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Gut Microbiota and Complications of Liver Disease

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Synopsis

The epidemic of chronic liver disease and how to combat its complications has been a challenging aspect for many years. New laboratory techniques have made analysis of the human intestinal microbiome easier and also more detailed. With insight into dysbiosis and how this dysbiosis impacts liver disease, scientists have new targets in the intestine and liver. Dysbiosis is associated with endotoxemia and propagates liver injury in NASH and alcoholic cirrhosis. The composition of the microbiota changes with the development of cirrhosis and decompensation. Apart from microbes, the role that bile acids play in this arena are also being discovered and newer treatments like FXR receptor agonists are coming into the picture. Altered gut microbiota plays an important role in cirrhosis and its modulation to prevent disease progression changes, and concomitantly bile acid physiology must be regulated or augmented to help prevent cirrhosis and its complications.

Keywords

Cirrhosis; Bile acids; Non-alcoholic steatohepatitis; Alcoholic Liver Disease; Dysbiosis; Microbiome; Firmicutes; Bacteroidetes

INTRODUCTION

Our fundamental understanding of liver disease, especially cirrhosis and its complications has changed dramatically over the last decade with the introduction of the culture-independent microbiome analysis. Cirrhosis is estimated to affect 0.27% of the general population¹. Hepatitis C cirrhosis, alcoholic cirrhosis and NASH-related cirrhosis are the most common etiologies accounting for 53.5%. With the changing demography and

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increasing obesity, NASH related cirrhosis is projected to be the most prevalent etiology of the future².

There are multiple initial insults spanning from viral hepatitis, fatty liver (alcoholic and nonalcoholic) and biliary stasis to name a few. These initial insult result in inflammation which are clinically detected by fatigue, malaise and elevated liver functions. With repeated insult the inflammation translates to fibrosis and with continued insults eventually cirrhosis. Clinically the pre-cirrhotic state is phenotypically different from the post-cirrhotic state with portal hypertension being the major driver of clinical manifestations later. Cirrhosis is associated with complications of hepatic encephalopathy (HE), Spontaneous bacterial peritonitis (SBP), variceal bleeding, ascites and other manifestations of volume overload and also renal complications. However all cirrhotics do not progress at the same pace and decompensation is unpredictable. The rate of decompensation for alcoholic cirrhosis is estimated to vary between 4~25%^{3,4}, for NASH cirrhosis ~2% over 5 years despite minimal histological progression⁵ and with Hepatitis C cirrhosis the cumulative probability for decompensation at 1 year is ~5%^{6,7}. Continuing with the initial insult definitely leads to decompensation but studies have noted progression of disease from fibrosis to cirrhosis despite cessation of the insulting factor⁸. To understand why certain patients remain stable, while others decompensate despite control of inciting insults, investigators have evaluated the gut microbiome and its associated changes in various stages and etiologies of liver disease to further explain this phenomenon.

The intestinal microbiome itself is a complex composition of microorganisms that is well known to be implicated in cirrhosis and its complications⁹. The metabolic neural pathway also known as the gut-liver-brain axis is a key player in cirrhosis and particularly in hepatic encephalopathy and this pathway is strongly regulated by the gut microbiome. Dysbiosis or an unfavorable change in the composition of the microbiome with a reduction in autochthonous (Firmicutes) bacteria and growth of other taxa (Bacteroidetes, Actinobacteria), and is well known to occur in advanced liver disease¹⁰ and other intestinal pathology^{11,12}. Dysbiosis is thought to be central to the proposed pathophysiology of the microbiota and gastrointestinal pathology for liver disease onset, progression and development of complication. Typically in dysbiosis there occurs a change in the balance of native Firmicutes to Bacteroidetes species with the former decreasing and the latter increasing. These native bacteria are important for the harmony of the gastrointestinal flora and as such for the wellbeing of the entire body, which is why science now considers the human microbiome as an organ in itself. The autochthonous bacteria produce short chain fatty acids (SCFA's) that nourish the colonic mucosal cells and reduce local colonic inflammation, and also produce anti-bacterial peptides and hence help maintain the intestinal barrier¹³. Hence dysbiosis is associated with increased inflammation and endotoxemia in multiple gastrointestinal pathology and in particular liver disease. The Cirrhosis Dysbiois Ratio (CDR) is the ratio of autochthonous to non-autochthonous taxa in cirrhosis. The lower the CDR the more the endotoxemia and more decompensated the cirrhosis¹⁴.

