sclerosing cholangitis'. Relevant articles were also selected from references of articles and review of the ClinicalTrials.gov website.

Results: Reduced bacterial diversity, alcohol sensitivity and the development of gut dysbiosis are seen in several chronic liver diseases, including non-alcoholic fatty liver disease, alcohol-related liver disease and primary sclerosing cholangitis. Perturbations in gut commensals could lead to deficient priming of the immune system predisposing the development of immune-mediated diseases. Furthermore, transfer of stool from an animal with the metabolic syndrome may induce steatosis in a healthy counterpart. Patients with cirrhosis develop dysbiosis, small bowel bacterial overgrowth and increased gut wall permeability, allowing bacterial translocation and uptake of endotoxin inducing hepatic and systemic inflammation.

Conclusions: Manipulation of the gut microbiota with diet, probiotics or faecal microbiota transplantation to promote the growth of "healthy" bacteria may ameliorate the dysbiosis and alter prognosis.

The Handling Editor for this article was Professor Peter Hayes, and this uncommissioned review was accepted for publication after full peer-review.

1 | INTRODUCTION

Our understanding of the gut microbiome in health and disease has increased significantly in recent years. The use of nonculture-dependent techniques, such as 16s rRNA sequencing and more recently metagenomic analysis, has been key in these advances, allowing sequencing of whole populations of microbiota in a short period of time. The advent of these techniques has allowed researchers to examine the impact of the microbiome on a host of different conditions. As the liver receives the majority of its blood supply directly from the microbiota laden gut, via the portal vein, inherently the host microbiome and the liver become intimately linked. This article will examine in detail the role of the gut microbiota in the pathogenesis and progression of chronic liver disease and tease out how modulation of the gut microbiome might offer novel therapeutic targets.

There is a wealth of data to link conditions such as non-alcoholic fatty liver disease (NAFLD), alcohol-related liver disease (ALD) and primary sclerosing cholangitis (PSC) with an altered and potentially "dysfunctional" gut microbiome. The term gut "dysbiosis" has been coined to encapsulate the perturbations in the structure of the complex commensal communities, which can lead to deficient priming of the host immune system leading to a predisposition to develop immune-mediated diseases. Interestingly, emerging evidence suggests that rather than 1 or 2 dominant organisms being responsible for host health, the composition of the entire microbial community contributes to a balanced immune response. The host has also developed a number of mechanisms to counteract changes in the gut microbial community structure. This has broad implications on the development of future therapies that goes beyond the introduction of a single organism to induce health such as introducing a probiotic agent to the diet. It ultimately means that we must identify mechanisms that reconstitute a healthy complex microbiome after dysbiosis has occurred, a process that could be referred to as "rebiosis," which will be fundamental to treating chronic liver disease especially those conditions which may be directly or indirectly immune mediated. The ultimate therapy may, thus, involve transplanting what might be perceived as a healthy gut microbiome from a non-obese healthy donor to a patient with chronic liver disease, an evolving therapy referred to as faecal microbiota transplantation (FMT).

2 | BACKGROUND

2.1 | The rise in chronic liver disease

Liver disease is the fifth biggest killer in England and Wales (British Liver Trust) and unlike mortality for cancer and ischaemic heart disease, mortality from liver disease continues to rise. In Western countries, alcoholic liver disease (ALD) and NAFLD make up the majority of the case-load. The prevalence of NAFLD varies and the prevalence of non-alcoholic steatohepatitis (NASH) is uncertain as it requires a liver biopsy for confirmation. An American study of 328 volunteers found the prevalence of NAFLD was 46% and NASH, which can progress to fibrosis and cirrhosis, was as high as 12%.[1] In diabetic patients, the prevalence was as high as 74% for NAFLD and 22% for NASH; NAFLD being strongly associated with obesity and "the metabolic syndrome." It is estimated that 10%-15% of those who drink to excess develop cirrhosis. The development of cirrhosis is thought to require alcohol intake of at least 50 g of alcohol per day for men or 30 g for women over a period of at least 5 years.[2] NAFLD and ALD together are a huge public health issue and have a considerable financial impact on health services.

2.2 | The gut-liver-immune system axis

The gut contains 100 trillion micro-organisms. These out-number somatic cells in the body by 10-fold.[3] The characterisation of gut flora has been greatly improved by 16S ribosomal RNA sequencing as the majority of gut bacteria are unculturable. The gut microbiota includes not only bacteria but also includes viruses and fungi. There are thousands of different bacteria, but the main phyla represented include Bacteroidetes, Firmicutes and Actinobacteria.[4] Many different factors influence microbiota composition, including diet, age and comorbid conditions. In children, the microbiota vary depending on mode of delivery and whether or not the child was breast-fed. Oligosaccharides from human breast milk promote Lactobacillus and Bifidobacteria, which are the predominant bacteria found in the intestines of breast-fed babies, but these bacteria decline following the introduction of a solid diet.[3]

The diverse and complex role played by the gut microbiome is central to the development and modulation of the innate and adaptive immune systems both locally within the intestinal mucosa facilitating defence against pathogenic invasion but also systemically. Their dietary and homeostatic functions complement those of the liver and include glucose metabolism, bile salts and xyloglucans, the liberation of short-chain fatty acids (SCFAs) from indigestible starches, and the biosynthesis of vitamins many of which the human host cannot perform independently. SCFAs are a vital source of energy for intestinal mucosal cells helping to maintain gut integrity and bolstering barrier function. The liver is an important immunological organ and is the first organ to challenge gut-derived bacteria, bacterial pathogen-associated molecular patterns (PAMPs) and ingested food products after they enter the systemic circulation. It is for this reason that 80% of the body's resident macrophages reside in the liver. The liver, thus, forms a firewall clearing circulating invading bacteria.[5] The healthy liver is also a site of active local regulation of immune responses favouring induction of T-cell tolerance by the presence of an unique microenvironment rich in tolerogenic organ-resident cells.[6] Any change, therefore, in the gut microbiome composition will impact on homeostatic functions which may induce perturbations in hepatic function and the development of liver disease.

