Review

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# Microbiome as a therapeutic target in alcohol-related liver disease

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## Summary

Alcohol-related liver disease is associated with significant changes in gut microbial composition. The transmissibility of ethanol-induced liver disease has been demonstrated using faecal microbiota transfer in preclinical models. This technique has also led to improved survival in patients with severe alcoholic hepatitis, suggesting that changes in the composition and function of the gut microbiota are causatively linked to alcohol-related liver disease. A major mechanism by which gut microbiota influence the development of alcohol-related liver disease is through a leaky intestinal barrier. This permits translocation of viable bacteria and microbial products to the liver, where they induce and promote inflammation, as well as contribute to hepatocyte death and the fibrotic response. In addition, gut dysbiosis is associated with changes in the metabolic function of the intestinal microbiota, bile acid composition and circulation, immune dysregulation during onset and progression of alcohol-related liver disease. Findings from preclinical and human studies will be used to demonstrate how alcohol causes intestinal pathology and contributes to alcohol-related liver disease and how the latter is self-perpetuating. Additionally, we summarise the effects of untargeted treatment approaches on the gut microbiota, such as diet, probiotics, antibiotics and faecal microbial transplantation in alcohol-related liver disease. We further discuss how targeted approaches can restore intestinal homeostasis and improve alcohol-related liver disease. These approaches are likely to add to the therapeutic options for alcohol-related liver disease independently or in conjunction with steroids.



**Key points**

Microbes in the human intestine play an important role during health and disease.

## Introduction

Over the past decade, intense efforts have been made to study the gut microbiota in order to understand disease mechanisms and explore the therapeutic potential of modulating it. The gut microbiome, also known as the intestinal microbiome, assumes increasing significance for mankind as it is probably the first metabolically active site of interaction of nature with host. The intestinal microbiota is central to the effects of diet, drugs and disease on the host.

Alcohol-related liver disease is a leading cause of morbidity and mortality, responsible for 0.9% of all global deaths and 47.9% of all liver cirrhosis-attributable deaths.[1] Severe alcoholic hepatitis, the most aggressive form of the disease, generally presents with acute hepatitis like illness, deep jaundice, over underlying cirrhosis, with a 30-day mortality rate ranging from 20–50%.[2] Corticosteroids remain the only available therapy, with a short-term response seen in about 60% of patients, but no long-term survival advantage over placebo.[2] Liver transplantation is the only curative option for these patients,[3] though recent alcohol consumption, sepsis and other ethical considerations often come in the way. Therefore, there is an urgent need to develop new therapies for patients with severe alcoholic hepatitis.

Since alcohol is consumed orally, the mechanisms by which it alters the gut microbiota, leading to disease progression, are incompletely understood. Alcoholics and patients with alcoholic hepatitis and liver cirrhosis display higher levels of bacterial products in their blood than healthy humans.[4,5] Therefore, modulation of gut microbial composition, including faecal microbiota transplantation (FMT) from healthy donors or precision microbiome therapies, is a logical treatment approach which is currently being evaluated for patientswith alcohol-related liver disease.

## Gut microbiome

The human intestine is home to a variety of microbes (bacteria, archaea, fungi and viruses). Bacteria consist of more than 10 different phyla. There is approximately the same number of bacterial cells as human cells,[6,7] but the human microbiome contains over 3 million genes, compared with only around 23,000 in the human genome.[8] Nearly 90% of the bacteria belong to 2 phyla, Firmicutes (gram positive) and Bacteroidetes (gram-negative); while the rest belong to 4 major phyla, Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia.[9,10] The phyla comprise of classes, that encompass orders which in turn have families, then genera and species of bacteria. There are >1,000 bacterial species in the human gut microbiota, which generally become stable in early childhood[11] depending on the dominant dietary pattern, such as Western (high animal fat and sugars) or Eastern (plant and vegetable) diets.[12] Though 60–80% of the gut bacteria cannot be cultured, we still diag-

nose intestinal bacterial overgrowth as at least $10^5$ cultured colony forming units of bacteria per ml in the jejunal aspirates.[13,14]

The gut microbiota plays crucial roles in: the maturation and continued stimulation of the host immune response;[15] the maintenance of intestinal barrier integrity, which limits pathogen perpetuation in the gut;[16] modulation of host-cell proliferation [17] and vascularisation;[18] regulation of intestinal, neurological[19] and endocrine functions,[20] and bone density.[21] The human gut microbiota provides a source of energy[22] (5 to 10% of daily host energy requirements); helps in the synthesis of vitamins[23] and neurotransmitters; metabolises bile salts;[24] reacts to or modifies specific drugs; and eliminates exogenous toxins.[25] In a healthy colon, the gut microbiota maintains a symbiotic relationship with the host and rapidly adapts to maintain eubiosis after an acute insult.

## Contribution of the gut microbiota to alcohol-related liver disease

### Transmissibility of alcohol-related liver disease via FMT

Alcohol ingestion causes intestinal bacterial overgrowth in preclinical models, predominantly in the upper small bowel,[26] as well as in humans.[27] In addition, changes in the microbial composition, commonly referred to as dysbiosis, are also present in rodent models[28] and patients with alcohol use disorder (see later for more details).[29] Chronic alcohol-associated changes in the intestinal microbiota appear to differ in animal models subjected to different chronic liver insults.[30] Thus, the question of whether changes to the gut microbiota are important for alcohol-related liver disease arises. A recent study addressed this important topic.[31] Germ-free mice – devoid of any microbes – fed with ethanol developed severe liver inflammation and necrosis when given gut microbiota from patients with severe alcoholic hepatitis. Subsequently, transfer of gut microbiota from patients with no alcoholic hepatitis led to less ethanol-induced liver injury and inflammation in these animals. The main deleterious bacterial species associated with transmission of the severe alcoholic hepatitis phenotype included altered Bacteroides phylum as well as *Bilophila, Alistipes, Butyricimonas, Clostridium, Proteus* and *Escherichia coli*.[31] In contrast, gut microbiota from patients with severe alcoholic hepatitis showed a relative lack of *Faecalibacterium prausnitzii*, a bacterial species known for its anti-inflammatory and mucosal-protective properties.[32] Taken together, the severity of ethanol-induced liver disease is transmissible via FMT in preclinical models using humanised mice. Additional evidence from human studies confirms these findings. FMT improves survival in steroid-ineligible patients with severe alcoholic hepatitis[33] (discussed in more detail later).

