Utilizing the gut microbiome in decompensated cirrhosis and acute-on-chronic liver failure

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Abstract | The human gut microbiome has emerged as a major player in human health and disease. The liver, as the first organ to encounter microbial products that cross the gut epithelial barrier, is affected by the gut microbiome in many ways. Thus, the gut microbiome might play a major part in the development of liver diseases. The common end stage of liver disease is decompensated cirrhosis and the further development towards acute-on-chronic liver failure (ACLF). These conditions have high short-term mortality. There is evidence that translocation of components of the gut microbiota, facilitated by different pathogenic mechanisms such as increased gut epithelial permeability and portal hypertension, is an important driver of decompensation by induction of systemic inflammation, and thereby also ACLF. Elucidating the role of the gut microbiome in the aetiology of decompensated cirrhosis and ACLF deserves further investigation and improvement; and might be the basis for development of diagnostic and therapeutic strategies. In this Review, we focus on the possible pathogenic, diagnostic and therapeutic role of the gut microbiome in decompensation of cirrhosis and progression to ACLF.

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Over the past decade, the human gut microbiome has emerged as a major component in human health and disease. It carries the largest pool of genetic material in the body, even larger than the human genome, and has metabolic activity[1]. Many estimates of human gut microbiome features come with large error margins and are controversial, but our gut seems to be inhabited by trillions of microbial cells, whose count is at least on par with the number of human cells[1,2]. Each of us harbours more than a thousand microbial species in the gut and, collectively across populations, the human gut microbiome consists of more than ten million non-redundant microbial genes, which is probably a vast underestimate[3–5]. Beyond the well-known metabolic functions such as vitamin provision and carbohydrate degradation, the gut microbiome also contributes to host defence mechanisms in close interaction with the immune system, with many additional roles continuously proposed[6].

The composition of the human gut microbiota has been associated with many diseases, such as gastroenterological, neurological, respiratory, metabolic, hepatic and cardiovascular disorders[6]. Based on these disease associations, changes in the gut microbiome are thought to influence disease development, although robust evidence for the gut microbiome directly causing a non-infectious disease is still lacking. The liver, as the first organ to encounter microbial products that cross the gut epithelial barrier and enter portal circulation, is probably affected by the gut microbiome and its changes in many ways. Components or metabolites of the gut microbiota interact with the liver through various mechanisms (FIG. 1). Some key players in this interaction are: a 'leaky gut' that enables increased translocation of bacteria or their metabolites to the portal blood circulation; circulating immune cells that the translocated bacteria encounter; liver cells that bacteria interact with upon reaching the liver; and bile acids produced by the liver, some of which alter the gut microbiome composition but also act as signalling molecules[7]. Thus, the gut microbiome might have a major role in development of liver diseases[8,9].

The liver has a huge regeneration capacity, yet chronic liver injury leads to scarring of the liver tissue, which can progress to clinically significant liver fibrosis[10,11]. The progressive accumulation of extracellular matrix together with the different inflammatory processes, depending on the aetiology of liver disease (for example, alcoholic hepatitis, chronic viral hepatitis, nonalcoholic fatty liver), induces profound changes in the liver architecture, such as fibrosis or hyperplastic nodules, which disrupts the bloodstream from the gut[12] and changes

Key points

- The gut microbiome is altered during development of liver cirrhosis, and these changes are associated with decompensation and development of acute-on-chronic liver failure (ACLF).
- Progression of liver cirrhosis towards decompensation and ACLF is mainly driven by the extent of systemic inflammation and associated with high short-term mortality.
- The gut microbiota can contribute to systemic inflammation and, thereby, to progression of cirrhosis towards decompensation and ACLF, directly via translocation or indirectly via their metabolites.
- Gut microbiota members or pathobionts might be helpful biomarkers to predict the presence and development of decompensation and ACLF, but the signatures are not consistent and more research is needed.
- Gut microbiome targeted therapies are promising strategies to improve the outcome of decompensated cirrhosis and ACLF, but better stratification for the existing drugs and novel, more effective strategies are needed.

the bile composition flowing into the gut[13] (reviewed elsewhere[14]). This end stage of liver disease is called cirrhosis. As a disease that severely affects the enterohepatic circulation, cirrhosis might influence or be influenced by both gut barrier function and the gut microbiome. During progression of cirrhosis, there is a substantial increase of blood pressure in the portal vein, known as portal hypertension, leading to further complications such as bleeding, ascites and infections[15] (TABLE 1). Portal hypertension leads to venous congestion and increased angiogenesis of intestinal vessels, both of which impair microcirculation and increase permeability of the gut barrier, and ascites formation, predisposing to spontaneous bacterial peritonitis (SBP) and other infections[16]. Indeed, profound differences in gut microbiome structure and function are found in patients with liver cirrhosis compared with healthy individuals[17]. However, it is still not clear whether these changes precede, coincide with or follow development of complications in cirrhosis, also defined as decompensating episodes[16] (TABLE 1), during which cirrhosis becomes a systemic disease. During acute decompensating episodes, a serious condition associated with systemic inflammation can occur, so-called acute-on-chronic liver failure (ACLF)[18,19]. This condition has up to 34% mortality in 28 days (ranging from 22 to 77% depending on the grade of ACLF)[20]. Bacterial infections, especially SBP, which mainly derive from the gut microbiota, might play an important role in the development of ACLF[21]. Moreover, data have demonstrated the association of gut microbiota metabolites with the presence of ACLF[22]. Thus, the influence of the gut microbiome on ACLF deserves investigation and improvement. Here, we review the role of the gut microbiome as a pathogenic factor, diagnostic tool and therapeutic target in decompensated cirrhosis and ACLF. Acute alcoholic hepatitis as a specific form of ACLF has been addressed elsewhere[23] and is not the main focus of this Review.

Cirrhosis and progression to ACLF

Globally, more than 800 million people are affected by chronic liver disease[24] and a further 6–7% of the adult population without known liver disease are thought to have undiagnosed liver fibrosis[25]. Chronic liver disease represents a major health concern as it can progress to liver cirrhosis and liver cancer. Cirrhosis is associated with high mortality (up to 57%)[12], with an increasing contribution towards total deaths globally from 1.9% in 1990 to 2.4% in 2017 (REF.[26]) (TABLE 1). Although the frequency of cirrhosis due to hepatitis B and hepatitis C is decreasing due to massive vaccination programmes against hepatitis B in children and the development of effective antiviral treatments, cirrhosis continues as a global health and economic challenge due to two major reasons[26]. First, the global pandemic of obesity has resulted in an increased prevalence of cirrhosis associated with nonalcoholic fatty liver disease and nonalcoholic steatohepatitis[26]. Second, in Europe, the USA and many Asian regions, alcohol consumption, which is the leading aetiology of cirrhosis[27–29], is increasing and has contributed to an increased prevalence of alcoholic cirrhosis.

Even though only 10–30% of patients with chronic liver diseases, irrespective of the underlying aetiology, progress to cirrhosis, its effect on global healthcare cannot be underestimated as cirrhosis has a high mortality and healthcare burden[30,31]. To make things worse, mortality due to liver cirrhosis has been rising: cirrhosis (not counting liver cancers) was responsible for 1.32 million deaths in 2017 compared with 1.2 million deaths in 2013 worldwide, resulting in a 10% increase in 4 years[26,30]. Although patients with cirrhosis initially do not have symptoms, decompensation of cirrhosis, defined as the development of ascites, hepatic encephalopathy, jaundice and/or gastrointestinal haemorrhage[16], is a turning point in the cirrhosis course (FIG. 2). Although the average life expectancy of a patient with compensated cirrhosis is 10–13 years, it reduces dramatically to 2 years if decompensation occurs[32]. Acute decompensation of cirrhosis can lead to ACLF, a syndrome characterized by failure of one or more major organs or systems (liver, kidney, brain, coagulation, circulation or respiration)[33]. Patients with acute decompensation of cirrhosis have a high short-term risk of developing ACLF (11% by day 28). Although acute decompensation itself has high mortality rates (5% by day 28, 14% by day 90), progression to ACLF increases this mortality dramatically (33% by day 28, 50% by day 90)[20]; ACLF is the main cause of death in decompensated cirrhosis[20,34]. In 2020, the PREDICT study — a large, prospective, multicentre study — confirmed this dangerous progression of decompensation to ACLF by identifying three different clinical courses of acute decompensation: stable decompensated cirrhosis, unstable decompensated cirrhosis and pre-ACLF[35]. Patients with pre-ACLF developed ACLF, and had 3-month and 1-year mortality rates of 53.7% and 67.4%, respectively. Patients with unstable decompensated cirrhosis required ≥1 readmission but they did not develop ACLF and had 3-month and 1-year mortality rates of 21.0% and 35.6%, respectively. By contrast, patients with stable decompensated cirrhosis neither developed ACLF nor were readmitted, and showed a 1-year mortality of only 9.5%[35]. It is important to note that the definition of ACLF varies between different regions of the world, as prescribed by The European Association for the Study of the Liver — Chronic Liver Failure (EASL-CLIF) Consortium in Europe, The North American Consortium for the Study of End-Stage Liver

Disease (NACSELD) and The Asian Pacific Association for the Study of the Liver (APASL)[20,36–38] and reviewed in detail by Hernaez et al.[39]. The main difference between the definitions is that cirrhosis is not a requirement for ACLF in the APASL definition[36,40]. In Asia, the most prevalent form of ACLF is that developing in patients with chronic hepatitis B virus (HBV) infection, which is associated with increased prevalence of liver failure and coagulation failure but reduced prevalence of other organ failures[40,41], thereby making the requirement of cirrhosis redundant. However, when discussing the role of the gut microbiome in ACLF, considering the presence of cirrhosis is important, given that even severe portal hypertension does not majorly impair the gut barrier in the absence of cirrhosis[42]. In cirrhosis, gut microbiome changes are probably associated with disease progression[22,43], making it a major target for diagnostic and therapeutic approaches (TABLE 1).