2.3 | Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease comprises simple steatosis and NASH, which can lead on to the development of cirrhosis and hepatocellular carcinoma (HCC). A study of 2287 people looking at the prevalence of hepatic steatosis in the United States revealed that 34% of Texans had steatosis, with higher rates in those from Hispanic backgrounds.[7] Another US study found the NAFLD prevalence to be 46% and the prevalence of biopsy-proven NASH was as high as 12%.[1] Rates were also found to be higher in diabetics and patients from Hispanic backgrounds. With rising rates of obesity, the issue of NAFLD is becoming more and more pressing, especially as there are currently no effective drug treatments for the condition.

Gut microbiota have been implicated in the development of obesity and the metabolic syndrome.[8] Germ-free mice who were fed a high-fat, high-sugar diet gained less weight than ordinary mice fed the same diet.[9] Germ-free mice were then given gut microbiota from normal mice, resulting in increased body fat. Le Roy et al took this one step further, identifying mice with the metabolic syndrome phenotype and treating germ-free mice with "metabolic syndrome" gut microbiota. They then fed the mice a high-fat diet. These mice were compared with germ-free mice treated with "normal" gut microbiota, from mice without the metabolic syndrome phenotype. Mice treated with the metabolic syndrome gut microbiota developed raised blood glucose and insulin levels and evidence of hepatic steatosis, suggesting that the metabolic syndrome phenotype can be transferred via transfer of stool microbiota.[10]

Gut microbiota produce SCFAs such as acetate, propionate and butyrate, from digestion of carbohydrates in the colon. Butyrate is the main source of nourishment for colonocytes.[11] Examples of butyrate-producing bacteria include members of the Firmicutes phylum, such as *Faecalibacterium prausnitzii*. Butyrate is thought to help modulate intestinal barrier integrity via modulation of expression of tight junction proteins and mucin.[11] This suggests that encouraging growth of butyrate-producing species could reduce gut permeability, thus reducing systemic inflammation. Inulin-type fructans promote the production of butyrate in vitro.[12]

Zhu et al examined the role of gut microbiota and endogenous alcohol production in NASH development. Twenty-five per cent of those with fatty livers progressed to fibrosis and eventually cirrhosis, while the remaining 75% had simple steatosis and did not progress. The aim was to discover which co-factors influenced this progression to NASH. Gut microbiota were examined from 3 groups—healthy children, children with biopsy proven NASH and children who were obese, but who had normal liver biochemistry.[13] Children were chosen to avoid alcohol consumption as a confounding factor. A statistically significant increase was noted in Bacteroidetes with an associated decrease in Firmicutes in obese subjects and NASH patients. Proteobacteria, Enterobacteriaceae and *Escherichia* were similarly represented between healthy and obese microbiomes, but were significantly elevated in NASH. Other studies have reported variable results with regard to Phyla alterations, due to the heterogeneity of study design and methodology in NAFLD trials.[8] Alcohol-producing bacteria were more abundant in NASH patients. Peripheral blood was obtained to assess blood ethanol concentrations in healthy subjects, obese subjects and NASH patients. Interestingly, children with NASH were noted to have significantly elevated blood ethanol concentrations when compared with healthy and obese controls. In health, alcohol is constantly produced in the gut by microbiota, but is usually completely metabolised by alcohol dehydrogenase (ADH) and other enzymes in the liver. It was postulated that gut microbiota enriched in alcohol-producing bacteria (eg *Escherichia* coli) constantly produce more alcohol than healthy microbiota (overwhelming the hepatic detoxification capacity) and therefore supply a constant source of reactive oxygen species (ROS) to the liver, which, in turn, induces hepatic inflammation, eventually resulting in steatohepatitis. Other studies have failed to confirm elevated blood alcohol levels in NAFLD. De Medeiros et al postulated that NAFLD could represent an "endogenous ALD" as patients with NAFLD may produce high levels of endogenous alcohol, but do not necessarily exhibit elevated blood alcohol levels. Endogenous ethanol is a prodrug, metabolised to acetaldehyde in the gut and liver. Acetaldehyde is hepatotoxic, with extrahepatic acetaldehyde far more hepatotoxic than acetaldehyde produced in the liver. Extrahepatic acetaldehyde causes steatosis and fibrosis in far lower concentrations than acetaldehyde produced in the liver. Alcohol dehydrogenase 4 (ADH4) metabolises ethanol to acetaldehyde and is up-regulated in NASH livers, suggesting higher alcohol exposure. Small bowel bacterial overgrowth may contribute to excess endogenous alcohol production and hepatic injury.[14]

2.4 | Alcohol-related liver disease

Not all patients who drink to excess go on to develop cirrhosis. The reasons for this are thought to relate to host genetic factors and environmental factors, such as diet and an altered gut microbiome. A growing evidence base suggests that gut-derived bacterial endotoxins may be co-factors for alcohol-induced tissue injury and organ failure that only occurs in a specific subset of those who drink alcohol to excess. Indeed, a recently published murine study examined the impact of alcohol on the gut microbiome[15] and observed that certain mice are resistant to the effects of alcohol while others are sensitive to it, resulting in the development of alcohol-related lesions, for example, steatosis. Alcohol-sensitive mice were noted to have reduced Bacteroidetes, with increased levels of Actinobacteria and Firmicutes. Alcohol-sensitive mice had 50% less Bacteroides than alcohol resistant mice. When FMT was performed from the alcohol-resistant mice to the alcohol-sensitive mice, FMT was found to protect against alcohol-induced Bacteroidetes depletion and to protect against the development of steatosis. The presence of reduced Bacteroidetes species in colonic biopsies in alcohol-exposed subjects has also been observed.[16] This was accompanied by increased levels of Proteobacteria. The alterations were correlated with a high concentration of serum endotoxin in a subset of patients, suggesting the development of bacterial translocation across a compromised intestinal barrier. Changes in microbial

function, rather than abundance, could also account for the increased levels of gut-derived pro-inflammatory factors such as endotoxin. There may also be bacterial groups that differ taxonomically yet are functionally equivalent, and this may differ from individual to individual. Interestingly, while a subset of both actively drinking and abstinent alcohol users had dysbiotic mucosal-associated microbiota, there was no correlation between the duration of sobriety and the presence of dysbiosis, suggesting that the effects of chronic alcohol consumption are not temporary but rather long-lasting.