To give a brief overview of the pathophysiology, the intestine and its barrier i.e epithelium, Peyer patches and its lymphoid tissue, acts as the first immune system to come into contact with bacteria endotoxins or lipopolysaccharides (LPS), also known as pathogen-associated

molecular patterns (PAMPs) that are produced by human microbiota. Due to changes in the intestinal barrier there is bacterial translocation (BT) which exposes the intestinal immune system to antigens. The intestinal cells have a system of receptors namely the membranous Toll-like receptors (TLR) and intracellular NOD-like receptors (NLR) that recognize bacterial LPS, bacterial DNA and peptidoglycans^{15,16}. Recognition of the bacterial product by its receptors leads to upregulation of inflammatory mediators like TNF- α ¹⁷. Another integral factor in this process is the portal vein that acts as the main conduit for transfer of LPS and other bacterial products from the intestines to the liver. The final step in this chain is that the metabolites interact with hepatocytes and Kupffer cells via the hepatic TLR and NLR and this results in changes that promote a cirrhotic morphology¹⁸.

In this chapter we shall touch upon the proposed pathophysiology of how the microbiome is associated with different liver disease stages and microbiome focusing mainly on human studies. In Table 1 and 2 we provide details about the main recent clinical studies that show dysbiosis in chronic liver disease (CLD). This review focuses primarily on human Non-alcoholic fatty liver disease (NAFLD)/Non-Alcoholic Steatohepatitis (NASH) related liver disease, alcohol related liver disease (ALD) as these etiologies are the paradigms for microbiome-related endotoxemia, dysbiosis and related changes in the liver. The onset of cirrhosis, regardless of etiology, results in changes to the microbiome, which is furthered by decompensation.

NAFLD

NAFLD and its more sinister evolution NASH is slated to be the most common cause for chronic liver disease (CLD) and liver transplantation in the near future¹⁹. It is estimated that one third of the general US population has a fatty liver²⁰. Upto 20–30 % of NAFLD patients progress to NASH²¹ and 30% of NASH cases progress to cirrhosis²². NAFLD and NASH are commonly associated with the metabolic syndrome and as noted in previous studies is a pro-inflammatory state that is associated with higher levels of serum TNF- α , IL-6, and adipokines^{23,24}. The linkages of the gut microbiome and changes with the microbiome with regards to the causation in NAFLD/NASH have been studied for a while now and the microbiome probably has a larger role in the causation than we know.

Pre-cirrhosis NAFLD microbiota changes—In order to understand why the microbiota is tied into NAFLD and NASH we need to first understand how obesity and the metabolic syndrome affect the human microbiome. The human microbiome has been noted to be altered in obesity and dysbiosis has been well documented^{25,26}. A study by Mouzaki et al looked at 50 patients (of which 22 had NASH) to understand the obesity and dysbiosis paradigm, showed an inverse relationship existed between percentage Bacteroidetes species and the presence of NASH, and this confirmed a possible role of dybiosis in NAFLD²⁷. An aspect of dysbiosis in NAFLD that is often overlooked is the effect of the microbiome in extraction of energy from the gut²⁸ where dysbiosis results in increased production of SCFA's in the intestine with increased monosaccharides, that on transport to the liver activate the proteins that promote hepatic lipogenesis and steatosis²⁹ Type 2 DM, an integral a component of the metabolic syndrome has no immediate effect on the microbiome per say,

but dysbiosis seems to effect the development of DM³⁰. However, once DM sets in it has a role in dysbiosis and pathogenesis of cirrhosis as mentioned in a subsequent section