The gut microbiome in decompensation

Under normal circumstances, we inherit most of our microbial diversity from our mother at birth. The microbiota is then subjected to major compositional changes during infancy, but also beyond infancy, with decreasing variation until adolescence and a reasonably stable state over time in adulthood[44]. In healthy conditions, the host

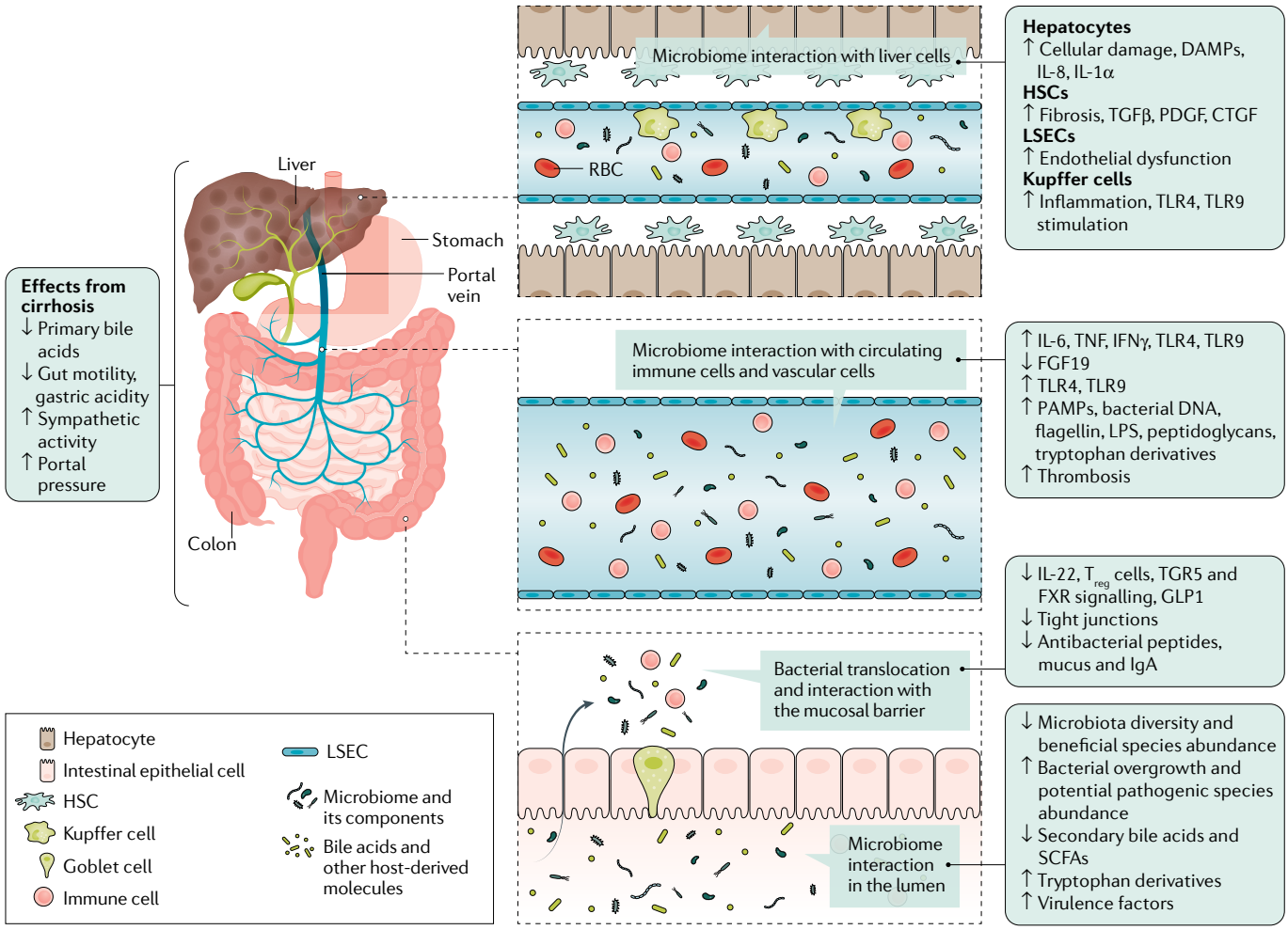

Fig. 1 | **Currently known and suggested microbiota–gut–liver interactions in cirrhosis.** Changes in the gut microbiome during the progression of liver cirrhosis are largely attributed not only to the composition of the gut microbiota but also to its products, which might have a pathogenic role. Short-chain fatty acids (SCFAs), secondary bile acids and tryptophan derivatives are increased, whereas changes in mucus proteins, dysfunctional tight junctions and decreased antibacterial peptides facilitate translocation of microbiota components and/or their metabolites (pathogen-associated molecular patterns (PAMPs)) across the intestinal barrier. The decreased gastrointestinal motility due to sympathetic overactivation and the decreased secretion of primary bile acids during cirrhosis progression facilitate changes in the microbiome and also promote translocation via, for example, decreased farnesoid X receptor (FXR) and/or TGR5 stimulation, which leads to disruption of the epithelial barrier. Intestinal inflammation promoted and aggravated by decreased IL-22, glucagon-like peptide 1 (GLP1), dysfunctional $CD4^+CD25^+$ regulatory T cells (T_{reg} cells) and increased interferon-γ (IFNγ) might lead to bursts of systemic inflammation, known to be associated with acute-on-chronic liver failure. On the other side of the intestinal barrier, translocated PAMPs on their way to the liver further aggravate systemic inflammation (upregulation of tumour necrosis factor (TNF), IL-6 and IL-1β), leading to activation of circulating and liver-resident immune cells, especially via Toll-like receptor 4 (TLR4) and TLR9 stimulation. Systemic and hepatic inflammation together with PAMPs aggravate fibrosis, endothelial dysfunction and hepatocellular function. CTGF, connective tissue growth factor; DAMP, danger-associated molecular pattern; FGF19, fibroblast growth factor 19; HSC, hepatic stellate cell; LPS, lipopolysaccharide; LSEC, liver sinusoidal endothelial cell; PDGF, platelet-derived growth factor; RBC, red blood cell; TGFβ, transforming growth factor-β; TGR5, Takeda G protein-coupled receptor 5. Image courtesy of MICROB-PREDICT.

Table 1 | **Cirrhosis and its progression**

Clinical condition	Definition	Clinical effect	Prognosis	Role of the gut microbiome
Fibrosis[11]	Excessive accumulation and changes of extracellular matrix in the liver	Key determinant of the evolution of chronic liver disease	Benign, but progression to cirrhosis occurs in at least 10%	Changes in the gut microbiome are influenced by the aetiology of liver disease[8,162]
Chronic advanced liver disease or cirrhosis[12,134]	Diffused nodular regeneration with dense fibrotic septae and parenchymal extinction and collapse of liver vascular structures	Wide clinical presentation as compensated or decompensated forms Compensated cirrhosis is usually silent for 3–10 years and might not be diagnosed in a large proportion of the patients	Decompensation 5–7% per year Survival >12 years	Gut microbiome contributes to progression of liver disease to cirrhosis, especially in alcoholic and nonalcoholic fatty liver disease but also in viral hepatitis, autoimmune and cholestatic diseases[8,162]
Portal hypertension[12,163,164]	HVPG >5 mmHg	HVPG higher than 10 mmHg is defined as CSPH CSPH drives development of varices, in general decompensation and even development of liver cancer	Absence of CSPH: 90% of the patients have no decompensation within 4 years	Presence of portal hypertension in cirrhosis facilitates microbial translocation[93] from the gut into the portal vein[57] and might predispose for the development of ACLF[165]
Decompensated cirrhosis[134]	Development of overt clinical signs	Patients with liver decompensation without an acute event are also called stable decompensated patients Acute decompensation is the acute development of ascites, overt encephalopathy, gastrointestinal haemorrhage, new onset of non-obstructive jaundice and/or bacterial infections	1-year mortality of stable decompensated patients is 20% 90-day mortality in acutely decompensated patients is 10%	Infections in particular, but also hepatic encephalopathy, variceal bleeding and development of ACLF might be a consequence of microbial translocation[8,14,162] Specific microbiome patterns are found to be diagnostic and predictive for hepatic encephalopathy and also other complications of cirrhosis[58]
ACLF[20,36–38,134]	APASL: acute deterioration of liver function in patients with chronic liver disease but without bacterial infection or previous decompensations of cirrhosis EASL-CLIF: cirrhosis with acute decompensation, organ failure(s) and high short-term mortality NACSELD: cirrhosis with or without previous decompensation of cirrhosis and infection at admission or during hospital stay, presenting with one or more organ failures	ACLF develops in 30% of hospitalized patients and in 25% of outpatients ACLF according to the EASL-CLIF definition is divided into three grades with increasing severity: Grade I is characterized by either renal failure (creatinine >2 mg/dl) or renal dysfunction and/or hepatic encephalopathy I and II together with another organ failure (liver, coagulation, circulation or respiratory); Grade II and III represent at least two and three organ failures, respectively Differences in APASL definition: circulatory and respiratory failure is disregarded; bilirubin ≥5 mg/dl and/or INR ≥1.5 constitutes liver and coagulation failure, respectively Differences in NACSELD definition: liver and coagulation failure are disregarded	90-day mortality in different grades of ACLF: Grade I 41%, Grade II 55%, Grade III 78%	Microbial metabolites are found to be associated with ACLF development[87] Specific microbiome patterns in stool and blood are found to be present in patients with ACLF[58,59]

ACLF, acute-on-chronic liver failure; APASL, Asian Pacific Association for the Study of the Liver; CSPH, clinically significant portal hypertension; EASL-CLIF, European Association for the Study of the Liver — Chronic Liver Failure; HVPG, hepatic-vein pressure gradient; INR, international normalized ratio; NACSELD, North American Consortium for the Study of End-Stage Liver Disease.

tightly regulates the gut microbial burden, distribution, composition and activity via the immune system, by secretion of bile acids and through antibacterial peptides released by intraepithelial immune cells[8]. An altered gut microbiome during disease development might affect the homeostasis between the host and the gut microbiome. An imbalanced gut microbiome is known as dysbiosis, and different dysbiotic compositions of the gut microbiota have been associated with different diseases[6].