Alcoholic hepatitis is the most florid form of alcoholic liver disease and has a 28 day mortality approaching 20%.[17] It has been shown by Llopis et al that alcoholic hepatitis is associated with significant gut dysbiosis. They reported patients with severe AAH had more Bifidobacteria and Streptococci than alcoholic patients without alcoholic hepatitis. Enterobacteria and Streptococci were positively correlated with AAH scores and Enterobacteria also correlated with serum bilirubin levels. Atopobium and *Clostridium leptum* (anti-inflammatory gut bacteria) were negatively correlated with blood bilirubin and fibrosis scores, respectively.[18] The group then went on to transplant intestinal microbiota (IM) from a patient with severe alcoholic hepatitis in to germ-free mice. They also used a control group of germ-free mice given IM from an alcoholic patient who did *not* have AAH. The mice received IM transplants and were then fed an alcohol-containing diet for 5 weeks. The mice who received microbiota transplants from the non-AAH donor gained significantly more weight than those who received microbiota from the severe AAH donor. Furthermore, liver inflammation was more severe in the severe AAH-treated mice, compared with the non-AAH-treated mice. Along with this, mice treated with the severe AAH IM had increased intestinal permeability and greater translocation of IM components than those treated with non-AAH microbiota. It was postulated that this may, in part, be explained by the reduction in Muc2 expression observed in the severe AAH-treated mice. Muc 2 is a mucin which provides a barrier to prevent translocation across the gut wall. Gut microbiota composition differed significantly in the severe AAH-treated mice when compared with non-AAH-treated mice. Bacteroides was the dominant bacteria in both groups, but was significantly more represented in severe AAH mice. Bilophila, Alistipes, Butyricimonas and Clostridium cluster XIVa were also significantly more abundant in the severe AAH mice, when compared with non-AAH mice. *Parasutterella excrementihominis* was found to make up 8.56% of non-AAH mouse microbiota, but represented only 0.001% in severe AAH donors and was absent in severe AAH-treated mouse microbiota, suggestive of a possible protective effect from this bacteria.

Metabolites in severe AAH-treated mice were also different, with alterations in bile acid composition. Ursodeoxycholic acid (UDCA), a secondary bile acid, and the primary bile acid chenodeoxycholic acid, CDCA, were noted to be increased in the faecal metabolome of non-AAH-treated mice, when compared with severe AAH-treated mice. Moreover, UDCA protects hepatocytes through its antioxidant properties and CDCA can be converted to UDCA by the action of gut bacteria.[19] CDCA also enhances production of alcohol dehydrogenase 1 (ADH1), enhancing alcohol breakdown (Figure 1).

Aside from alterations in the bacterial make-up of the gut, alcohol has also been shown to alter the composition of gut dwelling fungi. Yang et al assessed the impact of chronic alcohol administration on the murine mycobiota (fungal inhabitants) and noted fungal overgrowth.[20] Beta-glucan, a constituent of fungal cell walls, was found in the systemic circulation of the mice exposed to alcohol. Treatment with Amphotericin B reduced translocation of Beta-glucan and improved ALD. Alcohol-dependent human subjects were noted to have reduced fungal diversity and an overgrowth of Candida species.

2.5 | Primary sclerosing cholangitis

Primary sclerosing cholangitis is a chronic condition of unclear aetiology causing inflammation and subsequent stricturing of the bile ducts, leading to recurrent episodes of infection and eventually cirrhosis and liver failure. It is often associated with inflammatory bowel disease (IBD), more commonly ulcerative colitis (UC). When the bacterial composition of stool samples from patients with PSC was compared with healthy controls or patients with IBD,[21] 3 genera of bacteria were over-represented in PSC (*Enterococcus*, *Fusobacterium* and *Lactobacillus*) with overall reduced bacterial diversity. This finding was independent of co-existent IBD and UDCA treatment. These changes were most pronounced in those with cirrhosis and previous liver transplantation, compared with those with stable disease. Another study analysing the mucosa-associated bacteria, as opposed to stool, also found a lower bacterial diversity in patients with PSC with an underrepresentation of an uncultured Clostridiales II.[22] This was corroborated by a further study which noted Veillonella to be enriched in patients with PSC. Microbial communities were similar in PSC patients with and without IBD.[23] While the gut microbial profile in patients with PSC is distinct from patients with UC without biliary disease and healthy controls, another study assessing microbiota obtained from mucosal samples found an enrichment of Blautia and Barnesiellaceae.[24] Furthermore, several observations support the gut microbiota being directly involved in the pathogenesis of PSC with animal models of small bacterial overgrowth showing PSC-like changes in the liver, which can be counteracted by antibiotics and cultured cholangiocytes from patients with PSC that seem hypersensitive to PAMPs such as lipopolysaccharide (LPS). [25,26]

2.6 | Hepatocellular carcinoma

Fifteen patients with cirrhosis undergoing transplantation work up were matched with 15 patients with cirrhosis and HCC, also undergoing transplantation work up. The authors prospectively assessed the gut microbiota of all patients in the pre-transplant period and noted a marked increase in *E. coli* in those patients with HCC, compared with those with cirrhosis without HCC.[27] Patients were similar in age and sex and were matched according to aetiology of cirrhosis and model for end-stage liver disease scores. These results suggest a