In humans with NAFLD/NASH, higher levels of LPS in the serum³¹ and increased expression of the TNF- α gene in hepatic tissues have been noted²⁴ confirming the pro-inflammatory state associated with these pathologies²⁴. Disruption of the intestinal barrier i.e disruption of intercellular tight junctions has been seen in NAFLD and could directly contribute to LPS and other bacterial products reaching the liver³². The mechanism underlying disruption of the barrier is thought to be from local colonic inflammation, however the evidence for this is conflicting and indirect in adult humans as seen in a study by Pendyala et al where weight loss in obese individuals resulted in reduced colonic inflammation³³. Other studies to study colonic inflammation via fecal calprotectin and leptin showed no difference between obese and lean adults^{34,35}. Regardless of the functionality of the intestinal barriers, It has been shown that bacterial LPS are transported out of the intestine to the liver via the portal vein along with chylomicrons that are formed during consumption of high fat diets³⁶. This is a key step to aid in the passage of pro-inflammatory bacterial products past the intestinal barrier, to the liver, where these products interact with their corresponding TLR and induce production of pro-inflammatory mediators like TNF- α and then subsequent pro-cirrhotic changes^{37,38}. Hence we see multiple mechanisms that underlie the development of microbial dysbiosis in NAFLD.

Intestinal Barrier Dysfunction and the microbiome in NAFLD—Another prominent factor that could promote endotoxemia in NAFLD and NASH is small intestinal bacterial overgrowth (SIBO). For unclear reasons, the prevalence of SIBO has been found higher in obesity and NAFLD³⁹. The estimated prevalence of SIBO in NAFLD ranges from 50–70% from various studies^{39,40}. SIBO has been recognized as an independent risk factor for the severity of hepatic steatosis^{32,39} due to its role in the dysfunction of the intestinal barrier. SIBO in general results in a quantitative change in the microbiome, that is associated with dysbiosis, that leads to increased intestinal permeability and increased gut bacterial product translocation which are essential in the transition pathway from NAFLD to NASH to cirrhosis⁴¹. Intestinal microbiota, apart from providing bacterial byproducts, increasing intestinal barrier permeability also suppress small intestinal secretion of Fasting-induced adipocyte factor (Fiaf) which results in increased triglyceride deposition in the hepatocytes⁴². Studies that have looked at the microbiome change in NAFLD have not exactly been able to classify if the changes are SIBO related and as such there are no microbiome studies that document the exact changes in NAFLD/NASH from SIBO

Role of microbiota and their products as a “second hit” for NASH development—The human microbiome has a strong role in progression of NAFLD to NASH. The 2 hit hypothesis proposed by Day and James⁴³ puts hepatic steatosis as the first hit. Multiple second hits are potential with this model. Zhu et al studied blood-ethanol concentrations in pediatric NASH subjects and obese-non-NASH subjects and noted elevated levels in the former. They deduced that the production of endogenous alcohol by *E. coli*^{44,45} in the intestinal microbiome played a role in NASH development. Another study of 15 adult female patients placed on diets regulated choline content diets noted that liver fat

was inversely proportional to choline deficiency in the diets⁴⁶. Crespo et al studied 52 adult obese patients and looked the relationship between TNF- α p55 and p75 (TNF- α receptors) and noted overexpression of TNF- α mRNA in NASH patients with increased overexpression with increased severity of NASH⁴⁷. Lastly, TLR-mediated signaling in Kupffer cells⁴⁸ is another proposed second hit. Whatever being the second hit, given all the evidence we see that microbiome has an important role in NAFLD from the pre-cirrhotic to the cirrhotic stages.

Another important mode of evaluating bacterial products is the bile acid (BA) profile. BA's are thought to regulate the microbiome by a potential detergent effect on the cell walls of the intestinal microbacteria⁴⁹ and also by interacting with the Farnesoid X receptor (FXR) in the liver which not only induces excretion of BA's from the liver but also induces antimicrobial peptides production^{50,51}.