Interaction with the gut barrier. A dysbiotic microbiome can affect the gut epithelial barrier and lead to poorly controlled translocation of contents from the gut to the liver and beyond. Such impairment of the gut epithelial barrier is known as a leaky gut[8,45,46]. During liver disease, major changes occur in the host physiology and gut integrity due to changes in the enterohepatic circulation, intestinal inflammation and portal hypertension. At the same time, there are also major changes in the crosstalk

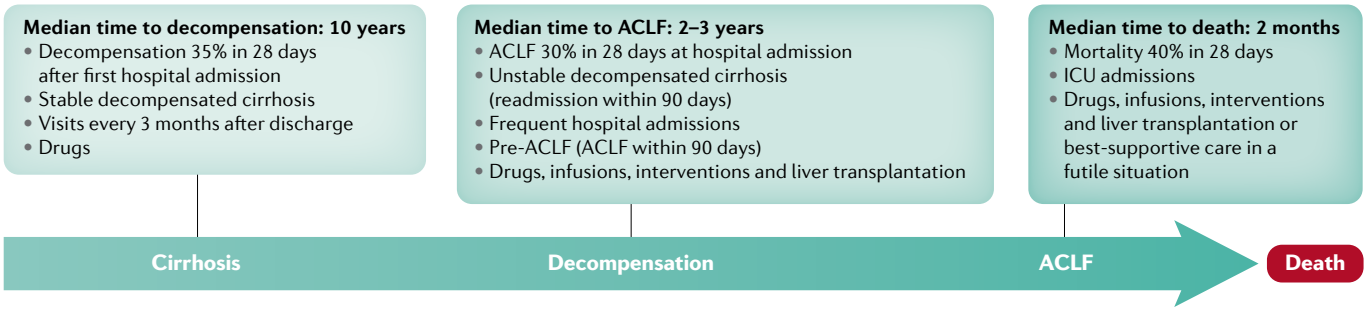

Fig. 2 | **Transition of liver cirrhosis to ACLF.** Patients with compensated cirrhosis might stay stable for years with a very low mortality of <1% per year. However, after the first hospitalization, 35% of the patients will develop subsequent decompensation in 28 days, defined by development of ascites. These patients will require frequent hospital admissions, their median time to acute-on-chronic liver failure (ACLF) is 2–3 years and their mortality would be around 10% each year. Upon each decompensation, the development of ACLF within 28 days is around 30% and at that stage the mortality climbs to 40% in 28 days. These patients with organ failures are typically treated in the intensive care unit (ICU) and liver transplantation is the only curative treatment[169].

between the gut microbiome and the liver: host mucosal proteins and pathways (for example, FXR signalling) in the gut are changed by gut microbiota metabolites (for example, short-chain fatty acids)[47] and might predispose for liver injury[48]; changes in intestinal innervation due to progression of liver disease might affect the gut barrier, promote intestinal inflammation[49,50] and reduce levels of antibacterial peptides[51,52]; and gut-associated lymphatic tissue might be involved in the intestinal barrier dysfunction by driving the intestinal inflammation and, thereby, increasing permeability of the barrier[47,53,54]. Progression of liver disease is associated with subclinical inflammation, which is extended to the gut mucosa, leading to impaired dendritic cell activity, expansion of TNF and IFNγ-expressing lymphocytes and IL-17-producing T helper cell depletion[47].

As bacterial components crossing the gut epithelial barrier trigger immune responses, a leaky gut is thought to increase chronic systemic inflammation. For example, elevated circulating levels of lipopolysaccharides (LPS) from bacterial cell walls, a condition also known as endotoxaemia, have been observed in patients with liver diseases, particularly cirrhosis[55,56]. A study in 2018 demonstrated that bacteria isolated from circulating blood and other 'sterile' compartments in patients with decompensated cirrhosis are viable, suggesting that even live bacteria could translocate across the intestinal barrier during decompensated cirrhosis[57].

Although the aetiology of cirrhosis can vary, in the end stage of the liver disease, the microbiome–liver interaction is largely aetiology independent[8]. Cirrhosis-associated dysbiosis (decreased diversity, an increase in potentially pathogenic species and a decrease in autochthonous species) and bacterial translocation are frequent phenomena closely associated with the development of decompensation in cirrhosis[56,58,59]. Bacterial translocation might also be associated with activation of platelets[56], which might lead to development of portal vein thrombosis, a sign of clinical progression of cirrhosis[60,61]. Although portal hypertension following cirrhosis leads to further complications, studies in rats showed that portal hypertension per se (in the absence of cirrhosis) does not cause major disruption to the gut barrier[42,62]. Thus, the presence of liver cirrhosis is the main factor predisposing the interaction of the gut microbiome with disease progression.

Interaction with the diseased liver. During their natural history, patients with cirrhosis experience deterioration of their underlying cirrhosis, called decompensation episodes, and usually require hospital admission[34]. In 30% of these patients, the clinical situation deteriorates and ACLF occurs within 3 months[20]. The progression from decompensation to ACLF is associated with an extensive activation of systemic inflammation, affecting many cytokines and inflammatory systems[18,19]. This understanding was clearly shown in the PREDICT study, in which patients who developed ACLF (pre-ACLF group) had very high systemic inflammation compared with patients with acute decompensation with an unstable or stable clinical course[35]. There are different inducers of inflammation in this context.

During development of ACLF, the dying cells of the different failing organs[63] and the remodelling of the extracellular matrix, especially in the liver[64], both boost decompensation and inflammation through the release of danger-associated molecular patterns, which ultimately might be a prerequisite for the development of ACLF[19]. This association is particularly common in patients with HBV-induced cirrhosis and/or ACLF[41,65]. Reactivation of HBV might induce the liver damage and, thereby, the release of danger-associated molecular patterns, which subsequently induce organ failure and ACLF. This event was implicated as the main precipitating factor in a study of Chinese patients[41,65]. Interestingly, superimposed hepatitis A virus and hepatitis E virus infections might also induce ACLF in patients with chronic liver disease and cirrhosis[65,66].

On the other hand, this burst of inflammation is preceded[19], and probably partly driven, by translocation of microorganisms or their components[34], also known as pathogen-associated molecular patterns (PAMPs). The immune response to PAMPs might induce organ dysfunction in a process called immunopathology, in which the immune response causes damage as a result of an infection[67]. Elevated plasma levels of systemic

inflammation markers (for example, IL-8 or IL-6), with or without obvious bacterial infection, were shown to be associated with acute decompensation of cirrhosis and ACLF[18,19]. The association between an altered gut microbiome and ACLF has been strengthened by novel data. In addition to the association between gut microbiota-derived metabolites and ACLF development[22], plasma metabolite signatures, potentially also deriving from gut microbiota, are also strongly associated with systemic inflammation and ACLF[68]. These findings might explain why an altered gut microbiome and increased bacterial translocation possibly prepare the milieu in different organs for development of organ failure following immunopathology, aggravating systemic inflammation and inducing ACLF.

In 2019, it was shown that circulating bacterial DNA was substantially increased in patients with HBV-associated ACLF and correlated to inflammatory markers[59] such as CXCL10, which is a chemokine known to also be associated with ACLF in non-HBV aetiologies, especially alcoholic cirrhosis[69]. Also, in acute alcoholic hepatitis, a subtype of ACLF, it was shown that patients with a specific cytolysin-producing *Enterococcus* strain had extremely high mortality compared with those without this specific strain (89% versus 3.8% mortality within 180 days)[70]. Acute alcoholic hepatitis is known to be closely associated with microbial changes, which might determine the clinical profile of the patients and their outcome[23,71,72]. Both decompensation and ACLF develop in almost half of patients with acute alcoholic hepatitis[20,35] without any identifiable precipitating events, suggesting that endogenous mechanisms are involved (for example, portal hypertension or bacterial translocation).

Role of portal hypertension and alcohol. Even if portal hypertension in cirrhosis is treated efficiently, for example, by placement of a transjugular intrahepatic portosystemic shunt (TIPS) stent, many patients (around 47% in 2 years)[73] still develop further decompensation and ACLF[74,75], especially due to systemic inflammation and subsequent organ failure[18,20,76]. Moreover, the presence of ascites, an indicator of decompensation[77], determines the composition of the circulating microbiota in the portal vein compared with the hepatic vein, right atrium and peripheral venous blood, and specific circulating microbiota members correlate with inflammatory markers[57] and the development of ACLF[59]. However, it should be stated that the TIPS stent itself might aggravate hyperdynamic circulation and thereby lead to increased levels of endotoxaemia[78]. In the long term, the TIPS stent decreases the rate of decompensation, probably mainly due to the decreased incidence of ascites in these patients with cirrhosis.

Decompensated cirrhosis and ACLF occur predominantly in alcoholic cirrhosis[20]. This finding might be important not only because alcohol is the most frequent aetiology in decompensated cirrhosis but, possibly, also due to direct effects of alcohol itself on gut microbiota and intestinal barrier function. In healthy individuals and animal models it has been shown that an acute alcohol binge increases endotoxin and bacterial DNA levels in the circulation[79,80]. Alcohol substantially changes the composition of the gut microbiota towards reduced relative abundances of Bacteroidetes and increased Proteobacteria[3,81,82]. Its metabolites, especially acetaldehyde, can disrupt tight junctions of the gut epithelium, inducing a leaky gut and facilitating the translocation of bacteria[83] and fungi[84], which might both be associated with progression of liver cirrhosis[85]. An altered microbiota composition as well as a leaky gut are also observed in different aetiologies of liver cirrhosis (for example, nonalcoholic steatohepatitis or hepatitis B-induced liver cirrhosis)[17,43].

Microbial metabolites and translocation. Dysbiosis and a leaky gut will increase with the number and severity of complications of cirrhosis and they might be an important variable of microbiome–liver interactions[8,45]. Ascites are associated with the presence of bacterial products in the circulation[86] of patients with cirrhosis and it was shown that the presence of decompensation is the most important determinant for the amount and composition of circulating bacteria in portal venous blood[57]. One study showed that the blood metabolite signature markedly changes during the progression of liver cirrhosis towards decompensation and ACLF[87]. Although this large study of 903 individuals brought important insight towards a microbial origin of some strongly predictive patterns of metabolites, it is difficult to make strong statements on those findings[87]. Another study with 602 patients demonstrated an association of microbiota-derived metabolites with the presence of ACLF[22]. Further microbial components such as secondary bile acids, short-chain fatty acids and tryptophan metabolites are also profoundly changed in cirrhosis and seem to be associated with progression of disease[88,89]. In particular, bile acids have been widely investigated and seem to alter the gut barrier function via downregulation of the FXR receptor, which opens novel treatment opportunities as FXR agonists are already available in the clinic[90–92].

There is indirect evidence that the gut microbiome might influence outcome in cirrhosis. First, studies have shown that treatment with non-selective β-blockers, a common choice to prevent decompensation of cirrhosis, improves the gut barrier[93] and decreases the gut transit time and, thereby, also the probability of bacterial translocation in cirrhosis[94–96]. Second, antibiotic treatment either during a variceal bleeding episode or as a long-term prophylaxis in decompensated cirrhosis is a recommended treatment as it improves outcome[16] and the mechanisms might be related to decreased bacterial translocation. Although this idea has not been conclusively demonstrated, knowing that a specific microbiome phenotype (so-called enterotype) induces pathologies in hepatic functions[8,17], and given the existence of a wide range of members of the microbiota such as fungi[84] or viruses[97], microbiome markers could be very useful clinical tools to identify patients at risk of decompensation and ACLF.

The gut microbiome as a biomarker

To prioritize and optimize treatment of cirrhosis and decompensation, the gut microbiome could serve as a biomarker for disease progression, severity and treatment response, as discussed for other diseases[98].