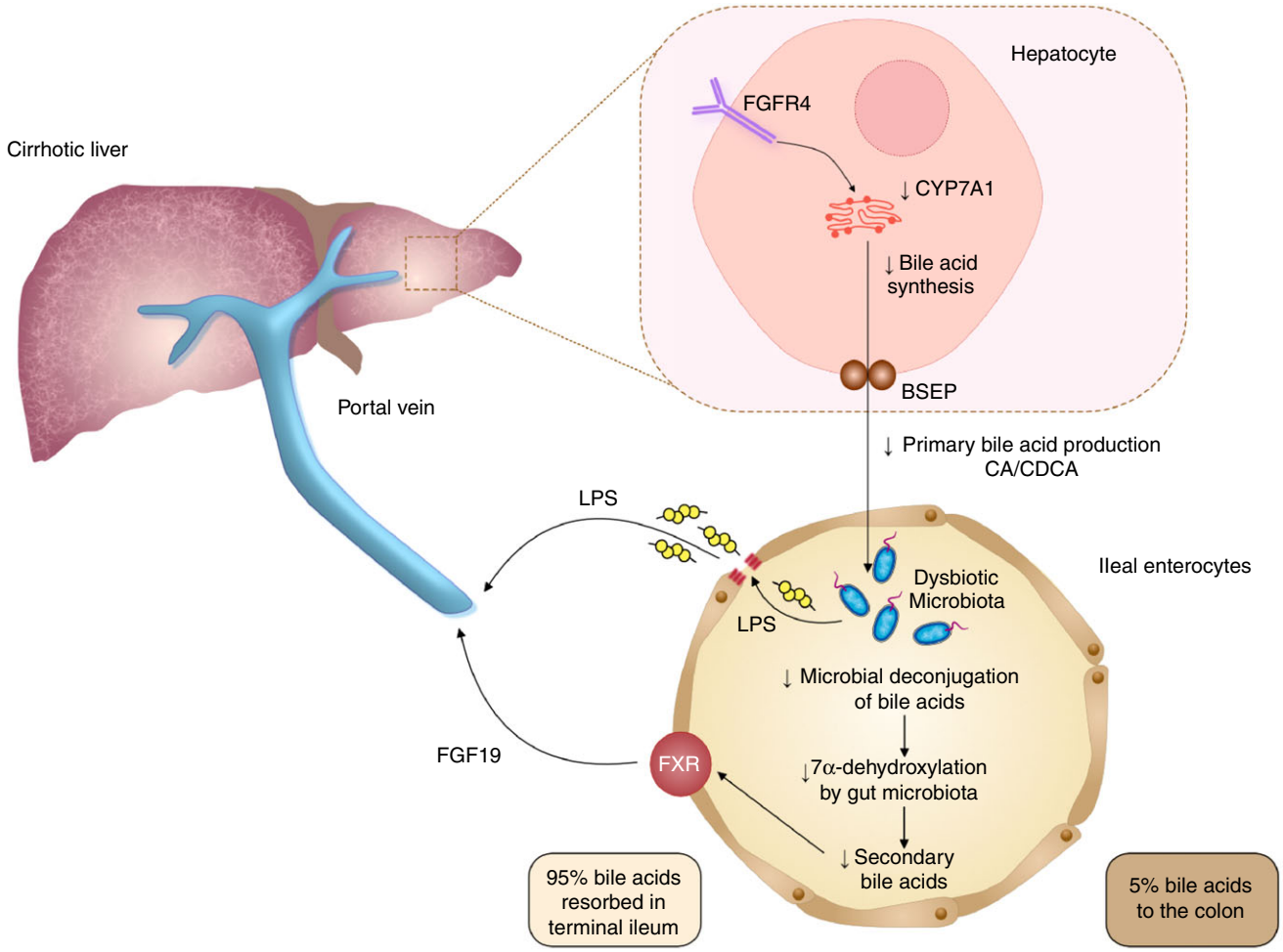

FIGURE 1 Diagrammatic representation of bile acid circulation. Cirrhosis alters bile acid homeostasis by disrupting the autochthonous gut flora. Cirrhosis reduces beneficial taxa, for example, Lachnospiraceae, Roseburia, Blautia and Ruminococcaceae, which make the SCFA, butyrate. Reduced butyrate disrupts tight junction integrity, allowing movement of LPS from the gut lumen into the portal circulation, where it acts on hepatic macrophages to cause pro-inflammatory cytokine release. Cirrhotic patients also possess increased numbers of pathogenic bacteria such as Enterobacteriaceae, which in turn produce more LPS, also up-regulating inflammation. Bile salt hydrolases expressed by gut bacteria deconjugate primary bile acids. Other bacteria expressing 7α-dehydroxylase convert these to secondary bile acids. These bacteria are altered in cirrhosis, reducing the conversion of primary to secondary bile acids. Bile acids produced in the gut lumen bind to the FXR, producing FGF19, which enters portal circulation and binds to the FGFR4, down-regulating CYP7A1 expressed on the endoplasmic reticulum within hepatocytes. Down-regulation of the CYP7A1 enzyme reduces the production of primary bile acids by the liver, further contributing to the disrupted microbiota. BSEP, Bile salt exporter protein; CDCA, chenodeoxycholic acid; CA, cholic acid; CYP7A1, cytochrome P450 family 7 subtype A polypeptide 1; FXR, farnesoid X receptor; FGF19, fibroblast growth factor 19; FGFR4, fibroblast growth factor receptor 4; LPS, lipopolysaccharide

potential role for *E. coli* in hepatocellular carcinogenesis. Further large-scale studies are needed to confirm this association and investigate the potential mechanisms associated with this correlation (Table 1).