The conjugated forms of primary acids BA's [cholic acid (CA) and chenodeoxycholic acid (CDCA)] have been found to be higher in the serum of NAFLD patients⁵² and similarly the conjugated form of primary and secondary BA's (lithocolic (LCA) and deoxycholic acid (DCA)) were noted to be 2.4 times higher in patients with NASH compared to controls⁵³. Lake et al studied the composition of BA's in the human livers of NAFLD patients and interestingly found a reduced level of CA and glycodeoxycholic acid and an increase in taurocholic acid and taurodeoxycholic acid⁵⁴. A recent study looking at the fecal composition of NAFLD and NASH adults confirmed a higher fecal bile acid level with a predominance of primary bile acids in the stool of NASH patients (NASH>NAFLD), further clarifying the mechanism by which BA's regulate dysbiosis⁵⁵. To further assess the interplay Tetri et al in a multicenter randomized trial studied the effect of obeticholic acid (BA derivative that activates FXR) in NASH patients and noted improvement in the histological features of NASH based on the NAFLD activity score on liver biopsies⁵⁶.

Alcoholic liver disease

ALD spans the spectrum of CLD from steatosis, steatohepatitis, liver fibrosis and eventually cirrhosis, to more acute manifestations like acute alcoholic hepatitis. Along with NAFLD it is one of the most leading etiologies for CLD⁵⁷. However, Only about 10% of chronic alcoholics end up with CLD⁵⁸ and only 15% of chronic alcoholics end up developing cirrhosis. Hence other factors such as host factors, immunity and the human microbiome could contribute to the disease progression.

Pre-cirrhosis changes to the microbiome in ALD—As with NAFLD/NASH the microbiome contributes to ALD via intestinal dysbiosis and increased BT. Bode et al first showed a significantly higher number of aerobic and anerobic bacteria on jejunal aspirates of chronic alcoholics⁵⁹ and this held true for alcoholic cirrhotics in a subsequent study⁶⁰. In ALD endotoxemia has also been noted well before the onset of cirrhosis supporting the theory of BT^{61,62}. The translocation of PAMPs out of the intestine in ALD would need a faulty intestinal barrier and Bjarnason et al showed that with chronic alcohol intake there was increased intestinal permeability that persisted upto 2 weeks after cessation of consumption⁶³. Studies have shown that even acute alcohol intake (single dose) can lead to

increased gastrointestinal permeability⁶⁴. This intestinal permeability has been explained by small bowel injury (Duodenal and Jejunal) injury which was noted to occur with ethanol intake⁶⁵ and further studies have shown enlarged intercellular spaces below the tight junctions of the distal duodenal mucosa⁶⁶. In vitro studies have shown that acetaldehyde, a metabolite of Ethanol is responsible for the tight junction disruptions^{67,68}. Acetaldehyde dehydrogenase which metabolizes acetaldehyde has a low activity in the colonic mucosa⁶⁹ and theoretically acetaldehyde could persist in the colon and cause local damage. No human or animal studies have been done to this effect yet.

Intestinal Barrier Dysfunction and the microbiome in ALD—As mentioned in the above NALFD section, SIBO is associated with dysbiosis and increased BT, dysmotility and eventual endotoxemia and is widely prevalent in ALD⁶⁵. Most of the evidence for SIBO changes to the microbiome in ALD are actually from animal model studies⁷⁰. Mutlu et al studied chronic alcoholics with no cirrhosis and in colonic microbiome analysis found dysbiosis with a relative lower median abundance of Bacteroidetes and higher abundance of Proteobacteria⁷¹. Human studies by Leclercq et al showed that there is increased intestinal permeability in alcoholics and this was associated with increased abundance of Ruminococcaceae family as well as some bacteria from the Lachnospiraceae family. Interestingly they also noted that increased intestinal permeability correlated negatively with total number of gut bacteria but the increased permeability was associated with dysbiosis⁷². As with NAFLD/NASH, with chronic alcoholism there is a change in the the intestinal and serum BA concentration. Kakiyama et al showed that chronic alcoholics had a higher concentration of intestinal secondary BAs and also primary BAs with a higher secondary to primary BA ratio⁷³. The same study noted that there was an increase in bacteria from Firmicutes and a reduction in phyla Bacteroidetes on comparison to the non-ALD cirrhosis subjects. Further studies regarding alcoholics and BA variation with the microbiome are needed.