### Mechanistic contribution of the gut microbiota to alcohol-related liver disease

During intestinal homeostasis multiple barriers protect the human body from invading microbes. Commensal bacteria are considered a barrier to the colonisation of pathogens. The mucus layer is the first physical barrier maintaining a distance between the gut lumen and the host. The mucus layer is divided into an outer and inner mucus layer. Although the outer mucus layer is colonised by bacteria, the inner mucus layer is largely devoid of bacteria. Intestinal epithelial cells and Paneth cells secrete antimicrobial proteins into the inner mucus layer to kill bacteria and prevent translocation of bacteria to the inner mucus layer. The importance of maintaining a "sterile" inner mucus layer has recently been demonstrated. Regenerating islet-derived 3 gamma (REG3G) is a c-type lectin produced by intestinal epithelial and Paneth cells, which maintains the spatial segregation of the microbiota and host.[34] REG3G expression is reduced in the jejunum of mice fed chronic ethanol and in humans with alcohol use disorder.[26] Most importantly, the reduction of intestinal REG3G is associated with an increased number of mucosa-associated bacteria in mice and humans.[35] *Reg3g* deficient mice showed increased bacterial colonisation of the mucus layer and epithelial cell surface, more translocation of bacteria to mesenteric lymph nodes and the liver, and increased ethanol-induced liver injury, inflammation and steatosis in mice. Intestinal specific overexpression of *Reg3g* prevents these deleterious effects of chronic ethanol administration in mice. Most importantly, ethanol-induced tight junction disruption was not affected, and surrogate markers of increased paracellular permeability (see later for more details) were not affected in *Reg3g* transgenic mice.[35] Thus, mice can be protected from ethanol-induced liver disease by reducing the translocation of viable bacteria, despite the same degree of increased intestinal permeability.[36] These results suggest that mechanisms for bacterial translocation involve translocation of microbial products (also termed pathogen-associated molecular patterns or PAMPs) such as lipopolysaccharide through the paracellular route and translocation of viable bacteria by currently undefined mechanisms. Whether translocation of viable bacteria occurs in humans with alcohol use disorder and mild alcohol-related liver disease is not known. Although REG3G is important for protection from alcohol-related liver disease, it is dispensable for the development of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis.[37] Deficiency of *Mucin-2* (*Muc2*), the most abundantly secreted mucin in the small and large intestine, leads to a compensatory increase in antimicrobial lectins, such as REG3B and REG3G, and is protective against ethanol-induced liver damage.[51]

**Key points**

Gut microbiota contributes to the development of alcohol-related liver disease via different mechanisms. The altered gut bacteria in alcoholic hepatitis lead to suppression of the immune system, increase in hepatic inflammation and changes in microbial metabolites.

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An additional layer of protection is the innate and adaptive immune system in the subepithelial space. Little is known about a possible impaired immune reaction in the lamina propria in patients with alcohol use disorder.

Another very important physical barrier for preventing translocation of bacterial products is intestinal epithelial cells, which are closely bound together by tight junction proteins. Disruption of tight junction proteins commonly occurs in humans consuming alcohol.[38] An opening of the paracellular space between intestinal epithelial cells allows bacterial products to translocate from the intestinal lumen to extraintestinal space, including the portal vein, enabling them to reach the liver. In the liver these bacterial products induce an inflammatory response in resident Kupffer cells and other hepatic immune cells, a fibrotic response in hepatic stellate cells and a possible direct injurious effect on hepatocytes.[39] Translocation is not only limited to bacterial-derived products, but fungal cell wall components are also increased in preclinical models of ethanol-induced liver disease.[40] The aetiology of tight junction disruption is likely multifactorial, including the direct effects of alcohol and its metabolite acetaldehyde on tight junction proteins.[41] The intestinal dysbiotic microbiota and dysregulated bile acid metabolism might trigger tight junction dysfunction by inducing subclinical intestinal inflammation.[42,43] A close interrelation of ethanol metabolism, the circadian clock, and intestinal permeability has been shown. Alcohol disturbs the circadian rhythm *in vivo* by increasing Per2 in the duodenum and colon of rats[44] via impaired occludin function in the colon; the disturbed circadian rhythm leads to increased bacterial permeability. Although increased intestinal permeability is commonly observed in preclinical models of ethanol-induced liver disease, a recent human study challenges this notion. Only a subset (43%) of patients with alcohol use disorder and mild liver disease showed evidence of gut barrier dysfunction, as measured by the $^{51}$Cr-EDTA method. Interestingly, gut barrier dysfunction was associated with an altered microbiota composition.[29] These results suggest that factors other than increased intestinal permeability might contribute to the onset and progression of liver disease in patients with alcohol use disorder.

**Alcohol and gut metabolome**

Many studies using high performance liquid chromatography and mass spectrometry have shown that intestinal, systemic and urine metabolites are affected by chronic ethanol feeding or consumption, including amino acids, steroids and their derivatives, fatty acids and conjugates.[45–49] The relevance of these changes to alcohol-related disease progression remains to be elucidated in future studies.

Chronic alcohol consumption affects vitamin metabolism. The gut microbiota synthesises and is a major source of vitamin B in humans.[50] Enteric dysbiosis has been found to be associated with vitamin B deficiency. Chronic alcohol feeding inhibits carrier-mediated intestinal vitamin uptake, which is often associated with a decrease in the respective vitamin carrier expression.[51]

Changes in faecal lipid metabolites like the short-chain fatty acids (SCFAs) butyrate and propionate have been reported in rats following chronic ethanol administration.[49] In a healthy colon, gut microbiota produce SCFAs, such as acetate, propionate and butyrate, from the digestion of carbohydrates. These fatty acids act as a source of energy for intestinal epithelia and are needed to maintain the integrity of the intestinal barrier and mucosal immune tolerance. Examples of butyrate-producing bacteria include members of the Firmicutes phylum, such as *Faecalibacterium prausnitzii*. Butyrate is thought to help modulate intestinal barrier integrity by modulating the expression of tight junction proteins and mucin.[52,53] This suggests that encouraging growth of butyrate-producing species could reduce gut permeability and systemic inflammation. Inulin-type fructans promote the production of butyrate *in vitro*.[54]