3 | THE ROLE OF THE GUT MICROBIOTA AS A DRIVER OF SYSTEMIC INFLAMMATION IN END-STAGE CHRONIC LIVER DISEASE

With the onset of cirrhosis, several intestinal factors contribute to downstream deleterious local and systemic effects that clinically drive several adverse outcomes including the development of hepatic encephalopathy (HE). This as discussed previously results from the delivery of gut-derived bacteria and their products directly to the liver via the portal vein, with the liver generating a robust innate immune response. Small bowel bacterial overgrowth in combination with translocation of these bacteria and their endotoxins (including LPS, flagellin, peptidoglycan and bacterial DNA) can cross a more permeable gut epithelial membrane exposing the liver to immune-activating bacterial degradation products. This is further exacerbated by underlying portal hypertension and endothelial dysfunction,[28] while portosystemic shunting increases the delivery of these bacterial

TABLE 1 Summary of changes in gut microbiota in liver disease

Condition	Predominant microbiota
Health	Bacteroidetes and Firmicutes, Actinobacteria
Cirrhosis	Reduced autochthonous bacteria such as Lachnospiraceae/Ruminococcus and Clostridiales XIV and increased pathogenic species, eg, Enterobacteriaceae, Staphylococcaceae and Enterococcaceae
Non-alcoholic fatty liver disease	Increased Bacteroidetes (some studies report reduction or no change),[8] reduced Firmicutes, increased Proteobacteria, Enterobacteriaceae and *Escherischia*
Alcohol-sensitive mice	Reduced Bacteroidetes, increased Firmicutes and Actinobacteria
Alcoholic hepatitis	Increased Bifidobacteria and Streptococci
Primary sclerosing cholangitis	Reduced diversity, over-representation of Enterococcus, Fusobacteria and Lactobacillus, enriched Veillonella
Hepatocellular carcinoma	Increased *Escherischia coli*

degradation products to the systemic circulation evading the reticuloendothelial system.[29]

Endotoxins activate hepatic macrophages via toll-like receptors (TLR), inducing the production of pro-inflammatory cytokines such as TNF-α and interleukin-8 (IL-8) which triggers the hepatic migration of neutrophils and monocytes.[30] This can ultimately culminate in hepatic injury and systemic inflammation contributing further to immune disarray predisposing individuals to infection and heralding the development of decompensating complications such as sepsis, bleeding, HE and acute-on-chronic liver failure.[31]

The evolution of gut dysbiosis has been causally linked to the pathogenesis of cirrhosis and the progression to end-stage liver disease.[32] Indeed, quantitative metagenomic analyses have demonstrated that 75 245 microbial genes differ in abundance between patients with cirrhosis and healthy controls.[33]

There is a growing evidence base that supports a relative decrease in the composition of potentially beneficial autochthonous taxa as well as a relative overgrowth of potentially pathogenic taxa (such as Staphylococcaceae, Enterobacteriaceae, and Enterococcaceae), which has been independently associated with severity of liver disease and the development of endotoxemia and multiple end-organ dysfunction.[31,34–37] The pathophysiological mechanisms underpinning this are likely to be complex and have been poorly characterised. It has been postulated that the reduced production of SCFAs, anti-bacterial peptides and changes in bile acid production arising from gut dysbiosis all contribute, in part, to worsening disease severity and intestinal decontamination with non-absorbable antibiotics such as rifaximin which has recently been demonstrated to be an effective treatment for HE,[38,39] implicates gut dysbiosis in contributing to the development of neurocognitive dysfunction. A reduction in the SCFA-producing Lachnospiraceae might also increase colonic pH, hence increasing ammonia production and absorption, resulting in HE.[35]

Salivary dysbiosis has also recently been reported to be present in patients with cirrhosis, with an associated systemic and buccal pro-inflammatory milieu.[40] Salivary dysbiosis was more pronounced in over a third of cirrhotic patients that went on to require liver-related hospital admissions during the 90-day follow-up period, suggesting that saliva may play a key role in maintaining intestinal barrier integrity and tempering oxidative stress. That said, the contribution of salivary dysbiosis compared to distal gut dysbiosis is likely to be trivial due to the quantum difference in the volume of bacteria between the two sites.

4 | THE GUT–LIVER–BRAIN AXIS IN CIRRHOSIS

For well over a century, ammonia has been considered central in the pathogenesis of HE in patients with cirrhosis. The majority of circulating ammonia arises from the intestinal breakdown of ingested amino acids and urea by resident gut bacteria. Increased enterocyte expression of enterocyte phosphate-activated glutaminase[41] further augments net intestinal ammonia production and probably also accounts for why germ-free dogs with a portocaval shunt still develop hyperammonemia and HE.[42] In health, ammonia is detoxified to urea in the liver in peri-portal hepatocytes and excreted via the kidneys, or alternatively converted to glutamine by glutamine synthetase (GS) expressing perivenous hepatocytes.[43,44] Skeletal muscles which richly express glutamine synthetase become the predominant detoxifier of ammonia in patients with cirrhosis especially those with large spontaneous or transjugular intrahepatic portosystemic shunts. Astrocytes rich in GS serve to protect neurones from the toxic effects of ammonia and can convert ammonia and glutamate into glutamine. Glutamine accumulation within astrocytes induces astrocyte swelling and, in excess, may be transported into mitochondria where it may be broken back down to ammonia again inducing free radical formation and oxidative stress.[45–48] Ammonia also induces circulating neutrophil dysfunction, causing release of ROS, up-regulating systemic inflammation and oxidative stress.[49]

4.1 | Therapeutic strategies targeting the gut-liver axis in chronic liver disease

4.1.1 | Non-absorbable disaccharides

Historically, the mainstay therapy for HE in patients with chronic liver disease has been to target the gut with the aim of reducing colonic bacterial generation and reducing blood ammonia. Non-absorbable disaccharides such as lactulose acidify the gut lumen deterring urease-producing gut bacteria from thriving coupled with inhibition of ammonia diffusion from the lumen into the bloodstream. While the utility of lactulose in acute HE has been hotly debated in the absence of any large multicentre randomised trials ever being performed,[50–52] there is a robust evidence base to support its use in the secondary prevention of overt HE.[53]