Post-Cirrhosis microbiome changes—Once cirrhosis has set in, the microbiome changes are often due to other mechanisms playing a larger role in promoting dysbiosis. These can have clinically relevant outcomes such as HE, infections such as SBP, acute-on-chronic liver failure (ACLF) and readmissions^{14,74,75}. In patients with cirrhosis and SBP or HE, lower levels of Firmicutes and higher levels of Bacteroidetes correlated with higher endotoxemia and clinically a higher MELD score correlated with lower levels of autochthonous bacteria. As the liver disease progresses and decompensation ensues the CDR ratio reduces further and dysbiosis plays a larger role¹⁴.

Intestinal Barrier Dysfunction is noted to be increased in cirrhosis and is also associated with endotoxemia similar to the pre-cirrhotic state^{76,77}. Interestingly the level of endotoxemia was noted to be higher in alcoholic cirrhosis as compared to other etiologies in early studies^{78,79}. The microbiome changes post cirrhosis in ALD are similar to that seen in any other etiology for cirrhosis excepting that as cirrhosis onsets the ratio of Bacteroidetes and Firmicutes changes with the former reducing. In most other etiologies the opposite happens. Mutlu et al also showed that among the Bacteroidetes it was the *Bacteriodaceae* that were reduced in chronic alcoholics and they had higher levels of Proteobacteria⁷¹. The

CDR similar to SIBO has a direct relationship with the severity of liver disease and interestingly alcoholic cirrhosis has been noted to have the lowest CDR¹⁴.

In understanding the role of BA's in cirrhosis Kakiyama et al showed an increase in BA's in the serum of cirrhotics (NASH related and Alcoholic) and that there was a reduced quantity of BAs entering the intestine from the liver as the severity of liver disease progressed. In another study the same group found that the fecal levels of total bile acids were higher in the stool in all alcoholic and non-alcoholic cirrhotics^{73,80}. Lastly, DM, irrespective of the metabolic syndrome has an increased prevalence in cirrhotic patients⁸¹, contributes to dysbiosis, and prognosticates complications in cirrhosis⁸². The presence of DM with insulin use in cirrhosis does alter the gut microbiome causing a relative increased in Bacteroidetes and other families but also a reduction in Firmicutes. This change is similar to what we see in NASH cirrhosis with DM not taking insulin, but does not confer an increased risk for readmissions as seen in a prospective study⁸³.

Hepatic Encephalopathy—With decompensation of cirrhosis come multiple microbiome changes. While looking at the studies one must keep in mind that decompensated patients are generally sicker and may also be on microbiome altering therapy (Rifaximin or lactulose). With the onset of HE there is an increased endotoxemia^{14,84} and the CDR reduces, signaling microbiome changes, though in the study by Bajaj et al the predominant change was increased in Bacteroidetes species with no changes in the autochthonous species. Changes to the microbiome have been noted to start with early HE or Minimal HE (MHE). Salivary microbiota changes have been shown to correlate with stool microbiota changes⁸⁵ and could be explored as a new frontier in immune profiling in cirrhosis. Stool microbiota studies in cirrhotics and MHE noted an increased in *Streptococcus salivarius* in patients with MHE and an increased blood ammonia level⁸⁶. In another study no difference was noted in the stool microbiome between MHE and Overt HE (OHE)⁷⁵, though in a similar study by the same group there was a significant difference in the colonic mucosal microbiome between OHE and healthy subjects with no difference in the MHE and OHE groups⁷⁴. In more studies Bajaj et al showed that certain bacterial taxa such as *Proteobacteria* correlated with endotoxemia and cognition⁷⁵. Ahluwalia et al looked at the correlation of intestinal bacteria, HE and Magnetic resonance spectroscopy (MRS) and found that pathogenic taxa (*Enterococcaeae*, *Staphylococcaeae*, *Porphyromonadaceae* and *Lactobacillaceae*) positively correlated with MRS and HE and autochthonous taxa correlated negatively⁸⁷. Lactulose withdrawal in HE patients did not bring about changes to the microbiome⁸⁸ but in another study treatment of HE with rifaximin also induces changes to the microbiome with improved endotoxemia and cognition⁸⁹. The changes correlated with changes in serum saturated and unsaturated fatty acids (UFA) with the UFAs increasing and possibly helping in improved brain function. The exact changes to the microbiome taxa by treatment have yet to be documented.