Metagenomic and metabolomic studies show that chronic alcohol administration reduces the capacity of the gut microbiota to produce saturated long-chain fatty acids (LCFA), leading to reduced intestinal levels of saturated LCFA. *Lactobacillus* species, which use saturated LCFA as an energy source, are consequently reduced, causing tight junction barrier disruption. Supplementing saturated LCFA restores eubiosis, stabilises the intestinal gut barrier, and reduces ethanol-induced liver disease in mice. Ethanol exerts a direct effect on the saturated fatty acid biosynthetic gene population in intestinal bacteria, independently of the host.[55]

Primary bile acids are synthesised in hepatocytes, secreted via the bile duct into the duodenum, where they facilitate digestion and absorption of lipids and lipid soluble vitamins. Bile acids can modulate the gut microbiota via direct and indirect mechanisms.[56] In turn, the gut microbiota metabolises primary bile acids into secondary bile acids.[57] Most bile acids ( >95%) are actively taken up by intestinal epithelial cells in the terminal ileum, where they are exported on the basolateral membrane into the portal vein. Bile acids are ligands for the nuclear receptor farnesoid X receptor (FXR) and induce FXR target gene fibroblast growth factor (FGF)-15 in mice and FGF-19 in humans. Gut-derived FGF15/19 is released into the portal circulation, inhibits hepatic bile acid synthesis and exerts beneficial effects on lipid metabolism in the liver.[43] Serum FGF19, total and conjugated bile acids were significantly increased in patients with alcoholic hepatitis com-

pared to controls.[58] Taken together, bile acids are important communicating molecules in the cross-talk between the gut and liver, representing an excellent target for therapy.

The mechanisms by which the intestinal microbiota contributes to alcohol-related liver disease are summarised (Fig. 1).

## Human gut microbiota studies

### Gut microbiome in patients with alcohol use disorder

A decrease in *Lactobacillus* species is seen with alcohol consumption/feeding and in alcoholic cirrhosis.[26,28,29,59] *Lactobacillus* species are good bacteria and help by producing bacteriocins, like antibiotics, which inhibit pathogens within the *Enterobacteriaceae* family, such as *Salmonella* or *Shigella*. Their peroxidase system inhibits other bacteria.[60,61] By adhering to intestinal epithelial cells, *Lactobacillus* species protect against pathogenic and invasive bacteria.[62,63] Their fermentation products include SCFAs, such as lactic acid, propionic acid, or butyric acid, which provide nutrition to the epithelial cells.[64] However, an increase in *Lactobacillus* species is also known to be associated with hepatic steatosis.[65–67]

Studies on mucosa-associated colonic microbiome in patients with alcohol use disorders, with and without liver disease, and in healthy individuals has shown dysbiosis with lower median abundances of Bacteroidetes and higher abundances of Proteobacteria. These changes correlate with endotoxemia. The gut-brain axis may be important in patients with alcohol use disorder based on evidence that the gut microbiota regulates behavioural disorders such as alcohol dependence.[29] Therefore, it has been increasingly recognised that the gut microbiota might be relevant in alcohol-related human disorders.

### Gut microbiome in patients with alcohol-related liver disease

In alcohol-related liver disease and other chronic liver diseases the gut microbiome has been well characterised, showing an increase in Proteobacteria and Fusobacteria and a reduction in Bacteroidetes and *Lactobacillus* species.[59] *Lachnospiraceae* and *Ruminococcaceae* families are decreased whereas *Enterobacteriaceae, Alcaligenaceae,* and *Fusobacteriaceae* are significantly increased in cirrhosis[68] (Fig. 2). The bacterial microbiota has been shown to change with progression and stage of liver disease. In addition, fungal dysbiosis has recently been described. There is a decrease in fungal diversity and an increase in *Candida* species in patients with alcohol use disorder, independent of liver disease stage.[40] It would be worthwhile to dissect the influence of gut microbiota on liver disease progression and the influence of progressive liver disease on gut microbiota.

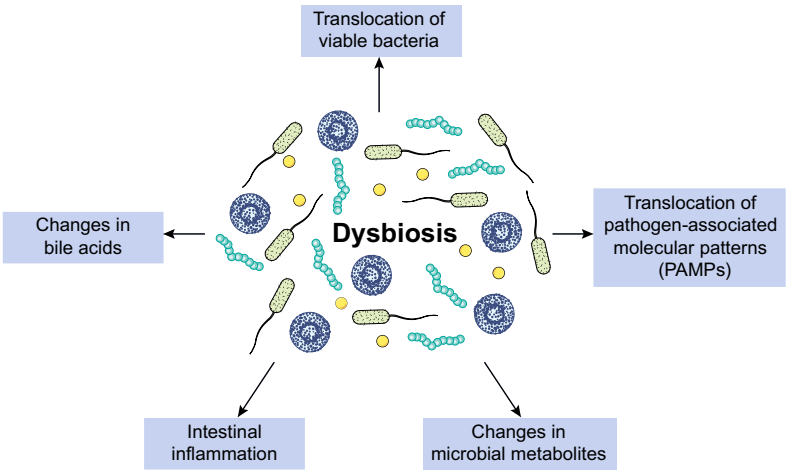


**Fig. 1. Contribution of the gut microbiota to alcohol-related liver disease.** Alcohol-associated dysbiosis contributes in multiple ways to the onset and progression of alcohol-related liver disease.

In alcohol-induced liver injury, the population of anaerobic and aerobic bacteria was found to be significantly higher in jejunal aspirates.[27] The degree of overgrowth correlates with the severity of cirrhosis.[69] The number of *Bacteroidaceae* was lower with a significant increase in the family of *Prevotellaceae* in the alcohol use disorder groups compared with healthy individuals.[59,70] A reduced concentration of *Coprococcus* and *Faecalibacterium prausnitzii* has been shown to be associated with gut integrity through butyrate production.[71,72]

Alcoholic hepatitis related dysbiosis was shown to be associated with an increase in *Bifidobacteria, Streptococci* and *Enterobacteria* and a decrease in anti-inflammatory *Clostridium leptum* or *Faecalibacterium prausnitzii*. The density of these protective strains inversely correlated with certain clinical parameters such as bilirubin levels.[31] Studies with FMT show that there is a clear microbiota signature in severe alcoholic hepatitis, a specific 'pathobiont' possibly exists.[33] Studies to identify these microbial species would identify new targets and therapies.