Table 2 | **Biomarkers and potential for microbiome-based tools**

Class of marker	Definition	Potential for microbiome-based tools
Diagnostic biomarker	A biomarker used to detect or confirm the presence of a disease or condition	Metagenomic signatures hold potential as non-invasive diagnostics for hepatocellular carcinoma[105,166,167]
Prognostic biomarker	A biomarker used to identify the likelihood of a clinical event, disease recurrence or progression in patients with the disease or medical condition of interest	Microbiome-based biomarkers to identify patients at high risk of developing acute-on-chronic liver failure and other complications of cirrhosis, and to enable early preventive interventions[58,106]
Predictive biomarker	A biomarker used to identify individuals who are more likely to experience a favourable or unfavourable effect from exposure to a medical product or an environmental agent	Microbiome signatures to identify patients with decompensated cirrhosis who are most likely to benefit from a particular therapeutic product (for example, long-term albumin therapy)
Monitoring biomarker	A biomarker measured serially for assessing the status of a disease or medical condition or for evidence of exposure to a medical product or an environmental agent	Monitoring effect of novel microbiome-based therapeutics such as faecal microbiota transplantation and more targeted approaches
Efficacy of intervention or pharmacodynamics of a biomarker	A biomarker used to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent	Changes in gut microbiome composition in response to a specific intervention
Companion diagnostic	A medical device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product	Microbiome signatures to identify patients who are most likely to benefit from a particular therapeutic product

Currently, several scores exist to predict patient survival in cirrhosis, but they all reflect hepatic, renal and other organ functions as well as general clinical features[99–101]. Given its close connection with the gut–liver axis, the gut microbiome represents a huge opportunity for diagnostic biomarkers for cirrhosis (TABLE 2).

Using the microbiome as a biomarker for complications arising from liver diseases is not a new concept. More than 15 years ago, bacterial DNA in ascites was proposed as a diagnostic marker of SBP[102]. This finding has been extensively investigated, but the evidence to propose its use as a diagnostic tool was not strong enough for recommendation in clinical practice guidelines[16]. Moreover, in an investigation of the microbiome in ascites in 2019, admittedly with only 33 patients, a clear relationship between SBP and bacterial DNA in ascites could not be found[103], casting doubts on the use of bacterial DNA in ascites to monitor or guide treatment.

The gut microbiome has shown promising potential for diagnostic biomarkers in many diseases[17,104,105]. Several studies characterized the gut microbiome in patients with cirrhosis using 16S ribosomal RNA (rRNA) gene amplicon sequencing technology[58,106–109] whereas very few studies used whole-metagenome shotgun sequencing, as summarized in TABLE 3. Even though it is often difficult to compare results from these two technologies, mostly due to the limitations of resolution achieved by amplicon sequencing, there are still common gut microbiome signatures of cirrhosis that can be identified by both. One 16S rRNA gene amplicon-based study in 244 patients[58] defined a cirrhosis dysbiosis ratio derived from abundances of several microbial families considered autochthonous and benign, including Ruminococcaceae and Lachnospiraceae, in comparison with others including Enterobacteriaceae and Bacteroidaceae. Progressive changes in the gut microbiome showed a deterioration in terms of the cirrhosis dysbiosis ratio with decompensation and development of ACLF[58]. Another study of 129 patients could identify the reduction in Lachnospiraceae as a characteristic of ACLF, whereas the relative abundance of Pasteurellaceae predicted mortality[106]. Other stool 16S rRNA gene amplicon-based studies have provided evidence that specific microbiome signatures can differentiate between cirrhosis and healthy controls[108], and that changes in the microbiome might be able to monitor the severity and progression of disease as shown by hospitalizations[108], development of extrahepatic organ failure, ACLF and death[107]. Indeed, evidence that the microbiome reflects decompensated liver cirrhosis was provided in a cohort of 45 liver transplant recipients, in whom liver transplantation changed the gut microbiome towards increased microbial diversity, increased autochthonous bacteria (for example, Lachnospiraceae) and decreased potentially pathogenic bacteria (for example, Enterobacteriaceae)[109] (TABLE 3). Although this study does not prove that the microbiome causes liver disease, it does underline that the diseased liver influences the gut microbiome[109].

The first whole-metagenome shotgun sequencing-based metagenome-wide association study (MWAS) of patients with liver cirrhosis from China (with or without alcohol exposure) showed an altered gut microbiota composition compared with healthy individuals[17], with >34 differentially abundant species. Among these species, *Veillonella* spp. and *Streptococcus* spp. were found in elevated levels in patients with cirrhosis, whereas butyrate-producing commensal bacteria including *Faecalibacterium prausnitzii* and *Coprococcus comes*

were depleted. Another study examined 99 patients from Russia with alcohol dependency and showed that, compared to patients without cirrhosis but with alcohol dependency, patients with alcoholic liver cirrhosis harboured 46 species and 13 genera that were differentially abundant[82], including elevated levels of *Streptococcus* spp. and depletion of *F. prausnitzii* and *Coprococcus eutactus*. Although each MWAS reported more than 30 differentially abundant or discriminative species (TABLE 3), depletion of *F. prausnitzii* as well as *Bacteroides uniformis* and enrichment of *Lactobacillus salivarius* were the only signatures at species level found in both

Table 3 | **Gut microbiome signatures of ACLF and cirrhosis**

Study	Comparison	Altered microorganisms	Altered phyla	Taxa enriched in ACLF/cirrhosis	Taxa depleted in ACLF/cirrhosis
16S ribosomal RNA gene amplicon sequencing technology					
Bajaj et al. (2014) [58]	Patients with liver cirrhosis, including those with ACLF, versus age-matched healthy controls	8 families	↑ Proteobacteria[a,b] ↓ Bacteroidetes[a,b]	Enterobacteriaceae[a], Enterococcaceae[a], Staphylococcaceae	Clostridiales Cluster XIV[a], Lachnospiraceae[a], Ruminococcaceae[a,b], Veillonellaceae, Porphyromonadaceae[a]
Chen et al. (2015) [106]	Patients with ACLF versus healthy controls	3 phyla 8 families	↑ Proteobacteria[a,b] ↑ Firmicutes[a] ↓ Bacteroidetes[a,b]	Enterecoccaceae[a], Pasteurellaceae, Streptococcaceae[b], Veillonellaceae	Bacteroidaceae[b], Lachnospiraceae[a], Ruminococcaceae[a,b], Porphyromonadaceae[a]
Bajaj et al. (2017)[109]	Patients with liver cirrhosis before versus after liver transplantation	0 phyla 7 families 3 genera	None	Enterobacteriaceae[a] (*Shigella*, *Escherichia*, *Salmonella*), Bifidobacteriaceae[b]	Clostridiales Cluster XIV[a], Lachnospiraceae[a], Ruminococcaceae[a,b], Streptococcaceae, Desulfovibrionaceae
Bajaj et al. (2018)[108]	Patients with liver cirrhosis with liver compensation versus liver decompensation	18 genera	Not reported	Enterococcaceae[a] (*Enterococcus*), Peptostreptococcaceae (*Clostridium* Group XI), Streptococcaceae (*Lactococcus*), Staphylococcaceae (*Staphylococcus*)	Lachnospiraceae[a] (*Anaerostipes*, *Blautia*, *Coprococcus*[b], *Dorea*[b], *Fusicatenibacter*, *Roseburia*[b], *Ruminococcus* Group 2), Ruminococcaceae[a] (*Faecalibacterium*[b], *Oscillibacter*, *Ruminococcus*[b]), Erysipelotrichaceae (*Clostridium* Group XVIII), Prevotellaceae (*Prevotella*)[b], Porphyromonadaceae[a] (*Barnesiella*)[b], Rikenellaceae (*Alistipes*)[b]
Bajaj et al. (2019)[107]	Patients with liver cirrhosis developing ACLF versus those not developing ACLF	2 classes 3 orders 6 families	Not reported	Epsilonproteobacteria, Campylobacterales, Campylobacteraceae; Cytophagia, Cytophagales, Cytophagaceae; Hydrogenophilales, Hydrogenophilaceae; Microbacteriaceae, Promicromonosporaceae, Pseudonocardiaceae	Not reported
Whole-metagenome shotgun sequencing studies					
Qin et al. (2014)[17]	Patients with liver cirrhosis versus healthy controls	3 phyla 22 genera >34 species	↑ Proteobacteria[a,b] ↑ Fusobacteria ↓ Bacteroidetes[a,b]	*Fusobacterium*, *Haemophilus*, *Lactobacillus*, *Megasphaera*, *Prevotella*, *Streptococcus*[a,b], *Veillonella*	*Alistipes*[a,b], *Bacteroides*[b], *Bilophila*, *Coprococcus*[a,b], *Dorea*[b], *Eubacterium*, *Faecalibacterium*[a,b], *Holdemania*, *Odoribacter*[a], *Parabacteroides*, *Phascolarctobacterium*[a], *Roseburia*[b], *Ruminococcus*[b], *Subdoligranulum*, *Tannerella*[a]
Dubinkina et al. (2017) [82]	In patients with alcohol dependency: patients with liver cirrhosis versus non-cirrhotic controls	13 genera 46 species	Not reported	*Bifidobacterium*[b], *Streptococcus*[a,b]	*Acidaminococcus*, *Alistipes*[a,b], *Anaerotruncus*, *Barnesiella*[b], *Coprococcus*[a,b], *Faecalibacterium*[a,b], *Odoribacter*[a], *Paraprevotella*, *Phascolarctobacterium*[a], *Prevotella*[b], *Tannerella*[a]

ACLF, acute-on-chronic liver failure. [a]Biomarkers confirmed independently by at least two studies within the same sequencing approach. [b]Biomarkers confirmed by both sequencing approaches.

studies. However, there was more agreement at the genus level: *Streptococcus* was enriched in both studies; and *Alistipes*, *Coprococcus*, *Faecalibacterium*, *Odoribacter*, *Phascolarctobacterium* and *Tannerella* were depleted in both studies. A lack of agreement at increased resolution (such as the species level) could be due to differences in data analysis procedures. Consistent analysis of these data sets could reveal even more overlapping gut microbiome signatures of liver cirrhosis.

Consistent signatures within the same technology and between different technologies are highlighted in TABLE 3. For example, while 16S rRNA gene amplicon-based studies consistently highlighted depletion of Lachnospiraceae and Ruminococcaceae at the family level, whole-metagenome shotgun sequencing-based studies consistently highlighted depletion of *F. prausnitzii* from the Ruminococcaceae and of *Coprococcus* spp. from the Lachnospiraceae. Given such agreement between technologies across multiple cohorts, the gut microbiome remains a promising avenue for biomarkers of liver cirrhosis. Indeed, the study by Dubinkina et al. showed that the effect of cirrhosis on gut microbiome community was different from that of alcohol dependence, suggesting that there are cirrhosis-specific microbiome biomarkers waiting to be discovered[82].

Analysing the gut microbiome is not as straightforward as it might seem, as most studies use the faecal microbiome as a proxy for the gut microbiome. Several studies have demonstrated that faecal microbiota and colonic mucosa-associated microbiota are different[81,110]. Indeed, the microbiota of ileal, jejunal and duodenal mucosal samples or aspirates are different[81,110], and they might hold important prognostic ability in cirrhosis, mainly shown for hepatic encephalopathy[110]. However, these samples are not easy to obtain, and it will be difficult to implement their routine point-of-care collection as a biomarker. One exception is the salivary microbiome, which is also very different from the faecal microbiome but might be able to predict hospitalization independent of cirrhosis severity, according to one study[111]. Further studies are required to draw firm conclusions.

