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The Microbiome in Cirrhosis and its Complications

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Abstract

The microbiome in cirrhosis is affected by multiple processes occurring at the level of the gut and systemically. This dysbiosis, or unfavorable microbiota profile, is present at several body sites and is associated with higher systemic inflammation, bacterial infections and poor outcomes. This increased morbidity in cirrhosis stems from an immune paralysis state that is partly related to the constant stimulation of the immune system by this dysbiosis. Dysbiosis as a dynamic event worsens with decompensation such as with hepatic encephalopathy, infections or acute-on-chronic liver failure (ACLF). These microbial patterns could be applied as diagnostic and prognostic measures in cirrhosis in the outpatient and inpatient setting. Current therapies for cirrhosis have differing impacts on gut microbial composition and functionality. Dietary modifications and the oral cavity have emerged as newer targetable factors to modulate the microbiome, which could affect inflammation and, potentially improve outcomes. Additionally, fecal microbial transplant is being increasingly studied to provide compositional and functional modulation of the microbiome. Ultimately, a combination of targeted therapies may be needed to provide an optimal gut milieu to improve outcomes in cirrhosis.

Keywords

Outcomes; Dysbiosis; Oral; Hepatic Encephalopathy; Fecal microbial transplant

Introduction:

Cirrhosis, the end stage of chronic liver disease, has a high morbidity and mortality due to complications that affect several organs outside the liver^{1, 2} The human microbiome which can be considered as a complex organ system in itself³ consists of 10-100 trillion bacteria, fungi and viruses. In healthy individuals, the microbiome is responsible for maintaining homeostasis at the intestinal level and systemically through various metabolites.

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Gut microbial analysis is more expansive compared to the culture-dependent techniques for analysis that were used in the past. The samples (fecal, oral etc.) can be analyzed for bacterial RNA or DNA (Figure 1). Once the samples are collected, samples scheduled for RNA analysis need to be placed in an RNA lysis solution for preservation. Samples undergo extraction of nuclear material and then undergo 16sRNA PCR amplification. The samples are then fed into an automated machine that compares the sample data to a large database of available microbial profiles. There are multiple available automated machines and software available for bioinformatics. Depending on the type of analysis and coverage depth, the results can be at genus level (16s) or at species level (metagenomics). Data can be compared using several methods (a) alpha- diversity: number of different types of microbial taxa within a group (analyzed using Simpson, chao1 or Shannon indices), (b) beta-diversity: differences in diversity between groups (can be analyzed using UNIFRAC), (c) individual taxa differences (e.g. by LEfSE Linear discriminant analysis effect size or by non-parametric tests), and (d) tests of function which can be indirect or direct. Indirect analysis could be the gene expressions based on the metagenomic data, and PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) which analyses 16sRNA raw sequence data for gene content prediction of KEGG pathways and COG scores among others. Direct tests are actual functional correlates of microbial function such as endotoxemia, secondary bile acid production etc. Ultimately the depth of coverage, functional assessment and costs need to be balanced with the question that needs to be answered.

The human intestinal bacterial microbiome is broadly classified into multiple phyla with the more clinically relevant ones in cirrhosis being Firmicutes, Bacteroidetes and the Proteobacteria. The autochthonous taxa i.e. taxa responsible for homeostasis at baseline consist primarily of the Firmicutes. These bacteria are relevant for synthesis of short chain fatty acids (SCFA) such as (butyrate, palmitate and acetate) from carbohydrates, and these SCFAs have local intestinal barrier stabilizing properties⁴ and also serve as nutrients for the colonocytes⁵. The autochthonous taxa contain bacteria that convert primary bile acids (BA) to secondary BAs via the enzyme 7- α dehydroxylase⁶. The other non-autochthonous taxa consist of multiple other gram-negative bacterial (GN) species such as *Escherichia Coli*, *Klebsiella* etc. that are usually associated with systemic infections such as spontaneous bacterial peritonitis (SBP)⁷. In cirrhosis there is a change in the existing balance within the intestinal microbiota. This change is referred to as dysbiosis, a pathological condition that results in a compositional and functional change in the intestinal microbiota that results in propagation and increased complications in cirrhosis⁸. Due to dysbiosis, homeostatic mechanisms described above are in disarray and pathogenic bacteria tend to dominate^{8, 9}. The etiology for dysbiosis has been proposed to involve (1) Reduced BA synthesis in cirrhosis and lower amounts reaching the duodenum. BAs have a detergent action and are toxic to bacteria¹⁰. BAs influence the intestinal mucosa and effect the synthesis of antibacterial peptides^{11, 12} (2). Small bacterial intestinal overgrowth (SIBO) which is wide spread in cirrhosis and in the pre-cirrhosis state¹³⁻¹⁶. SIBO results in an increased quantity of bacteria, functional changes in the bacteria and increased intestinal permeability¹⁴.