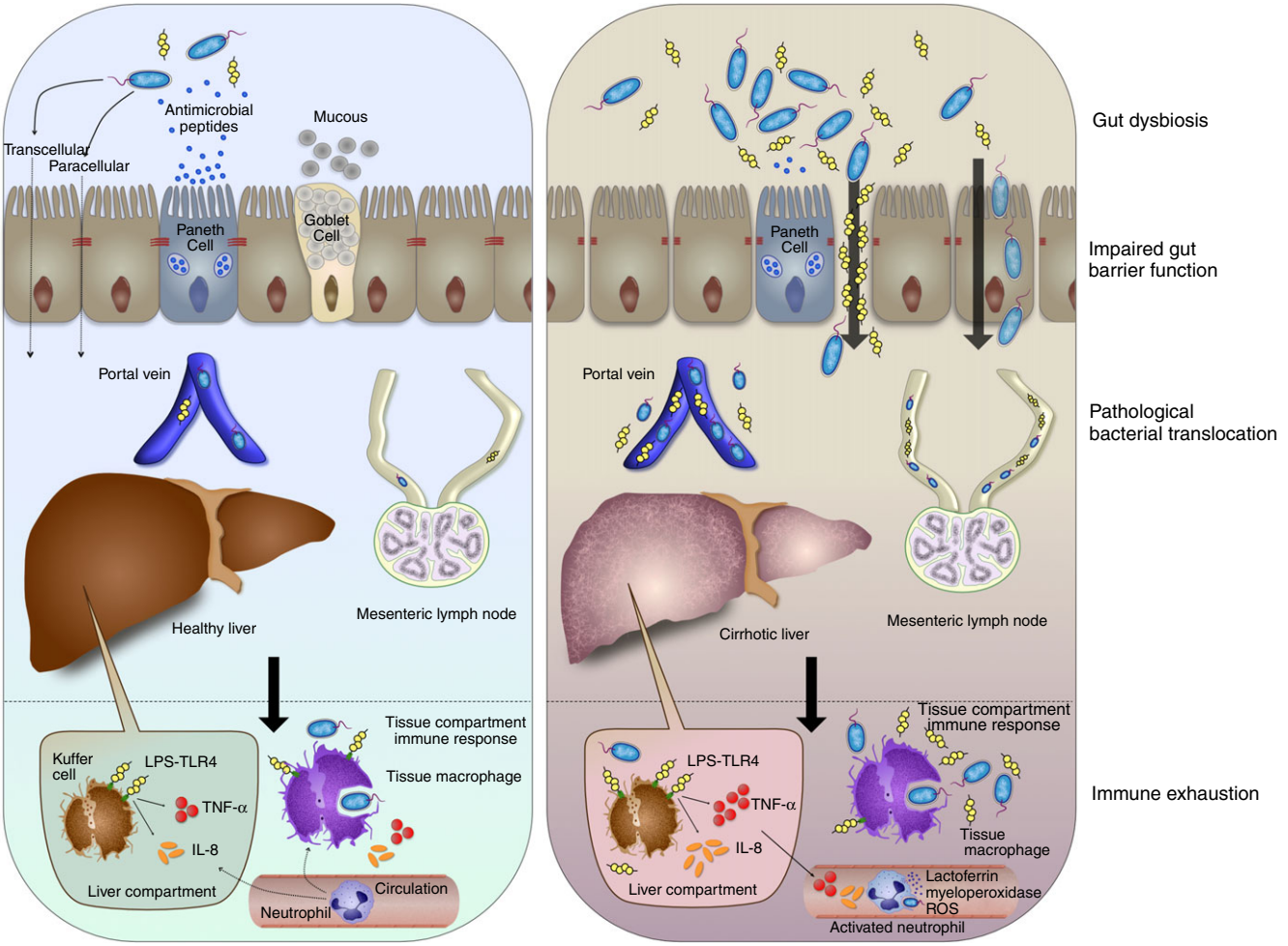

FIGURE 2 Movement of bacteria from the gut lumen to the liver in health and in cirrhosis. Figure 2 shows movement of bacteria from the gut lumen to the liver in health and in cirrhosis. In health gut barrier, integrity is maintained by a combination of factors including mucus from goblet cells, secretory IgA, bile acids and tight junctions. This prevents unregulated movement of bacteria and endotoxin from the gut to the liver via the portal vein. In cirrhosis, gut dysbiosis and bacterial overgrowth disrupt the usual mechanisms of protection, resulting in increased pathological bacterial translocation (via trans- and para-cellular routes) and endotoxin uptake. These products reach the liver and mesenteric lymph nodes, activating immune cells and causing pro-inflammatory cytokine release (eg TNF-α and IL-8). Circulating neutrophils degranulate in response to systemic TNF-α and IL-8 released from hepatic macrophages (Kupffer cells) in response to the endotoxin exposure (via TLR4 receptors on the macrophages). These neutrophils release a variety of granules including lactoferrin, myeloperoxidase and ROS into the circulation contributing to a systemic inflammatory response. Neutrophils also avidly phagocytose any circulating bacteria as will tissue macrophages. Abbreviations: interleukin-8 (IL-8), Lipopolysaccharide (LPS), reactive oxygen species (ROS), toll-like receptor 4 (TLR4), tumour necrosis factor alpha (TNF-α).

4.1.2 | Antibiotics

A Cochrane systematic review and meta-analysis favoured antibiotics such as vancomycin, neomycin and metronidazole over non-absorbable antibiotics; however, their oto-, nephro- and neurotoxicities have precluded their long-term use.[50] The broad-spectrum antibiotic rifaximin which has minimal systemic absorption has recently come to the forefront after a large double-blinded randomised controlled trial demonstrated a significant improvement in maintained remission from HE and a reduction in hospitalisations due to HE.[39] Its mechanism of action is not yet fully understood as it does not convincingly lower blood ammonia levels and is thought to be multi-factorial. It may ameliorate the cirrhosis dysbiosis ratio, that is, the ratio of beneficial to harmful bacteria and may improve the function of beneficial gut microbiota. Rifaximin may reduce the amount of toxic metabolites produced by the gut microbiota and appears to reduce serum pro-inflammatory cytokine levels. It may also exert an effect on secondary bile acids in the bowel and their impact on gut microbiota.[54] A 2013 study looked at the impact of rifaximin upon gut microbiota and serum and urine metabolome in 20 cirrhotic patients with minimal HE. Subjects were treated with rifaximin 550 mg BD for 8 weeks. Rifaximin improved cognition and endotoxemia. Serum saturated and unsaturated fatty acids were significantly increased following rifaximin treatment. There was no significant change in the composition of the gut microbiome in response to rifaximin treatment, aside from a small increase in Eubacteriaceae and reduction in Veillonellaceae.[55] These results suggest that rifaximin does not alter the composition of gut bacteria, rather it alters the metabolites of

the resident bacteria. Arachidonic and linoleic acids were increased by rifaximin and are known to have a beneficial effect upon brain function, and cognition was improved by rifaximin in this study.