Patients with different conditions and diseases exhibit both disease-specific changes as well as non-specific shared responses in their gut microbiome[112]. Identifying robust disease-specific gut microbiome signatures is therefore essential before its potential as a biomarker is realized. Accurate identification of microbiome changes during disease, independent of other confounding factors, still remains elusive and so does estimation of the magnitude of effects. The effect sizes of different known technical and biological factors on microbiome composition seem to be in the range of 10–15%[98]. This relatively low estimate already includes microbiome variation associated with disease status, raising concerns of whether microbiome research has been overhyped and whether microbiome-based biomarkers are indeed possible. Although the feasibility of gut microbiome biomarkers in general has been demonstrated for colorectal cancer, obtaining robust results across large geographical areas and different studies[113,114], how broadly applicable this approach is remains an open question. A more accurate estimate of the magnitude of effect of diseases on the gut microbiome needs robust large-scale longitudinal data from across the world, taking confounding factors into account. For example, when we obtained gut microbiome profiles corresponding to the cirrhosis MWAS from China[17] from the curated Metagenomic Data resource[115] and performed a permutational multivariate analysis of variance test, only 6.8% of variation in the genus composition of the gut microbiota can be explained by the cirrhosis status. Removing just one confounding factor (alcohol exposure) increases the explained variance to 7.8%. Understanding and accounting for other relevant confounding factors in microbiome studies will bring us closer to microbiome-based biomarkers for cirrhosis.

A major confounder in previous MWAS seems to be drug treatment. The gut microbiota composition is altered not only by antibiotic drugs but also by non-antibiotic drugs. One study showed that 25% of all non-antibiotic drugs change at least one gut species, and this value is likely an underestimate[116]. Specifically, proton-pump inhibitors, which have been associated with increased risk of developing hepatic encephalopathy and SBP in patients with ascites[117], change the gut microbiota towards bacterial overgrowth of enterococci and facilitate cirrhosis progression in experimental and human alcoholic liver disease[108,118]. The use of proton-pump inhibitors might also be the reason for the invasion of the gut by buccal microbiota strains, as the acidic barrier in the stomach is missing and the buccal strains can migrate unhampered into the gut and change its composition[17,119]. This situation argues for careful accounting of medication use in biomarker discovery studies. Proper consideration of, and stratification for, known microbiome covariates as potential confounders will not only greatly improve the accuracy of MWAS but also inform the interpretation of longitudinal and interventional data sets.

An integral view of the microbiome including microbial gene expression and microbial-derived metabolites or proteins might lead to more accurate and holistic biomarkers, analogous to the robust disease associations when integrating host genetics with gene expression and epigenetics. Such a holistic view encompassing the host–microbial holobiont might also underpin individualization of diagnosis, stratification and treatment, and could usher in a new era of holobiome-wide association studies, expanding MWAS.

Targeting the gut microbiome–liver axis

Several current treatments for liver cirrhosis, such as non-absorbable antibiotics, already target the gut microbiome–liver axis and other approaches, such as faecal microbiota transplantation (FMT), are now in clinical testing (FIG. 3; TABLE 4).

Probiotics and diet. Probiotics have been the subject of several clinical studies in cirrhosis, particularly their effect on brain function and the risk of hepatic encephalopathy. Even though most studies were small (between 20 and 70 individuals) and potentially associated with

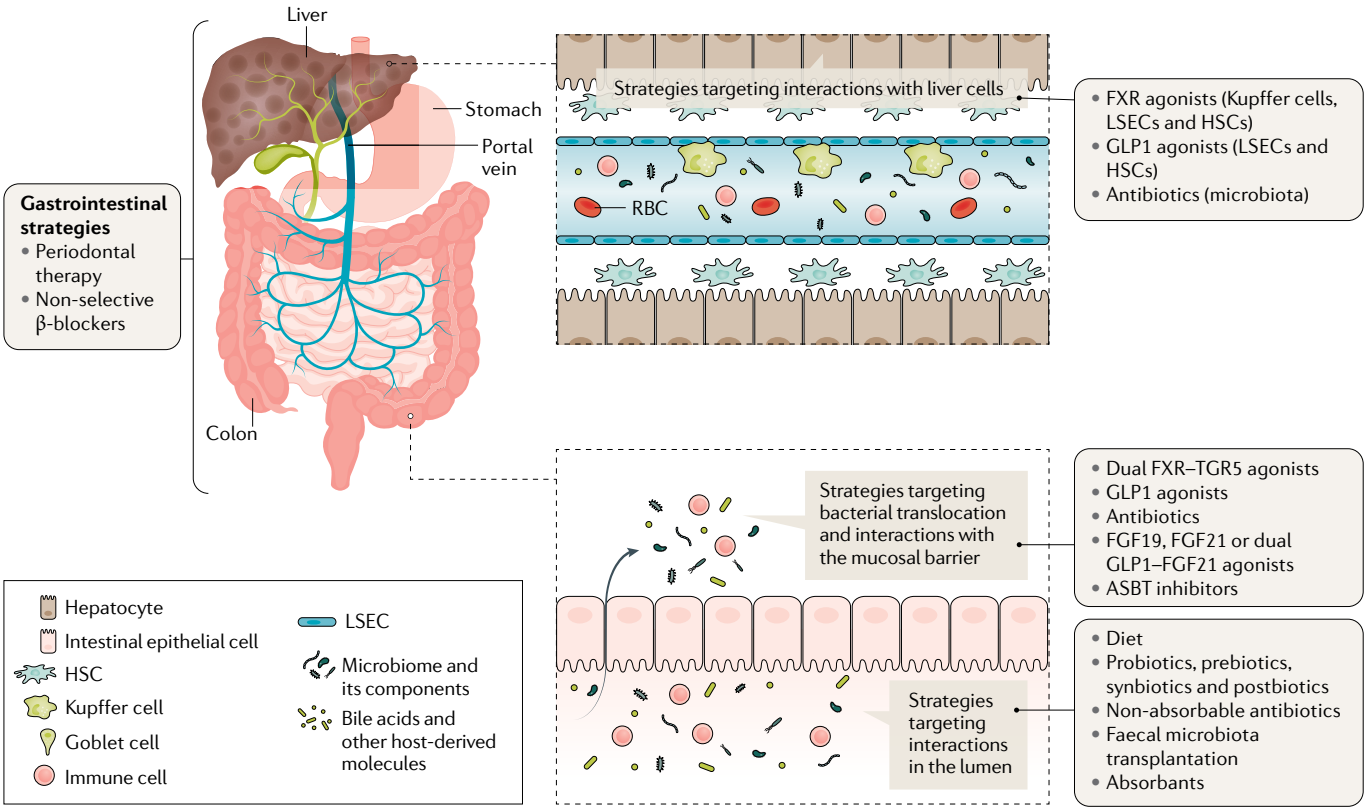

Fig. 3 | **Strategies to target the gut microbiome in cirrhosis.** Modulation of the gut microbiome with treatments (partly established and partly experimental) might improve the outcomes of patients with cirrhosis and microbiome biomarkers might reflect response to those treatments. Different strategies are shown (TABLE 4), but there might be some other strategies, not yet tested in decompensated cirrhosis and acute-on-chronic liver failure (ACLF) in humans (for example, agonists for glucagon-like peptide 1 (GLP1), farnesoid X receptor (FXR), Takeda G protein-coupled receptor 5 (TGR5), fibroblast growth factor 19 (FGF19), FGF21 and apical sodium-dependent bile acid transporter (ABST) inhibitors), which might be promising according to experimental evidence. HSC, hepatic stellate cell; LSEC, liver sinusoidal endothelial cell; RBC, red blood cell. Image courtesy of MICROB-PREDICT.

some methodological flaw and risk of bias, a meta-analysis indeed suggests that probiotics do have clinically meaningful effects for the treatment of hepatic encephalopathy[120], and a randomized controlled trial showed a benefit in overall liver disease severity and hospitalizations[121].

Diet has a major effect on, among other things, the gut microbiome and has been studied in the context of various conditions and diseases[98]. One study of an international cirrhosis cohort reported that coffee, tea, vegetables, chocolate and fermented milk intake were all associated with increased diversity of microbiome species and a reduced rate of hospitalizations[122], suggesting promising avenues for modulating the gut microbiome for liver health.

Antibiotics and statins. Rifaximin, a poorly absorbable antibiotic, seems to be a good treatment option in many gastrointestinal diseases such as inflammatory bowel diseases[123]. Several uncontrolled studies assessing the effect of rifaximin on liver disease have delivered some evidence that rifaximin might halt the progression of liver disease and decrease portal pressure[124,125]. Unfortunately, other studies showed not only that rifaximin has no effect on progression of liver disease but also that the changes induced in the gut microbiome profile of these patients were not profound[126–128], which is quite unexpected for an antibiotic that primarily stays in the gastrointestinal tract and is approved for the treatment of hepatic encephalopathy. In addition, a randomized controlled trial has previously shown that statins might improve survival of patients with cirrhosis after variceal haemorrhage[129], by mechanisms beyond a portal pressure decreasing effect[130]. One could speculate that statin therapy might prevent ACLF, as shown in animals[131], by improvement of the microbiome profile as shown in a study in 2020 (REF.[132]). The LIVERHOPE consortium, following a safety study combining rifaximin and statin[133], is recruiting for an efficacy trial and will also analyse the gut microbiome in those patients.

Antibiotics as prophylactics. Antibiotics, in particular quinolones, have also been used as prophylactic therapy in decompensated cirrhosis[134]. Patients with ascites have an increased risk for the development of SBP and frequently receive poorly absorbable antibiotic prophylaxis (norfloxacin) to prevent development of SBP. This primary prophylaxis of SBP has been shown to improve survival in selected patients, especially those with a low albumin concentration in ascites[135].

Table 4 | **Interventions in cirrhosis that target the gut microbiome**

Clinical condition	Intervention	Suggested mechanism of action	Clinical effect
Hepatic encephalopathy[168]	Rifaximin	Reduces ammonia production from gut microbiota	Reduces the risk of recurrent episodes of hepatic encephalopathy
Spontaneous bacterial peritonitis[135]	Long-term quinolone prophylaxis	Direct effect on bacteria and associated reduced bacterial translocation and risk of spontaneous infections	Improves survival; reduces the risk of hepatorenal syndrome and spontaneous bacterial peritonitis
Oesophageal varices[93,95]	Non selective β-blockers (propranolol, nadolol and timolol)	Improve gut barrier function and improve defence against bacterial translocation	Improve survival; reduce risk of bleeding from oesophageal varices and episodes of spontaneous bacterial peritonitis
Hepatic encephalopathy[155]	Oral and periodontal hygiene	Decreases the invasion of gut microbiome by buccal and oral microbiome	Improves endotoxaemia and cognitive brain function; reduces episodes of hepatic encephalopathy
Hepatic encephalopathy[156,157]	Faecal microbiota transplantation	Improves the gut microbiome profile	Improves cognitive brain function and reduces episodes of hepatic encephalopathy

In a multicentre, randomized controlled trial using norfloxacin in patients with severe liver cirrhosis (Child–Pugh score C), there was no benefit in the overall survival, but, again, patients with a low albumin concentration in ascites showed improved survival when receiving norfloxacin[136].