**Key points**

Patients with alcohol use disorder have changes in the gut microbiota composition, which leads to increased gut permeability and bacterial translocation.

## Modulation of the gut microbiota

The gut microbiota is probably the most pliable human organ. Achieving eubiosis is the aim of therapeutic gut modulation, be it by untargeted or targeted approaches. The former includes, modulation by diet, antibiotics, prebiotics, probiotics and FMT, while the latter includes using bacterial and host metabolites as targets[57] (Fig. 3).

**Key points**

The goal of microbiome centered therapies is restoring microbial ecology. This can be achieved by untargeted approaches including changes in diet, supplementation with beneficial bacteria, antibiotics or faecal microbiota transfer.

## Untargeted gut microbiota modulation

### Diet

One of the simplest and most effective approaches to modulate the gut microbiota is through diet, as microbial communities and the gut metabolome can rapidly shift with dietary changes. The gut microbiota can be modulated rapidly, within a day, by introducing a specific diet. However, the

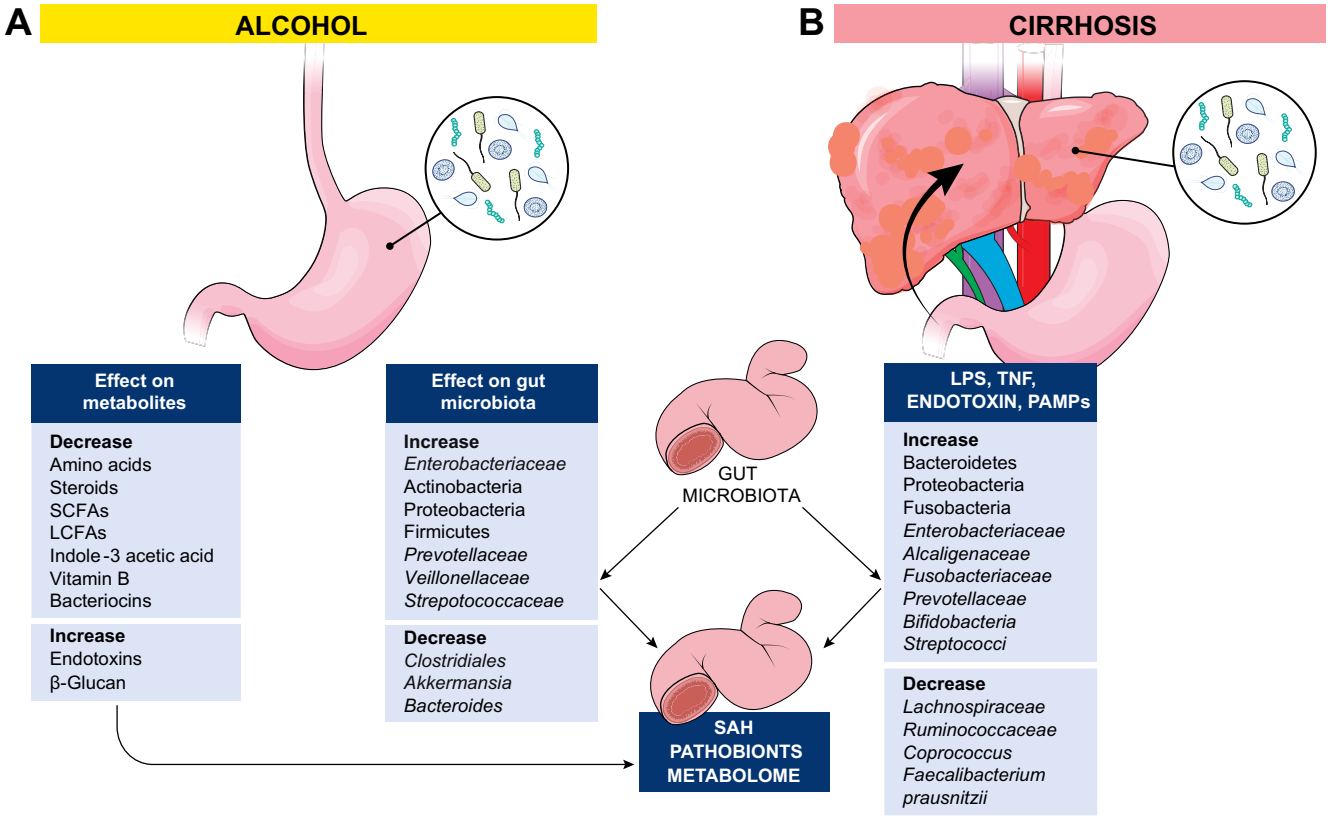


**Fig. 2. The effects of alcohol and chronic liver disease on the gut microbiota.** (A) The effects of alcohol on gut microbiota and metabolites, and (B) of chronic liver disease on the gut microbiota. The state of SAH generally has a distinct profile of gut microbiota (pathobionts) and metabolome, which is toxic may produce features of alcoholic hepatitis when transferred to a healthy animal. Attempts to modulate and reverse pathobionts and the metabolome, may improve the clinical condition. LCFAs, long-chain fatty acids; LPS, lipopolysaccharide; PAMPs, pathogen-associated molecular patterns; SAH, severe alcoholic hepatitis; SCFAs, short-chain fatty acids.

effects do wane 2 days after withdrawal of the introduced diet.[73]

Western diets which are rich in animal proteins and fats are associated with an abundance of *Bacteroides* and greater concentration of the products of amino acid fermentation.[73] In an elaborate study, using an animal-based diet, 3 of the bacteria associated with cheese and cured meats (*Lactococcus lactis, Pediococcus acidilactici* and *Staphylococcus*) became significantly more prevalent in faecal samples. The genus *Prevotella*, sensitive to long-term fibre intake[74,75] was reduced on an animal-based diet.[73] The plant based diets on the other hand, increased the concentrations of SCFA, such as butyrate, acetate and lactate on the same day.[73] Dietary fibres help to maintain the intestinal mucus layer, and fibre-deprived microbiota have been shown to use the colonic mucus layer as an alternative food source.[76]