4.1.3 | Probiotics

A recent meta-analysis assessed the impact of probiotics ("healthy" or beneficial bacteria) on HE. The investigators looked at 9 high-quality randomised controlled trials assessing the effects of probiotics. They found that probiotics improved both minimal and overt HE.[56] Not only were probiotics noted to reduce arterial ammonia concentrations, but they also reduced the frequency of hospital admissions, rates of infection and progression of minimal to overt HE. Only trials that had confirmed the presence of minimal HE on psychometric testing were included. There were no significant differences in overall mortality or rates of minor adverse events, but probiotics were associated with reduced frequency of severe adverse events. This meta-analysis only included 9 trials that were considered to be of a high standard based on their Jadad scores, but may have excluded trials that had low scores, but were not subject to bias. The probiotics used in each study also varied widely. The need for large-scale high-quality studies of probiotics in HE is clearly warranted.

A phase I study published in 2014 examined the effect of probiotics in cirrhosis.[57] Thirty cirrhotic patients with minimal HE were randomised to receive Lactobacillus GG (LGG) or placebo for 8 weeks. There was no difference between the active treatment group and placebo group with respect to serious adverse events, but the LGG group did experience more diarrhoea (self-limiting). The LGG group was noted to have altered microbiome, reduced endotoxemia and TNF-α levels, but there was no change in cognition. Dysbiosis was defined as having a low ratio of beneficial taxa (Lachnospiraceae, Clostridiales XIV, Ruminococcaceae and Veillonellaceae) to other taxa (Enterobacteriaceae and Bacteroidaceae). The LGG-treated group experienced a reduction in dysbiosis. Interestingly, the investigators were not able to appreciate an increase in stool Lactobacillus levels at the thresholds measured. This was postulated to be due to Lactobacillus promoting the growth of other beneficial bacteria. An alternative mechanism postulated that Lactobacillus used their pili to attach to the intestinal mucosa, displacing pathogenic bacteria and hence preventing them from stimulating the host immune system. Further larger scale studies are needed to judge the effectiveness of probiotic treatment upon patient symptoms, but this study suggests that they are safe for use in patients with cirrhosis. Unfortunately to date, the majority of studies of probiotics have been disappointing. The 2015 meta-analysis reviewed 135 studies, but was only able to draw conclusions from 9 studies that they deemed to be of sufficiently high quality. In principle, probiotics should help reconstitute a healthy complex microbiome after dysbiosis has occurred in the cirrhotic gut; however, owing to the trillions of resident bacteria in the human colon, the number of inoculated bacteria given as a probiotic is likely to be wholly insufficient to displace all of the resident dysbiotic bacteria. Cirrhotic subjects often have small bowel bacterial overgrowth, which increases the number of bacteria present in the gut, and when dosed with probiotics, the "healthy bacteria" are required to compete with virulent resident species. The probiotic bacteria may not reach the colon, due to altered intestinal motility or may be destroyed by gastric acid upon ingestion. Cirrhotic patients are often treated with antibiotics for presumed infection, and these drugs may kill off the fragile probiotic species, resulting in as yet disappointing outcomes for probiotics in liver disease.

4.1.4 | Synbiotics

A synbiotic is a healthy bacteria or probiotic, combined with a prebiotic, that provides fuel for the probiotic. A study published in 2004 assessed the impact of a synbiotic in cirrhotic patients with minimal HE. Twenty patients received the synbiotic (a combination of 4 non-urease-producing bacteria with pectin, inulin, beta-glucan and resistant starch). Of the remaining subjects, 20 patients received the fermentable fibre on its own and 15 were given a matched placebo for 30 days. Synbiotic treatment was shown to increase non-urease-producing Lactobacillus species for 14 days following cessation of treatment, leading to a reduction in minimal HE in half of the subjects and to reduced blood ammonia concentrations and endotoxemia. Those given the fermentable fibre alone also had an improvement in minimal HE, reduced blood ammonia levels and increased Bifidobacterium in stool, with reduced pathogenic bacteria, as seen in the synbiotic group.[58] Further studies have not been performed to assess whether synbiotics have a durable effect upon symptoms.

4.1.5 | Diet

A recent study assessing the impact of iso-caloric high-fat, high-sugar and high-protein diets, as well as an unlimited high-fat intake diet, upon the development of NAFLD in rats, showed that diets high in fat and sugar were detrimental to health. The authors also examined the effects of these diets upon gut microbiota composition.[59] Rats fed a high-fat diet (restricted and unrestricted calorie intake) had higher portal LPS levels than the other dietary groups. Unrestricted high-fat diet fed mice also exhibited higher hepatic triglyceride concentrations. Compared with the control group, free fat intake mice had more abundant Firmicutes, with a reduction in Bacteroidetes. Those who consumed a high-fat, but iso-caloric diet only exhibited an increased Firmicutes abundance. Those fed a high-sugar diet and high-protein diet experienced the opposite effect (a reduction in Firmicutes and increase in Bacteroidetes). It was postulated that high-fat and high-sugar diets impact upon development of NAFLD, independently of caloric intake and alter the gut flora in different ways. The beneficial effects of a high protein diet may relate to increased levels of Prevotella and Oscillospira.[59]

There is a complex interplay between host genetics (in particular the PNPLA3 and TM6SF2 gene mutations), diet (with high fructose and high-fat diets being particularly effective at inducing de novo

hepatic lipogenesis, potentially leading to NAFLD), and gut microbiota in NAFLD.[60] Deficiency of dietary choline (found in eggs and meat as well as cruciferous vegetables) can lead to reversible hepatic steatosis. Choline may exert its effect by removing fat from hepatocytes. Gut microbiota metabolise choline to methylamine, which not only reduces effective concentrations of choline, but methylamine is itself toxic and pro-inflammatory. Gammaproteobacteria are thought to prevent steatosis in women on a choline-deficient diet.[60] Excess fructose intake may also correlate with NAFLD. A number of studies have found evidence to suggest that excess fructose intake up-regulates de novo lipogenesis and inhibits beta fatty acid oxidation, leading to hepatic steatosis and initiating inflammation via TLR signalling and release of inflammatory cytokines.[60,61] Fructose also reduces insulin sensitivity.[62]