Prophylactic antibiotic treatment is also recommended in several other clinical situations, such as variceal bleeding, previous SBP and recurrent overt hepatic encephalopathy[134]. However, although the short-term effects in the most vulnerable patients might be beneficial, in many other patients these treatments cannot prevent further decompensation and ACLF. Perhaps this situation is also partly due to the decrease in diversity of the microbiome induced by antibiotics. Additionally, an increased rate of antibiotic-resistant bacteria is also found in this population of patients[137,138].

The possible role of antibiotic prophylaxis in the increase of multidrug-resistant bacteria is controversial, but a large international study suggests that prophylaxis does not increase the rate of multidrug-resistant infections in cirrhosis[139]. Non-selective antibiotic strategies might still lead to increased resistance in bacteria towards the most commonly used antibiotics. Untargeted treatment with antibiotics, either using broad-spectrum antibiotics or as prophylactics, could lead to serious healthcare challenges in the future. In humans, antibiotic treatment during early life has been associated with obesity[140]. Unnecessary use of antibiotics will also lead to widespread resistance among bacteria, leading to multidrug-resistant pathogens. Broad-spectrum antibiotics amplify this problem by spreading resistance at an increased rate[139]. Thus, a more targeted modulation of the gut microbiome towards improving liver health needs to be developed.

Albumin as potential therapy. When the intestinal barrier is weakened, it leaks both ways, which also leads to leaking of important host molecules from the blood compartment into the gut lumen. For example, increased intestinal permeability leads to elevated faecal albumin concentrations in animal models of alcoholic liver disease[48]. In patients with decompensated cirrhosis, it can lead to enteral loss of more than 2 g of albumin per day, as assessed in a study from the 1960s[141]. Additionally, albumin synthesis in the liver is impaired with progression of cirrhosis: on one hand, the hepatic injury leads to loss of hepatocyte functional mass; on the other, increased liver stiffness decreases albumin synthesis[142]. Interestingly, not only do the levels of albumin decrease but also its potential to bind toxins and other damaging substances[143–145]. These observations have positioned albumin level as a key parameter for severity of liver disease[100]. Albumin has also been investigated as a therapy based on different beneficial properties including plasma expansion[146], and is recommended for several situations in decompensated cirrhosis[146], such as hepatorenal syndrome, SBP and large-volume paracentesis (removal of several litres of ascites)[134]. Albumin administration was also shown to improve immune B cell function by binding prostaglandin E_2 (REF.[147]), decreasing the flares of immune response in cirrhosis and, thereby, halting the bursts of systemic inflammation[148,149] that are tightly associated with development of ACLF and death[19]. Although not yet recommended in the clinical guidelines, long-term albumin treatment of patients with liver decompensation[150] and non-SBP infections[148,151,152] improved clinical outcome by, respectively, improving survival and resolving ACLF[153]. Thus, it is tempting to hypothesize that albumin leaking into the gut lumen might influence and be metabolized by the gut microbiota, thereby possibly altering the microbiome composition and, indirectly, the host. Currently, no evidence linking albumin to the gut microbiome exists, neither regarding potential therapeutic effects nor as predictive biomarkers for albumin response. Ongoing large-scale international multicentre studies (for example, MICROB-PREDICT) are currently investigating albumin's therapeutic effects.

Periodontal hygiene. Published human MWAS suggest that during cirrhosis the gut microbiome changes towards an oral microbiome profile[17,82,154], and different medications such as proton-pump inhibitors might be responsible for this change. Diminishing the acidic milieu in the stomach that acts as a natural barrier might facilitate translocation of the oral microbiota into the gut, where the epithelial barrier is adapted to a different microbiome composition. These changes might facilitate translocation of the gut microbiota across the gut barrier and then lead to complications as discussed earlier. Thus, preventing the unwanted translocation of oral microbiota into the gut could form the basis of future treatment of cirrhosis. Although intuitive and tempting, this hypothesis has still not been fully proven. Nevertheless, there is some support for this hypothesis, as a study showed that intervention improving periodontal hygiene in 30 patients with cirrhosis compared with 20 non-cirrhotic controls changed the gut microbiome and improved hepatic encephalopathy[155].

Faecal microbiota transplantation. Pilot studies on FMT in decompensated cirrhosis are emerging with promising safety profiles, paving the way for larger studies[156]. One open-label randomized trial including 20 patients with recurrent hepatic encephalopathy observed reduced numbers of hospitalizations and improved cognition and dysbiosis using an FMT enema[157], which was also demonstrated in the long term (12–15 months) by another study[158]. Novel applications of FMT using an oral capsule formulation also show a similar safety profile and therapeutic effects in cirrhosis and hepatic encephalopathy[159,160]. However, caution is required due to the reporting of some fatalities associated with FMT resulting from drug-resistant bacteria transfer, including in some patients with cirrhosis[161]. Thus, better understanding and comprehensive characterization of such studies could lead to defined microbiome-modulating interventions to treat decompensated cirrhosis (FIG. 3).

Conclusions

Although the description, functionality and role of the gut microbiome in human health and disease have advanced, facilitated by fast technological development, important confounders prevent the implementation of this knowledge into clinical tools and practice[98]. The factors influencing the gut microbiome can be host-intrinsic (for example, genetics, biological sex, immunity), host-extrinsic (for example, lifestyle, alcohol, diet, medication) and environmental (regional, household, family). Clearly, these categories overlap, and many factors are also associated with each other and determine the role of the microbiome in the development of a specific disease[98]. As liver cirrhosis presents a large and important interaction of the gut microbiome with the host, microbiome diagnostics and treatments are almost mandatory to treat the progression of disease and development of decompensation. Unfortunately, the tools currently used to diagnose cirrhosis and measure its progression do not reflect the gut microbiome and are mainly focused on the host. Future studies should explore and deepen understanding of the dynamics and mechanisms of gut microbiome changes affecting cirrhosis progression and development of decompensation. Moreover, strategies targeting the interaction of the microbiome with the host at different levels might improve the outcome of the patients. Finally, the interaction of the microbiome with different treatments might also help us in the future to guide treatment and monitor treatment effects.

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1. Sender, R., Fuchs, S. & Milo, R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cell humans. *Cell* **164**, 337–340 (2016).
2. Sender, R., Fuchs, S. & Milo, R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* **14**, e1002533 (2016).
3. Qin, J. et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
4. Li, J. et al. An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014).
5. Almeida, A. et al. A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-020-0603-3 (2020).
6. Lynch, S. V. & Pedersen, O. The human intestinal microbiome in health and disease. *N. Engl. J. Med.* **375**, 2369–2379 (2016).
7. Ridlon, J. M., Kang, D. J., Hylemon, P. B. & Bajaj, J. S. Gut microbiota, cirrhosis, and alcohol regulate bile acid metabolism in the gut. *Dig. Dis.* **33**, 338–345 (2015).
8. Tilg, H., Cani, P. D. & Mayer, E. A. Gut microbiome and liver diseases. *Gut* **65**, 2035–2044 (2016).
9. Tripathi, A. et al. The gut–liver axis and the intersection with the microbiome. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 397–411 (2018).
10. Tsuchida, T. & Friedman, S. L. Mechanisms of hepatic stellate cell activation. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 397–411 (2017).
11. Parola, M. & Pinzani, M. Liver fibrosis: pathophysiology, pathogenetic targets and clinical issues. *Mol. Aspects Med.* **65**, 37–55 (2019).
12. Tsochatzis, E. A., Bosch, J. & Burroughs, A. K. Liver cirrhosis. *Lancet* **383**, 1749–1761 (2014).
13. Vlahcevic, Z. R., Buhac, I., Bell, C. C. Jr & Swell, L. Abnormal metabolism of secondary bile acids in patients with cirrhosis. *Gut* **11**, 420–422 (1970).
14. Acharya, C., Sahingur, S. E. & Bajaj, J. S. Microbiota, cirrhosis, and the emerging oral–gut–liver axis. *JCI Insight* **2**, e94416 (2017).
15. Bosch, J. & Garcia-Pagan, J. C. Complications of cirrhosis. I. Portal hypertension. *J. Hepatol.* **32**, 141–156 (2000).
16. European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J. Hepatol.* **69**, 406–460 (2018).
17. Qin, N. et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* **513**, 59–64 (2014).
18. Claria, J. et al. Systemic inflammation in decompensated cirrhosis: characterization and role in acute-on-chronic liver failure. *Hepatology* **64**, 1249–1264 (2016).
19. Trebicka, J. et al. Addressing profiles of systemic inflammation across the different clinical phenotypes of acutely decompensated cirrhosis. *Front. Immunol.* **10**, 476 (2019).
20. Moreau, R. et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* **144**, 1426–1437 (2013).
21. Fernandez, J. et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. *Gut* **67**, 1870–1880 (2018).
22. Bajaj, J. S. et al. Serum levels of metabolites produced by intestinal microbes and lipid moieties independently associated with acute on chronic liver failure and death in patients with cirrhosis. *Gastroenterology* https://doi.org/10.1053/j.gastro.2020.07.019 (2020).
23. Bajaj, J. S. Alcohol, liver disease and the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **16**, 235–246 (2019).
24. Byass, P. The global burden of liver disease: a challenge for methods and for public health. *BMC Med.* **12**, 159 (2014).
25. Gines, P. et al. Screening for liver fibrosis in the general population: a call for action. *Lancet Gastroenterol. Hepatol.* **1**, 256–260 (2016).
26. Collaborators, G. B. D. C. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol. Hepatol.* **5**, 245–266 (2020).
27. Stein, E. et al. Heavy daily alcohol intake at the population level predicts the weight of alcohol in cirrhosis burden worldwide. *J. Hepatol.* **65**, 998–1005 (2016).
28. Kim, D. et al. Changing trends in etiology-based annual mortality from chronic liver disease, from 2007 through 2016. *Gastroenterology* **155**, 1154–1163. e3 (2018).
29. Singh, S. P., Panigrahi, S., Mishra, D. & Khatua, C. R. Alcohol-associated liver disease, not hepatitis B, is the major cause of cirrhosis in Asia. *J. Hepatol.* **70**, 1031–1032 (2019).
30. Allen, A. M. & Kim, W. R. Epidemiology and healthcare burden of acute-on-chronic liver failure. *Semin. Liver Dis.* **36**, 123–126 (2016).
31. Zutshi, Y. *Liver Disease Treatments: The Global Market* (BCC, 2015).
32. Ge, P. S. & Runyon, B. A. Treatment of patients with cirrhosis. *N. Engl. J. Med.* **375**, 767–777 (2016).
33. Arroyo, V., Moreau, R. & Jalan, R. Acute-on-chronic liver failure. *N. Engl. J. Med.* **382**, 2137–2145 (2020).