To alter the microbiome to prevent decompensation prebiotics and probiotics have been studied. In healthy adults there is no evidence of probiotics resulting in changes⁹⁰, however in cirrhotics probiotics belonging to Firmicutes and Actinobacteria phyla have been studied in RCT's and have been proven to be beneficial. VLS#3(*mixture of multiple probiotic strains*) given daily has been proven to reduce the severity of cirrhosis and reduce HE-related

admissions in cirrhotics of alcoholic, NASH and Hepatitis C related etiologies, though the exact microbiome changes were not noted in this study as well⁹¹ Lactobacillus GG (LGG) use in a RCT for cirrhotics who were diagnosed to have MHE, showed those randomized to LGG reduced dysbiosis through an increased relative abundances of autochthonous taxa and a reduced relative abundances of potentially pathogenic taxa *Enterobacteriaceae* and *Porphyromonadaceae*), along with reduction in endotoxemia⁹².

Spontaneous Bacterial Peritonitis (SBP)—Bacterial translocation and SIBO are integral to SBP onset in cirrhosis and hence dysbiosis plays an important role here. A higher prevalence of Gram negative bacteria of the *Enterobacteriaceae* family has been noted in cirrhotics with and without decompensation^{14,93} and it is bacteria from this family that are predominantly noted in SBP ascitic fluid cultures⁹⁴. As seen earlier, with advance in liver disease the dysbiosis typically worsens¹⁴. In patients with SBP or infections, the CDR ratio was noted to be lower, there was a higher degree of endotoxemia and the CDR negatively correlated with endotoxemia. The dysbiosis however remained stable for cirrhotics who had not decompensated, indicating that the microbiome changes likely start after decompensation starts.

Prediction of Admissions in Cirrhosis—Bajaj et al studied 278 cirrhotics (all etiologies) looking at diabetes mellitus as a factor for readmission. In their prospective study with a median readmission time of 90 days they looked at the stool and sigmoid colon microbiome and noted that cirrhotics that had a non-elective readmission had a reduction in 2 families in the Bacteroidetes phyla⁸³. Reduction of Bacteroidetes is associated with increased risk of infection⁹⁵ and hence on comparing this phyla between alcoholic cirrhotics and NASH cirrhotics given the relative abundance of this phyla in NASH cirrhotics they have a lesser rate of infection compared to alcoholic cirrhotics.

Acute-on-chronic-Liver Failure and death—ACLF is defined as the presence of failure of 2 or more organs in cirrhotic patients. It portends a poor prognosis and has a high mortality^{96,97}. Patients with ACLF have been noted to have a higher level of endotoxemia and in a large study, patients who developed ACLF and organ failure 30 days after admission could be differentiated from those who did not based on microbiota¹⁴. This was confirmed by Chen et al who also noted that this dysbiosis is marked in ACLF and can independently predict mortality⁹⁸. Bajaj et al also noted that there was an increase in gram negative bacteremia on stool microbiome analysis, greater endotoxemia and these patients had a lower CDR compared to the infected cirrhotics in their study in those who survived. These changes likely occurred well before death and decompensation and may definitively play a role in disease.

CONCLUSIONS

We conclude that dysbiosis correlates with endotoxemia, starts early in NAFLD and ALD and progressively worsens with increasing severity of liver disease. BA's are major proponents of dysbiosis and alteration in BA profile could profoundly impact liver disease progression. Once cirrhosis sets in, the microbial composition and function alterations

worsen and contribute to complications such as HE, ACLF and can be a predictor of readmissions and mortality.

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Key Points

1. Intestinal microbial dysbiosis has a large role in the progression of liver disease towards cirrhosis via endotoxemia, intestinal barrier dysfunction and bile acid changes.
2. Decompensation of cirrhosis results in significant changes to the gut microbiome that correlates with complications.
3. Dysbiosis predicts predicts decompensation and acute on chronic liver failure and hence every attempt has to be made to modulate this dysbiosis to prevent these outcomes.
4. Bile acid changes are an important tool to study microbial function and the microbiota-modulated bile acid profile plays an important role in progression of human liver disease.