Previously, it was postulated that protein restriction in cirrhosis could reduce nitrogenous load and hence reduce ammonia production, in turn reducing episodes of HE. A Spanish study published in 2004 showed that protein restriction had no impact upon HE in cirrhotic patients.[63] Twenty cirrhotic patients admitted with HE were randomised to receive a low protein diet or a normal diet for 14 days. Protein synthesis and catabolism were measured using radiolabelled glycine-N^{15}. The low protein group exhibited higher protein breakdown, with no major benefit upon HE outcomes. Indeed, most patients with cirrhosis are malnourished and protein restriction will undoubtedly worsen their catabolic state; therefore, protein intake should never be restricted in these patients.

4.1.6 | Faecal microbiota transplantation

Faeces from healthy volunteers (FMT) have been used with excellent success rates in recurrent *Clostridium difficile* infection, to re-populate the gut with healthy intestinal flora, and are approved by NICE in the UK for this indication (Faecal microbiota transplant for recurrent *C. difficile* infection, Interventional procedures guidance [IPG485] Published date March 2014). There have to-date been only a handful of human studies examining FMT in liver disease. A Canadian group is conducting a pilot study assessing the impact of FMT upon patients with cirrhosis and breakthrough HE in patients on standard therapy (lactulose, rifaximin, or metronidazole). They will administer FMT via an initial colonoscopy and weekly enemas.[64] Kao et al have also published a case report of a 57-year-old patient with cirrhosis secondary to alcohol and hepatitis C treated with FMT when he was no longer able to access rifaximin for his HE. He was treated with 5 weekly doses of FMT, initially at colonoscopy and then administered by enema. The patient experienced subjective and objective improvements in his HE, but these were only for the duration of the treatment and he subsequently developed gallstone pancreatitis, resulting in worsening of his HE [65]

Bajaj et al have also undertaken a safety study assessing the impact of FMT compared with no treatment in patients with recurrent HE. They assessed the safety of FMT provided by a rationally selected single donor and examined the impact of FMT upon faecal microbiota composition and urinary metabolome. Subjects were pre-treated for 5 days with broad-spectrum antibiotics and 90 ml of FMT was instilled by retention enema. The impact of FMT upon cognitive function was also assessed and found to have a significant positive impact.[66]

Our group will be commencing a feasibility study in early 2018 [PROFIT Trial] looking at the administration of FMT to patients with cirrhosis to assess the safety and tolerability of the intervention. The ultimate aim of the PROFIT trial is to assess the impact of FMT upon patients with cirrhosis and whether or not this intervention positively alters the gut microbiota and ultimately improves outcomes in this difficult to manage group. This trial differs from those previously undertaken in that the FMT will be administered by upper GI endoscopy in order to deliver microbiota directly to the small bowel where dysbiosis is believed to be greatest. PROFIT will compare FMT to placebo in a single-blinded fashion with a plan to recruit 32 patients, 24 of whom will receive FMT and 8 placebo.

A consensus report was recently published setting out guidance for the use of FMT in recurrent *C. difficile* infection, IBD, irritable bowel syndrome and the metabolic syndrome.[67] Recommendations were made for the screening of donors and for the archiving of donor and patient samples for review in the case of transmissible infection. Although FMT in liver disease was not explicitly addressed, the recommendations are applicable to our patients, who are relatively immunosuppressed and thus at increased risk of infection due to altered gut permeability.

5 | THE FUTURE

Our evolving knowledge of the dysbiosis which develops in chronic liver disease may help to develop more targeted manipulation of the gut microbiota. Saiedi et al were able to genetically engineer an *E. coli* strain to specifically target the pathogenic bacteria *Pseudomonas aeruginosa*.[68] The *E. coli* vessel was designed to produce a pyocin (a bacterial killing protein that kills *P. aeruginosa* and not *E. coli*) in response to detection of the pathogen via quorum sensing. The *E. coli* cells then auto-lysed, releasing the pyocin and killing any *P. aeruginosa* in the vicinity. Better understanding of the unique changes seen in cirrhosis may help develop targeted therapies to remove pathogenic strains and selectively re-populate the gut with favourable species.

6 | CONCLUSIONS

As the liver receives the majority of its blood supply directly from the microbiota laden gut, inherently the host microbiome and the liver are intimately linked. This review has examined in detail the role gut microbiota serve in the pathogenesis and progression of chronic liver disease and explored how modulation of the gut microbiome might offer novel therapeutic targets. There is a wealth of data that now link NAFLD, ALD and PSC with an altered and potentially "dysfunctional" gut microbiome. Advanced liver disease is associated with the development of gut dysbiosis, with an over-representation of pathogenic bacteria and small bowel bacterial overgrowth. Altered gut permeability, with resultant translocation of

endotoxins and bacteria into the portal (and via shunting into the systemic) circulation, results in immune activation and eventual hepatic injury as well as systemic inflammation. Amelioration of the dysbiosis or "rebiosis" could potentially be achieved by manipulation of microbiota through dietary changes and non-absorbable antibiotics such as rifaximin. In an era of multi-drug resistant bacteria, the need for therapies to alter microbiota, without resorting to broad-spectrum antibiotics, is strong. Direct manipulation of the microbiota without drugs may be achieved via probiotic or synbiotic therapy or eventually whole microbiota transplants in the form of FMT. There is an urgent need for large-scale, high-quality studies in this area to assess the most effective way to achieve rebiosis. FMT has the advantage of repopulating the whole dysbiotic gut milieu, but the optimal route of administration, duration of treatment, and durability of response remain to be determined.

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AUTHORSHIP

Guarantor of the article: Debbie Shawcross.

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