It is important to know the functional characteristics of constituents of gut microbiota. Certain microbes, such as *Prevotella copri* and *Bacteroides vulgatus*, contribute to insulin resistance, while *Akkermansia muciniphila*,[77,78] and *Faecalibacterium prausnitzii*, are associated with increased insulin sensitivity. Metformin improves glucose homeostasis by increasing the population of *Akkermansia* species.[79]

An animal-based diet has been shown to increase the expression of bacterial genes encoding bile salt hydrolases, required for gut microbial production of deoxycholic acid.[80] Increased deoxycholic acid levels can influence the gut microbiota profile by inhibiting the growth of members of the Bacteroidetes and Firmicutes phyla and increasing the risk of hepatocellular carcinoma development.[81]

The animal-based diets increase the abundance of bile-tolerant microorganisms (*Alistipes, Bilophila* and *Bacteroides*) and decrease the levels of Firmicutes that metabolise dietary plant polysaccharides (*Roseburia, Eubacterium rectale* and *Ruminococcus bromii*).[82] Further, genes for vitamin biosynthesis and beta-lactamase are increased.

Long-term fibre intake supports lower levels of the products of carbohydrate fermentation and carbohydrate diets and is associated with a dominance of *Prevotella*.[83] Notably, the genus *Prevotella*, one of the leading sources of inter-individual gut microbiota variation[83] and hypothesised to be sensitive to long-term fibre intake,[84] is reduced by the consumption of an animal-based diet.

A recent study showed that a diet rich in fermented milk products, coffee, tea and chocolates is associated with higher microbial diversity and a lower risk of hospitalisation in patients with cirrhosis.[85] Coffee and tea provide dietary phenols which could modulate the gut microbiota[86] and reduce hepatic steatosis. This effect could be caused by polyphenols and is not related to the caffeine *per se,* as caffeinated beverages do not have similar effects. Phenols have beneficial effects through a mutual interaction with the microbiota, which promotes their effects on human health.[87] Rhubarb extract can improve the microbial ecosystem by enhancing concentrations of *Akkermansia muciniphila* and *Parabacteroides goldsteinii* and ameliorating alcohol-induced hepatic injury by downregulating inflammatory and oxidative injuries.

In a recent study, dietary saturated and unsaturated fats have been shown to differentially modulate the gut microbiota, intestinal barrier and liver injury in a mouse model of alcohol-related liver disease.[88] Compared to saturated fat and ethanol, unsaturated fat and ethanol administration produced more hepatic steatosis (micro- and macrovesicular), inflammation (determined by elevated levels of pro-inflammatory markers).

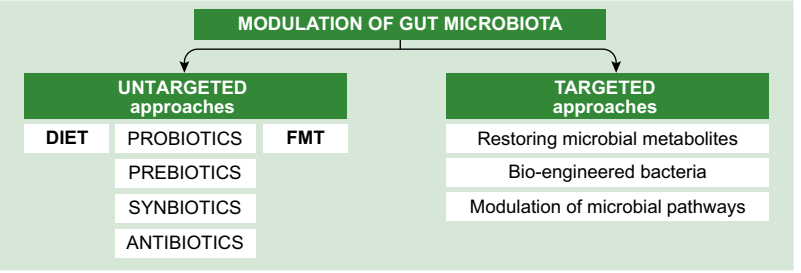


**Fig. 3. Therapeutic gut modulation.** Modulation of the gut microbiota can be achieved by untargeted or targeted approaches. Precision medicine approaches can be considered to restore eubiosis. Such approaches include efforts to restore levels of microbial metabolites that are changed during alcohol-related liver disease. Bio-engineered bacteria can deliver therapeutics to the microbiota or host. Alternatively, drugs can be used to change and modulate bacterial enzymes or pathways. FMT, faecal microbiota transplantation.

## Probiotics, prebiotics and synbiotics

Probiotics are cultures of single or multiple microbes which can modulate the properties of the existing gut microbiota. These microbes can promote the anti-inflammatory environment within the gut, thereby promoting the integrity of the intestinal barrier and preventing bacterial translocation and endotoxin production.[89] A potential mechanism by which probiotics act is via the production of antimicrobial agents, which inhibit the epithelial binding, growth and invasion of pathogenic bacteria.[90] Probiotics might promote intestinal barrier function by promoting intestinal epithelial growth and survival.[91] They might modulate the immune system, inhibiting the release of pro-inflammatory cytokines like TNF-α[92] and inducing the release of anti-inflammatory cytokines like IL-10[93] and TGF-β.

Probiotics were used in a rodent alcohol intake model using *Lactobacillus GG*, which improved gut leakiness and liver inflammation.[94] The addition of oat fibre or a supernatant of *Lactobacillus GG* induced similar results. VSL#3, a mixture of 8 probiotic strains (mainly *Lactobacillus* and *Bifidobacterium*), also improved liver lesions.[95] *Akkermansia muciniphila* administration prevented hepatic injury, steatosis, and neutrophil infiltration in a mouse model of alcohol-related liver disease.[96] Among species associated with the resistance to alcohol, *Lactobacillus, Bifidobacterium,* and *Roseburia* were identified as targets of interest. In a recent meta-analysis, probiotics were shown to improve minimal and overt hepatic encephalopathy[97] and reduce arterial ammonia concentrations.

Prebiotics are defined as substances that are helpful in promoting the growth and activity of specific microbes in the gastrointestinal tract which are beneficial to the host.[98] Prebiotics reach the large intestine and act as substrates for beneficial bacteria like *Bifidobacteria* and *Lactobacillus* species.[99] Prebiotic use has also been tested, and showed that fructooligosaccharides improved rodent alcohol-induced liver damage in a mouse model of ethanol-induced liver disease.[26] The most recent data concern the preventive role of pectin in alcohol-related liver disease, in which pectin treatment of alcohol-fed mice restored the level of *Bacteroides* and completely prevented the development of liver injury.[100] These results need to be tested in human beings, before further recommendations. Moreover, cirrhotic patients have bacterial overgrowth and dysbiosis in the small bowel, with high concentrations of pathogenic bacteria. The precise dosage, duration and type of pre- and probiotics needed to achieve eubiosis in severe alcoholic hepatitis is not known.