34. Arroyo, V. et al. Acute-on-chronic liver failure in cirrhosis. *Nat. Rev. Dis. Primers* **2**, 16041 (2016).
35. Trebicka, J. et al. The PREDICT study uncovers three clinical courses in acutely decompensated cirrhosis with distinct pathophysiology. *J. Hepatol.* **73**, 842–854 (2020).
36. Sarin, S. K. et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014. *Hepatol. Int.* **8**, 453–471 (2014).
37. Bajaj, J. S. et al. Survival in infection-related acute-on-chronic liver failure is defined by extrahepatic organ failures. *Hepatology* **60**, 250–256 (2014).
38. O'Leary, J. G. et al. NACSELD acute-on-chronic liver failure (NACSELD-ACLF) score predicts 30-day survival in hospitalized patients with cirrhosis. *Hepatology* **67**, 2367–2374 (2018).
39. Hernaez, R., Sola, E., Moreau, R. & Gines, P. Acute-on-chronic liver failure: an update. *Gut* **66**, 541–553 (2017).
40. Kim, T. Y. et al. Characteristics and discrepancies in acute-on-chronic liver failure: need for a unified definition. *PLoS ONE* **11**, e0146745 (2016).
41. Li, H. et al. Characteristics, diagnosis and prognosis of acute-on-chronic liver failure in cirrhosis associated to hepatitis B. *Sci. Rep.* **6**, 25487 (2016).
42. Garcia-Tsao, G., Albillos, A., Barden, G. E. & West, A. B. Bacterial translocation in acute and chronic portal hypertension. *Hepatology* **17**, 1081–1085 (1993).
43. Oh, T. G. et al. A universal gut-microbiome-derived signature predicts cirrhosis. *Cell Metab.* **32**, 878–888.e6 (2020).
44. Korpela, K. et al. Selective maternal seeding and environment shape the human gut microbiome. *Genome Res.* **28**, 561–568 (2018).
45. Acharya, C. & Bajaj, J. S. Gut microbiota and complications of liver disease. *Gastroenterol. Clin. North Am.* **46**, 155–169 (2017).
46. Davis, B. C. & Bajaj, J. S. The human gut microbiome in liver diseases. *Semin. Liver Dis.* **37**, 128–140 (2017).
47. Albillos, A., Gottardi, A. & Rescigno, M. The gut–liver axis in liver disease: pathophysiological basis for therapy. *J. Hepatol.* **72**, 558–577 (2020).
48. Hartmann, P. et al. Deficiency of intestinal mucin-2 ameliorates experimental alcoholic liver disease in mice. *Hepatology* **58**, 108–119 (2013).
49. Henriksen, J. H., Moller, S., Ring-Larsen, H. & Christensen, N. J. The sympathetic nervous system in liver disease. *J. Hepatol.* **29**, 328–341 (1998).
50. Wiest, R., Albillos, A., Trauner, M., Bajaj, J. S. & Jalan, R. Targeting the gut–liver axis in liver disease. *J. Hepatol.* **67**, 1084–1103 (2017).
51. Teltschik, Z. et al. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* **55**, 1154–1163 (2012).
52. Wang, L. et al. Intestinal REG3 lectins protect against alcoholic steatohepatitis by reducing mucosa-associated microbiota and preventing bacterial translocation. *Cell Host Microbe* **19**, 227–239 (2016).
53. Du Plessis, J. et al. Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J. Hepatol.* **58**, 1125–1132 (2013).
54. Munoz, L. et al. Intestinal immune dysregulation driven by dysbiosis promotes barrier disruption and bacterial translocation in rats with cirrhosis. *Hepatology* **70**, 925–938 (2019).
55. Trebicka, J. et al. Soluble TNF-α-receptors I are prognostic markers in TIPS-treated patients with cirrhosis and portal hypertension. *PLoS ONE* **8**, e83341 (2013).
56. Queck, A. et al. Role of portal venous platelet activation in patients with decompensated cirrhosis and TIPS. *Gut* **69**, 1535–1536 (2020).
57. Schierwagen, R. et al. Circulating microbiome in blood of different circulatory compartments. *Gut* **68**, 578–580 (2019).
58. Bajaj, J. S. et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J. Hepatol.* **60**, 940–947 (2014).
59. Zhang, Y. et al. Characterization of the circulating microbiome in acute-on-chronic liver failure associated with hepatitis B. *Liver Int.* **39**, 1207–1216 (2019).
60. Villa, E. et al. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* **143**, 1253–1260 (2012).
61. Nery, F. et al. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study. *Hepatology* **61**, 660–667 (2015).
62. Garcia-Tsao, G., Lee, F. Y., Barden, G. E., Cartun, R. & West, A. B. Bacterial translocation to mesenteric lymph nodes is increased in cirrhotic rats with ascites. *Gastroenterology* **108**, 1835–1841 (1995).
63. Macdonald, S. et al. Cell death markers in patients with cirrhosis and acute decompensation. *Hepatology* **67**, 989–1002 (2018).
64. Praktiknjo, M. et al. Acute decompensation boosts hepatic collagen type III deposition and deteriorates experimental and human cirrhosis. *Hepatol. Commun.* **2**, 211–222 (2018).
65. Shi, Y. et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. *Hepatology* **62**, 232–242 (2015).
66. Sarin, S. K. & Choudhury, A. Acute-on-chronic liver failure: terminology, mechanisms and management. *Nat. Rev. Gastroenterol. Hepatol.* **13**, 131–149 (2016).
67. Medzhitov, R. Inflammation 2010: new adventures of an old flame. *Cell* **140**, 771–776 (2010).
68. Moreau, R. et al. Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. *J. Hepatol.* **72**, 688–701 (2020).
69. Lehmann, J. M. et al. Circulating CXCL10 in cirrhotic portal hypertension might reflect systemic inflammation and predict ACLF and mortality. *Liver Int.* **38**, 875–884 (2018).
70. Duan, Y. et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* **575**, 505–511 (2019).
71. Llopis, M. et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. *Gut* **65**, 830–839 (2016).
72. Puri, P. et al. The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. *Hepatology* **67**, 1284–1302 (2018).
73. Jansen, C. et al. Left ventricular longitudinal contractility predicts acute-on-chronic liver failure development and mortality after transjugular intrahepatic portosystemic shunt. *Hepatol. Commun.* **3**, 340–347 (2019).
74. Trebicka, J. Emergency TIPS in a Child–Pugh B patient: when does the window of opportunity open and close? *J. Hepatol.* **66**, 442–450 (2017).
75. Trebicka, J. Predisposing factors in acute-on-chronic liver failure. *Semin. Liver Dis.* **36**, 167–173 (2016).
76. Jansen, C. et al. Increase in liver stiffness after transjugular intrahepatic portosystemic shunt is associated with inflammation and predicts mortality. *Hepatology* **67**, 1472–1484 (2018).
77. D'Amico, G., Garcia-Tsao, G. & Pagliaro, L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J. Hepatol.* **44**, 217–231 (2006).
78. Jalan, R. et al. Acute endotoxemia following transjugular intrahepatic stent-shunt insertion is associated with systemic and cerebral vasodilatation with increased whole body nitric oxide production in critically ill cirrhotic patients. *J. Hepatol.* **54**, 265–271 (2011).
79. Bala, S., Marcos, M., Gattu, A., Catalano, D. & Szabo, G. Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. *PLoS ONE* **9**, e96864 (2014).
80. Voigt, R. M. et al. Diurnal variations in intestinal barrier integrity and liver pathology in mice: implications for alcohol binge. *Am. J. Physiol. Gastrointest. Liver Physiol.* **314**, G131–G141 (2018).
81. Mutlu, E. A. et al. Colonic microbiome is altered in alcoholism. *Am. J. Physiol. Gastrointest. Liver Physiol.* **302**, G966–G978 (2012).
82. Dubinkina, V. B. et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome* **5**, 141 (2017).
83. Bjarnason, I., Peters, T. J. & Wise, R. J. The leaky gut of alcoholism: possible route of entry for toxic compounds. *Lancet* **1**, 179–182 (1984).
84. Yang, A. M. et al. Intestinal fungi contribute to development of alcoholic liver disease. *J. Clin. Invest.* **127**, 2829–2841 (2017).
85. Bajaj, J. S. et al. Fungal dysbiosis in cirrhosis. *Gut* **67**, 1146–1154 (2018).
86. Albillos, A., de-la-Hera, A. & Alvarez-Mon, M. Serum lipopolysaccharide-binding protein prediction of severe bacterial infection in cirrhotic patients with ascites. *Lancet* **363**, 1608–1610 (2004).
87. Moreau, R. et al. Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. *J. Hepatol.* **72**, 688–701 (2020).
88. Ridlon, J. M., Alves, J. M., Hylemon, P. B. & Bajaj, J. S. Cirrhosis, bile acids and gut microbiota: unraveling a complex relationship. *Gut Microbes* **4**, 382–387 (2013).
89. Kakiyama, G. et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **306**, G929–G937 (2014).
90. Verbeke, L. et al. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. *Am. J. Pathol.* **185**, 409–419 (2015).
91. Ubeda, M. et al. Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. *J. Hepatol.* **64**, 1049–1057 (2016).
92. Sorribas, M. et al. FXR modulates the gut–vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis. *J. Hepatol.* **71**, 1126–1140 (2019).
93. Reiberger, T. et al. Non-selective β-blocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. *J. Hepatol.* **58**, 911–921 (2013).
94. Perez-Paramo, M. et al. Effect of propranolol on the factors promoting bacterial translocation in cirrhotic rats with ascites. *Hepatology* **31**, 43–48 (2000).
95. Senzolo, M. et al. β-Blockers protect against spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *Liver Int.* **29**, 1189–1193 (2009).
96. Senzolo, M. et al. Oral propranolol decreases intestinal permeability in patients with cirrhosis: another protective mechanism against bleeding? *Am. J. Gastroenterol.* **104**, 3115–3116 (2009).
97. Lang, S. et al. Intestinal virome signature associated with severity of nonalcoholic fatty liver disease. *Gastroenterology* https://doi.org/10.1053/j.gastro.2020.07.005 (2020).
98. Schmidt, T. S. B., Raes, J. & Bork, P. The human gut microbiome: from association to modulation. *Cell* **172**, 1198–1215 (2018).
99. Malinchoc, M. et al. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* **31**, 864–871 (2000).
100. Pugh, R. N., Murray-Lyon, I. M., Dawson, J. L., Pietroni, M. C. & Williams, R. Transection of the oesophagus for bleeding oesophageal varices. *Br. J. Surg.* **60**, 646–649 (1973).
101. Jalan, R. et al. The CLIF consortium acute decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. *J. Hepatol.* **62**, 831–840 (2015).
102. Such, J. et al. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, nonneutrocytic ascites. *Hepatology* **36**, 135–141 (2002).
103. Alvarez-Silva, C. et al. Compartmentalization of immune response and microbial translocation in decompensated cirrhosis. *Front. Immunol.* **10**, 69 (2019).
104. Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).
105. Yu, J. et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* **66**, 70–78 (2017).
106. Chen, Y. et al. Gut dysbiosis in acute-on-chronic liver failure and its predictive value for mortality. *J. Gastroenterol. Hepatol.* **30**, 1429–1437 (2015).
107. Bajaj, J. S. et al. Association between intestinal microbiota collected at hospital admission and outcomes of patients with cirrhosis. *Clin. Gastroenterol. Hepatol.* **17**, 756–765.e3 (2019).
108. Bajaj, J. S. et al. Gut microbial RNA and DNA analysis predicts hospitalizations in cirrhosis. *JCI Insight* **8**, e98019 (2018).
109. Bajaj, J. S. et al. Liver transplant modulates gut microbial dysbiosis and cognitive function in cirrhosis. *Liver Transpl.* **23**, 907–914 (2017).
110. Bajaj, J. S. et al. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **303**, G675–G685 (2012).
111. Bajaj, J. S. et al. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* **62**, 1260–1271 (2015).
112. Duvallet, C., Gibbons, S. M., Gurry, T., Irizarry, R. A. & Alm, E. J. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nat. Commun.* **8**, 1784 (2017).