Table 1

List of human studies for NASH and ALD

Human studies in NAFLD/NASH			
Authors and year	Groups compared	Sample used for analysis	Results
Raman et al 2013	Healthy controls vs NASH	Stool	Increased <i>Ruminococcaceae</i> with reduced <i>Lactobacillae</i> and <i>Lachnospiraceae</i>
Zhu et al 2013	Adolescent health control vs obese vs NASH	Stool	Increased <i>Bacteroidaceae</i> and <i>Bifidobacterium</i> with reduced <i>Ruminococcaceae</i> and <i>Lachnospiraceae</i>
Mousaki et al 2013	Controls vs Simple steatosis vs NASH	Stool	NASH had a lower (<i>Bacteroidetes</i> to total bacteria counts) compared the rest and higher fecal <i>C. coccoides</i>
Bajaj et al 2014	Controls vs NASH cirrhosis	Stool	Increased <i>Porphyromonadaceae</i> , <i>Bacteroidaceae</i> and reduced <i>Veillonellaceae</i>
Boursier et al 2016	Non Cirrhotics-NASH vs No NASH	Stool	Increased <i>Porphyromonadaceae</i> , <i>Ruminococcaceae</i> and <i>Enterobacteriaceae</i> with reduced <i>Veillonellaceae</i> and <i>Ruminococcaceae</i>
Human studies in ALD			
Authors and year	Groups compared	Sample used for analysis	Results
Mutlu et al 2012	Healthy controls vs Alcohol cirrhotics	Sigmoid mucosal biopsies	Increased <i>Bacteroidaceae</i>
Bajaj et al 2014	Controls vs Alcohol cirrhotics	Stool	Increased <i>Enterobacteriaceae</i> and <i>Halomonadaceae</i> , reduced <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> and <i>Clostridiales XIV</i>
Kakiyama et al 2014	Nonalcoholic cirrhotics vs Alcoholic cirrhotics	stool	Increased <i>Bacteroidaceae</i> and reduced <i>Veillonellaceae</i>

Table 2

List of human studies that correlated microbiome changes to HE and ACLF

Authors and year	Groups compared	Sample used for analysis	Results
Bajaj et al 2012	Controls vs HE and No HE	Stool	Increased <i>Veillonellaceae</i> in OHE compared with no OHE cirrhotics, <i>Porphyromonadaceae</i> correlated with cognition
Bajaj et al 2012	Controls vs HE and No HE	Sigmoid mucosal biopsy	Increased <i>Enterococcus</i> , <i>Megasphaera</i> , and <i>Burkholderia</i> linked to poor cognition and higher inflammation. Reduced <i>Enterococcus</i> , <i>Megasphaera</i> , and <i>Burkholderia</i> . <i>Alcaligenaceae</i> and <i>Porphyromonadaceae</i> were associated with poor cognition
Zhang et al 2013	Controls vs minimal HE vs without MHE	Stool	<i>Veillonella parvula</i> and <i>Streptococcus salivarius</i> correlated negatively with cognitive function
Bajaj et al 2014	Cirrhotics of all etiologies studied prospectively	Stool	Increased pathogenic bacteria (<i>Porphyromonadaceae</i> , <i>Bacteroidaceae</i>) with no real change in autochthonous bacteria in OHE. ACLF was associated with increased <i>Propionibacteriaceae</i> and <i>Halomonadaceae</i> but reduced <i>Lachnospiraceae</i> and <i>Veillonellaceae</i>
Chen et al 2015	Controls vs ACLF cirrhotics all etiologies	Stool	ACLF was associated with increased <i>Pasteurellaceae</i> , <i>Streptococcaceae</i> and <i>Enterococcaceae</i> with decreased <i>Bacteroidaceae</i> , <i>Lachnospiraceae</i> and <i>Ruminococcaceae</i>
Ahluwalia et al 2016	Controls vs cirrhotics all etiologies	Stool	HE patients had higher relative abundance of autochthonous bacteria and higher abundance of <i>Enterococcaceae</i> , <i>Staphylococcaceae</i> , <i>Porphyromonadaceae</i> and <i>Lactobacillaceae</i> . The autochthonous families correlated negatively with MRI brain and positively with pathogenic bacteria.