Synbiotics are a combination of healthy bacteria or probiotic with a prebiotic, that provides fuel for the probiotic.[101] In a small study in 2004, synbiotic treatment was shown to increase non-urease-producing *Lactobacillus* species for 14 days following cessation of treatment, leading to a reduction in minimal hepatic encephalopathy.[102] Studies are lacking in patients with alcohol-related liver disease.

## Antibiotics

Patients with alcoholic cirrhosis have bacterial overgrowth in the small intestine,[69,103] which worsens in advanced disease. While selective intestinal decontamination with antibiotics alleviated liver damage in rodents,[42,104] the same was not observed in a randomised, double-blind, placebo-controlled trial in patients with alcohol-related liver disease using the non-absorbable antibiotic paromomycin for 3–4 weeks.[105] This could be due to dysbiosis[21] and selection of pathogenic bacteria by treatment with paromomycin, or due to the short treatment period. In another

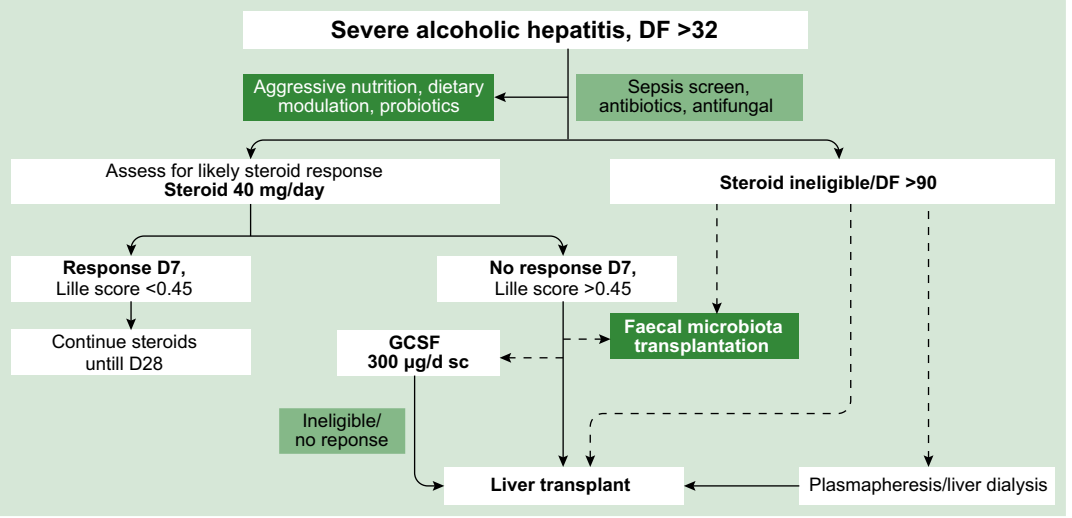


**Fig. 4. The role of gut microbiota modulation in the current management approaches of severe alcoholic hepatitis.** While the modulation of the gut microbiota by diet, probiotics and antibiotics has a distinct role, faecal microbiota transplantation at present is only an experimental therapy. Other emerging and support therapies have also been depicted. Dotted lines denote experimental therapies.

series, fluoroquinolones increased Bacteroidetes and Proteobacteria, without modifying Firmicutes.[106]

Some antibiotics could be beneficial. Rifaximin has been demonstrated to influence microbiota functions in patients with minimal hepatic encephalopathy despite non-significant changes in microbiota composition (higher abundance of *Eubacteriaceae* and reduction of *Veillonellaceae*). Treatment with rifaximin enhanced serum saturated and unsaturated fatty acids, while reducing microbiome–metabolome network connections, in particular those involving *Enterobacteriaceae, Porphyromonadaceae* and *Bacteroidaceae*.[107] Rifaximin increases the abundance of *Faecalibacterium prausnitzii* and *Lactobacillus*,[108,109] as well as arachidonic and linoleic acids, which have a beneficial effect upon brain function.[107]

In another study, ampicillin given with aspirin induces a significant increase in bleeding time in comparison to aspirin given alone in animals.[110] This effect was suggested to be mediated by modification in the gut microbiota, as administration of ampicillin significantly reduced microbial aspirin-metabolising activity.

Antibiotic administration is often associated with the spread of resistant microorganisms. The term "gut resistome" describes the collective resistance genes of the gut microbiota[111] and the genes could be transferred to other gut pathogens. Therefore, development of a resistome might prevent treatment of a chronic disease like alcohol use disorder with antibiotics for a prolonged period.

## Faecal microbiota transplantation

There are reports from as far back as the fourth century in China, of using the stool of healthy individuals or children to treat serious human diseases.[112] In the past few years FMT has resurfaced as an option for the treatment of human disease.

### Type of faecal preparation

Freshly collected faeces are preferred over the stored or frozen material. While there are some reports that the efficacy of the fresh and frozen faecal materials is comparable,[113] many investigators feel that the frozen materials loose a large proportion of bacteria and have reduced efficacy, so prefer fresh faecal preparations.[114–118] In fact, "fresh one hour defecation to transplant FMT" protocols have been proposed, using an automated purification system based on GenFMTer.[33,114–119] The 'ILBS FMT Protocol' of 2015[120] has been revised and now includes a 3 hour turnaround time. Whether an oxygen depleted environment is helpful to improve microbial functionality even for this short window, has not been studied.

Selective faecal transplant, using a select group of desired bacteria is emerging as a new avenue for specific disease groups.[120,121]

### Routes of delivery of FMT

There are several routes for administration of FMT, oral, nasogastric, nasoduodenal, nasojejunal,[113,114] endoscopic, rectal, colonoscopic or mid-gut transendoscopic enteral tubing.[122] It is not clear which route is preferable and in which disease state. The rectal route has been found to be effective in patients with *Clostridium difficile* induced diarrhoea, Crohn's disease and ulcerative colitis. On the other hand, in patients with cirrhosis the dysbiosis probably mainly affects the small bowel. Though there is no head to head comparison of oral or rectal routes, oral delivery of faecal concentrates is used more frequently.