113. Wirbel, J. et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nat. Med.* **25**, 679–689 (2019).
114. Thomas, A. M. et al. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. *Nat. Med.* **25**, 667–678 (2019).
115. Pasolli, E. et al. Accessible, curated metagenomic data through ExperimentHub. *Nat. Methods* **14**, 1023–1024 (2017).
116. Maier, L. et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* **555**, 623–628 (2018).
117. Dam, G., Vilstrup, H., Watson, H. & Jepsen, P. Proton pump inhibitors as a risk factor for hepatic encephalopathy and spontaneous bacterial peritonitis in patients with cirrhosis with ascites. *Hepatology* **64**, 1265–1272 (2016).
118. Llorente, C. et al. Gastric acid suppression promotes alcoholic liver disease by inducing overgrowth of intestinal *Enterococcus*. *Nat. Commun.* **8**, 837 (2017).
119. Bajaj, J. S., Betrapally, N. S. & Gillevet, P. M. Decompensated cirrhosis and microbiome interpretation. *Nature* **525**, E1–E2 (2015).
120. Holte, K., Krag, A. & Gluud, L. L. Systematic review and meta-analysis of randomized trials on probiotics for hepatic encephalopathy. *Hepatol. Res.* **42**, 1008–1015 (2012).
121. Dhiman, R. K. et al. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* **147**, 1327–1337.e3 (2014).
122. Bajaj, J. S. et al. Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. *Hepatology* **68**, 234–247 (2018).
123. Sartor, R. B. Review article: the potential mechanisms of action of rifaximin in the management of inflammatory bowel diseases. *Aliment. Pharmacol. Ther.* **43**, 27–36 (2016).
124. Kalambokis, G. N. et al. Rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites. *Clin. Gastroenterol. Hepatol.* **10**, 815–818 (2012).
125. Vlachogiannakos, J. et al. Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. *J. Gastroenterol. Hepatol.* **28**, 450–455 (2013).
126. Lutz, P. et al. Impact of rifaximin on the frequency and characteristics of spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites. *PLoS ONE* **9**, e93909 (2014).
127. Kimer, N. et al. Rifaximin has no effect on hemodynamics in decompensated cirrhosis: a randomized, double-blind, placebo-controlled trial. *Hepatology* **65**, 592–603 (2017).
128. Kimer, N. et al. Rifaximin has minor effects on bacterial composition, inflammation and bacterial translocation in cirrhosis; a randomized trial. *J. Gastroenterol. Hepatol.* **33**, 307–314 (2018).
129. Abraldes, J. G. et al. Addition of simvastatin to standard therapy for the prevention of variceal rebleeding does not reduce rebleeding but increases survival in patients with cirrhosis. *Gastroenterology* **150**, 1160–1170.e3 (2016).
130. Abraldes, J. G. et al. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. *Gastroenterology* **136**, 1651–1658 (2009).
131. Tripathi, D. M. et al. Simvastatin prevents progression of acute on chronic liver failure in rats with cirrhosis and portal hypertension. *Gastroenterology* **155**, 1564–1577 (2018).
132. Vieira-Silva, S. et al. Statin therapy is associated with lower prevalence of gut microbiota dysbiosis. *Nature* **581**, 310–315 (2020).
133. Pose, E. et al. Safety of two different doses of simvastatin plus rifaximin in decompensated cirrhosis (LIVERHOPE-SAFETY): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Gastroenterol. Hepatol.* **5**, 31–41 (2020).
134. European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J. Hepatol.* **69**, 406–460 (2018).
135. Wiest, R., Krag, A. & Gerbes, A. Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut* **61**, 297–310 (2012).
136. Moreau, R. et al. Effects of long-term norfloxacin therapy in patients with advanced cirrhosis. *Gastroenterology* **155**, 1816–1827.e9 (2018).
137. Fernandez, J., Tandon, P., Mensa, J. & Garcia-Tsao, G. Antibiotic prophylaxis in cirrhosis: good and bad. *Hepatology* **63**, 2019–2031 (2016).
138. Fernandez, J. et al. Multidrug-resistant bacterial infections in patients with decompensated cirrhosis and with acute-on-chronic liver failure in Europe. *J. Hepatol.* **70**, 398–411 (2019).
139. Piano, S. et al. Epidemiology and effects of bacterial infections in patients with cirrhosis worldwide. *Gastroenterology* **156**, 1368–1380.e10 (2019).
140. Cox, L. M. & Blaser, M. J. Antibiotics in early life and obesity. *Nat. Rev. Endocrinol.* **11**, 182–190 (2015).
141. Kerr, R. M., Du Bois, J. J. & Holt, P. R. Use of 125-I- and 51-Cr-labeled albumin for the measurement of gastrointestinal and total albumin catabolism. *J. Clin. Invest.* **46**, 2064–2082 (1967).
142. Gortzen, J. et al. Interplay of matrix stiffness and c-SRC in hepatic fibrosis. *Front. Physiol.* **6**, 359 (2015).
143. Klammt, S. et al. Albumin-binding function is reduced in patients with decompensated cirrhosis and correlates inversely with severity of liver disease assessed by model for end-stage liver disease. *Eur. J. Gastroenterol. Hepatol.* **19**, 257–263 (2007).
144. Jalan, R. et al. Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality. *Hepatology* **50**, 555–564 (2009).
145. Domenicali, M. et al. Posttranscriptional changes of serum albumin: clinical and prognostic significance in hospitalized patients with cirrhosis. *Hepatology* **60**, 1851–1860 (2014).
146. Bernardi, M., Ricci, C. S. & Zaccherini, G. Role of human albumin in the management of complications of liver cirrhosis. *J. Clin. Exp. Hepatol.* **4**, 302–311 (2014).
147. O'Brien, A. J. et al. Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E_2. *Nat. Med.* **20**, 518–523 (2014).
148. Fernandez, J. et al. Efficacy of albumin treatment for patients with cirrhosis and infections unrelated to spontaneous bacterial peritonitis. *Clin. Gastroenterol. Hepatol.* **18**, 963–973.e14 (2020).
149. Fernandez, J. et al. Effects of albumin treatment on systemic and portal hemodynamics and systemic inflammation in patients with decompensated cirrhosis. *Gastroenterology* **157**, 149–162 (2019).
150. Caraceni, P. et al. Long-term albumin administration in decompensated cirrhosis (ANSWER): an open-label randomised trial. *Lancet* **391**, 2417–2429 (2018).
151. Guevara, M. et al. Albumin for bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. A randomized, controlled study. *J. Hepatol.* **57**, 759–765 (2012).
152. Thevenot, T. et al. Effect of albumin in cirrhotic patients with infection other than spontaneous bacterial peritonitis. A randomized trial. *J. Hepatol.* **62**, 822–830 (2015).
153. Bernardi, M. et al. Albumin in decompensated cirrhosis: new concepts and perspectives. *Gut* **69**, 1127–1138 (2020).
154. Loomba, R. et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab.* **25**, 1054–1062.e5 (2017).
155. Bajaj, J. S. et al. Periodontal therapy favorably modulates the oral–gut–hepatic axis in cirrhosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **315**, G824–G837 (2018).
156. Braham, Y., Meunier, G. & Meunier, B. [Demonstration of an oxidative biotransformation of 9-methoxyellipticine. Comparison with the case of 9-hydroxyellipticine] [French]. *C. R. Acad. Sci. III* **304**, 301–306 (1987).
157. Bajaj, J. S. et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. *Hepatology* **66**, 1727–1738 (2017).
158. Bajaj, J. S. et al. Long-term outcomes of fecal microbiota transplantation in patients with cirrhosis. *Gastroenterology* **156**, 1921–1923.e3 (2019).
159. Bajaj, J. S. et al. Fecal microbial transplant capsules are safe in hepatic encephalopathy: a phase 1, randomized, placebo-controlled trial. *Hepatology* **70**, 1690–1703 (2019).
160. Bajaj, J. S. et al. Microbial functional change is linked with clinical outcomes after capsular fecal transplant in cirrhosis. *JCI Insight* **4**, e133410 (2019).
161. DeFilipp, Z. et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N. Engl. J. Med.* **381**, 2043–2050 (2019).
162. Hartmann, P., Chu, H., Duan, Y. & Schnabl, B. Gut microbiota in liver disease: too much is harmful, nothing at all is not helpful either. *Am. J. Physiol. Gastrointest. Liver Physiol.* **316**, G563–G573 (2019).
163. Burroughs, A. K. & Thalheimer, U. Hepatic venous pressure gradient in 2010: optimal measurement is key. *Hepatology* **51**, 1894–1896 (2010).
164. Ripoll, C. et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* **133**, 481–488 (2007).
165. Trebicka, J., Reiberger, T. & Laleman, W. Gut–liver axis links portal hypertension to acute-on-chronic liver failure. *Visc. Med.* **34**, 270–275 (2018).
166. Cho, E. J. et al. Circulating microbiota-based metagenomic signature for detection of hepatocellular carcinoma. *Sci. Rep.* **9**, 7536 (2019).
167. Ren, Z. et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut* **68**, 1014–1023 (2019).
168. Bass, N. M. et al. Rifaximin treatment in hepatic encephalopathy. *N. Engl. J. Med.* **362**, 1071–1081 (2010).
169. Arroyo, V., Claria, J. & Trebicka, J. in *Encyclopedia of Gastroenterology* 2nd edn (ed. Gerbes, A. L.) 436–443 (Elsevier, 2020).

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