**Key points**

Faecal microbial transplantation (FMT) in pilot studies has been shown to restore the gut flora, decrease disease severity and improve survival in a select group of patients with severe alcoholic hepatitis. Compared to steroids FMT has minimal side-effects.

### Donor screening

Donors are selected from healthy individuals. Detailed protocols are available for donor screening. In brief, they are negative for ova & cyst, *Clostridium difficile* toxin; *Helicobacter pylori* stool antigen; *Cryptosporidium* & *Isospora* (acid fast stain); *Rotavirus* antigen; hepatitis B surface antigen, anti-hepatitis C virus antibody; HIV 1 & 2; venereal disease research laboratory test (VDRL); human leukocyte antigen (HLA)-A1, abnormal bowel motions, chronic alcohol intake, active substance abuse or failed to provide consent; less than 18 or more than 60 years of age; any chronic disease; and history of antibiotic usage within past 3 months.[120] Though there is little evidence, we generally prefer a young, lean, healthy, first degree relative of the recipient. This may have the added advantage of similar dietary pattern and an HLA-A2 match.

### Possible mechanisms of action of FMT

The proposed mechanism of action involves the establishment of non-pathogenic microbes in the gut and production of antimicrobial substances like bacteriocins produced by these microbes.[123] The mucosa-associated invariant T (MAIT) cells have been shown to be altered in patients with severe alcoholic hepatitis, which is likely to play a role in promoting bacterial infection in these patients. Circulating MAIT cells have been found to be depleted and have shown a defective antibacterial cytokine/cytotoxic response in alcoholic cirrhosis and severe alcoholic hepatitis, which is probably an important mechanism contributing to the increased risk of bacterial infection in these patients. An alteration in MAIT cells induced by faecal microbiota is a likely mechanism for the beneficial effects of FMT.[124]

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, it protected against alcohol-induced Bacteroidetes depletion and development of steatosis.[100]

### Clinical trials on FMT in alcoholic hepatitis

With the enormous potential of modulating the gut microbiota and consequently improving homeostasis, investigators have considered FMT in a number of clinical conditions (Box 1). Several randomised controlled trials addressing the therapeutic modulation of intestinal microbiota in hepatic encephalopathy, fibrosis progression, metabolic consequences of liver disease and in the outcome of severe alcoholic hepatitis are currently underway (trials number NCT02485106, NCT02862249, NCT01069133, NCT02400216, NCT02496390, NCT02424175 and NCT01968382 at www.clinicaltrials.gov) (Table 1). In a pilot study, Bajaj *et al.* have recently shown that FMT improves cognitive function in patients with minimal hepatic encephalopathy.[125] There are limited studies in patients with alcohol-related liver disease.

### FMT in steroid-ineligible severe alcoholic hepatitis

Only a proportion of patients with severe alcoholic hepatitis are eligible for corticosteroid therapy. In a pilot study of FMT in steroid eligible patients, Philips *et al.* demonstrated an improvement in 1-year survival rate in FMT-treated patients compared to historical controls (87.5% *vs.* 33.3%). The FMT was given daily for 7 consecutive days in 8 patients. The encouraging results of these trials paved the way for FMT as a treatment for alcoholic hepatitis (Fig. 4). In patients with severe alcoholic hepatitis, a relatively high abundance of Proteobacteria and low abundance of Actinobacteria was seen compared to controls. A year after FMT, there was a reduction in Proteobacteria and an increase in Actinobacteria. Coexistence of donor and recipient species was seen 6–12 months post-FMT.[33] This has also been reported by Li *et al.*[126] This indicates that new species from the donor, which are less pathogenic and beneficial, coexist with pre-existing bacterial communities of the recipient. It is likely that the latter is substantially modified by the donor species.

The relative abundance of several species was altered, notably including the pathogenic species *Klebsiella pneumoniae* (10% to <1% at 1 year), and non-pathogenic species, such as *Enterococcus villorum* (9% to 23% at 6 months), *Bifidobacterium longum* (6% to 50% at 6 months), and *Megasphaera elsdenii* (10% to 60% at 1 year). The baseline deranged metabolic milieu of patients with severe alcoholic hepatitis; the upregulated methane metabolism, fluorobenzoate acid degradation pathway (mediated by *Pseudomonas* and *Escherichia coli* groups) and bacterial invasion of the epithelial cells were downregulated 1-year post-FMT. Similarly, bile secretion, carotenoid biosynthesis, and pantothenate biosynthesis pathways, which were downregulated at baseline, improved to near normal levels following FMT.[33]

Moreover, the pathways associated with inflammation (arachidonic acid metabolism), cellular toxic and oxidative stress (geraniol and styrene degradation), endogenous toxic alcohol and volatile organic compound burden (caprolactam, toluene, and propanoate metabolism), and aromatic amino acid generation (tryptophan and

**Box 1. Liver diseases in which FMT may be useful.**

- Severe alcoholic hepatitis
- Hepatic encephalopathy
- Hepatitis B related chronic liver disease
- Non-alcoholic fatty liver disease
- Primary sclerosing cholangitis

**Table 1. Ongoing clinical trials on FMT in liver diseases.**

| Patient group | Trial design/modality | Trial status | Trial No. |
|---|---|---|---|
| Decompensated cirrhosis | Randomised controlled trial | Recruiting | NCT03014505 |
| Primary sclerosing cholangitis | Single group assignment | Completed | NCT02424175 |
| Severe alcoholic hepatitis | Randomised controlled trial | Recruiting | NCT03091010 |
| Non-alcoholic steatohepatitis | Single group assignment | Recruiting | NCT02469272 |
| Cirrhosis | Randomised parallel assignment | Recruiting | NCT02862249 |
| Management of hepatic encephalopathy | Single group assignment | Recruiting | NCT02255617 |
| Chronic hepatitis B | Randomised parallel assignment | Recruiting | NCT03429439 |
| Obesity | Randomised parallel assignment | Recruiting | NCT02741518 |
| Type II diabetes mellitus | Randomised parallel assignment | Unknown | NCT02346669 |

FMT, faecal microbiota transplantation.

phenylalanine metabolism) which were highly active at baseline in patients with severe alcoholic hepatitis, were markedly reduced and modified into beneficial pathways – non-aromatic amino acid biosynthesis, nitrogen metabolism, anti-inflammatory pathways (thiamine metabolism, peroxisome, and peroxisome proliferator-activated receptor pathway), and cellular regeneration.

In another anecdotal study of FMT in patients with severe alcoholic hepatitis, the abundance of *Bilophila, Citrobacter, Enterobacter,* and *Klebsiella* was found to be significantly higher at baseline. One week after FMT, less pathogenic bacteria such as *Bacteroides, Parabacteroides,* and *Porphyromonas* predominated.[127]

### Steroid *vs.* FMT

A randomised clinical trial (NCT03091010) comparing FMT with steroid therapy in patients with severe alcoholic hepatitis is being conducted at our centre. A total of 82 patients have been enrolled so far. Preliminary results of the trial have shown a survival benefit among the patients enrolled in the FMT arm compared to those receiving steroid therapy. The encouraging results of these trials pave the way for FMT as a potential therapeutic option for alcoholic hepatitis.

## Targeted gut microbiota modulation

**Key points**

Restoring microbial ecology can also be achieved by very specific, microbiota centered approaches including engineered bacteria or supplementation of beneficial microbial metabolites.

Translating microbiome research into clinical therapies is in its infancy. FMT, pro-, pre-, syn- and antibiotics broadly target the intestinal microbiota and do not enable pinpointing of specific changes in the microbiome. A better understanding of the interaction between gut microbial communities and the host would allow for more targeted therapies, paving the way towards a precision medicine approach. Such treatments could overcome possible adverse effects. Unfortunately, no targeted approach has reached clinical trials yet.

As described previously, a combined metagenomic and metabolomic approach revealed a relative deficiency in luminal saturated LCFA after chronic ethanol administration in mice. Targeted supplementation with saturated LCFA restored this deficiency, supported growth of *Lactobacillus* species, and reduced ethanol-induced liver disease in mice.[55] Similarly, reduced intestinal levels of butyrate[49] were restored by supplementation with the prodrug glyceryl tributyrate, which stabilises the gut barrier and improves ethanol-induced liver disease.[52,53]

Bile acid metabolism is dysregulated in mice subjected to ethanol-induced liver disease as demonstrated by metagenomic and metabolomic analysis. To restore an ethanol-associated reduction of intestinal FXR activity, we used an intestine-restricted FXR agonist. Fexaramine reduced intestinal inflammation, restored gut barrier function and reduced ethanol-induced hepatic steatosis and injury in mice.[43] Intestine-restricted FXR agonism might overcome adverse effects of systemic FXR agonists. In addition, overexpression of a human FGF19 variant in mice showed similar beneficial effects on ethanol-induced hepatic steatosis in mice.[43]

Bio-engineering bacteria is another way of precisely modulating the effects of intestinal dysbiosis. The bacteria-derived antioxidant pyrroloquinoline quinone reduced liver injury by counteracting the oxidative stress caused by acetaldehyde.[128] Gram-negative bacteria secrete pyrroloquinoline quinone, which reduces blood levels of acetaldehyde in a rat model of alcohol feeding.[129,130] Supplementation of *E. coli Nissle* 1917 transfected with a pyrroloquinoline quinone-producing plasmid reduced ethanol-induced hepatic oxidative stress and hyperlipidaemia in rats.[128] Levels of faecal SCFA, including acetate, propionate, and butyrate, were increased in supplemented rats.

We have recently bio-engineered *Lactobacillus reuteri* to produce and secrete interleukin 22 (IL-22). Using metabolomic analysis we demonstrate that intestinal tryptophan metabolites such as indole-3 acetic acid are reduced in mice subjected to ethanol feeding and in patients with alcoholic hepatitis. Indole-3 acetic acid is a ligand for the Aryl hydrocarbon receptor to induce IL-22 in innate lymphoid cells type 3 (ILC3). Consequently, IL-22 production by intestinal ILC3 was suppressed after ethanol feeding in mice. Supplementation of either indole-3 acetic acid or IL-22

producing *Lactobacillus reuteri* induced the IL-22 target gene *REG3G* in intestinal epithelial cells, reduced bacterial translocation and ameliorated ethanol-induced liver disease in mice.[131]

This data suggests that use of a precision microbiome approach and genetically engineered bacteria to precisely target acetaldehyde or overcome IL-22 deficiency might be sufficient to decrease alcohol-related liver disease.

## Summary, conclusion and outlook

Alcohol-related liver disease is associated with changes in the gut microbiota. Given the transmissibility of alcohol-related liver disease via FMT, dysbiotic changes seem to contribute to hepatic disease. Although we have been gaining some insight into the interactions between gut microbial communities and host, a comprehensive understanding of the gut microbiota's function during alcohol-related liver disease is lacking. In fact, these initial studies identify a large area for future basic and clinical research in hepatology (Box 2). A better characterisation of the intestinal microbiome, metabolome and host response using different preclinical models, stages of liver disease and larger cohorts of patients is required. This will allow us to determine subtypes or groups of patients that would benefit most from therapies targeting the gut microbiota. Microbiome and metabolome analysis in patients with alcohol-related liver disease might become a routine diagnostic test to stratify patients for tailored microbiome treatment approaches. Untargeted therapies using antibiotics, probiotics, or FMT will be replaced by personalised and precision medicine approaches, such as bio-engineered bacterial strains or drugs that modulate specific bacterial enzymes and metabolic pathways.

**Box 2. Future research areas in gut microbiota modulation for treatment of liver diseases.**

1. Quantification of bacterial diversity and need for reversal
2. Functional derangements (metabolic and immunological) and degree of reversal
3. Dose, frequency, route of modulation of gut microbiota
4. Assessment of efficacy/harmful effects of gut microbiota modulation
   - Intestinal permeability, degree and potential of reversal
   - Circulating pathogen associated molecular patterns
   - Regeneration pathways - markers of fibrosis regression
   - Effects on hepatic parenchyma
5. Biomarkers of injury of enterocytes, MAIT and other intestinal cells,
   - Cell turn-over rates due to changed gut microbiota
   - Markers of hepatic parenchymal cell injury due to altered gut microbiota
6. Influence on bile acids and enterohepatic circulation dynamics

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## Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contribution

SKS, AP and BS were responsible for writing the manuscript.

## Supplementary data

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