It is important to understand that dysbiosis truly begins before development of cirrhosis during progression of chronic liver disease. This pre-cirrhosis microbial change is largely

driven by the underlying disease etiology. Boursier et al showed that there was a significant difference in the abundance of genera *Bacteroides* and *Ruminococcus* in patients with biopsy proven non-alcoholic steatohepatitis (NASH) with and without liver fibrosis (F2). Patients with NASH (no fibrosis) had a predominance of *Bacteroides* and those with NASH and fibrosis had a higher relative abundance of *Bacteroides* and *Ruminococcus*. The predicted microbial functionality between NASH and non-alcoholic fatty liver disease (NAFLD) were different and *Bacteroides* associated functionality probably promoted NASH¹⁷. Loomba et al utilized the microbial profile for distinguishing between advanced fibrosis in NAFLD. They included 86 biopsy-proven NAFLD patients (14 with stage 3 or 4 fibrosis). Using a metagenomic profile of 37 selected species along with age, BMI and diversity, they generated a model with an AUC of 0.936 to differentiate between early and advanced fibrosis in NAFLD¹⁸. The predicted metabolite functional change was not significant between groups but advanced fibrosis NAFLD patients had higher 3-phenylpropanoate, a product of anaerobic bacterial metabolism.

Another study showed that certain families (*Bacteroidaceae* and *Porphyromonadaceae*) from the Bacteroidetes phyla were more enriched while *Veillonellaceae* were less common in patients with NASH cirrhosis compared to those with cirrhosis from other etiologies⁸. Similarly, in alcoholic liver disease, gut microbial changes are driven early by alcohol itself. Chronic alcohol use results in increased intestinal permeability¹⁹. Bala et al studied the effects of acute alcohol consumption on serum endotoxin in healthy volunteers and noted that acute alcohol intake resulted in serum endotoxemia even in this population. More interestingly, they noted alcohol induced endotoxemia resulted in a prolonged increase in lipoprotein binding protein (LBP) and CD14 with increased bacterial 16S rDNA at 24 hours which reflected gut bacterial translocation²⁰. Mutlu et al showed that serum endotoxin levels were not different between alcoholics with and without liver disease suggesting that alcohol use resulted in dysbiosis and endotoxemia. The relative abundance of stool *Bacteroidaceae* was reduced in alcoholics with and without liver disease compared to controls but not within alcohol use groups²¹. This suggests that microbial changes were not able to differentiate between alcoholic patients who developed liver disease compared to those who remained free of it. In another study on the effects of alcohol on intestinal permeability and dysbiosis, microbiota composition influenced gut barrier function; intestinal permeability negatively correlated with the total quantity of bacteria and specifically with bacteria in the *Ruminococcaceae* family in non-cirrhotic individuals. Alcohol abstinence increased the abundance of genus *Ruminococcus* in those with increased intestinal permeability²². Similar changes are seen in patients with alcoholic liver disease after they progress to cirrhosis. A study showed that multiple genera from the phyla Firmicutes were less abundant while *Enterobacteriaceae* were more abundant in the alcoholic cirrhosis group compared to the non-alcoholic cirrhosis patients⁸. Hence, dysbiosis starts early in liver disease, progresses as the disease progresses, and is largely initiated by the insulting etiology itself.

Progression from compensated to decompensated state does not have a set course/timeline. Multiple conditions result in acute decompensation, such as ongoing alcohol use, development of hepatocellular conditions, acute portal venous thrombosis, infections etc. The role of gut microbiota is highly relevant [with respect to infections and hepatic encephalopathy (HE)] for progression to chronic decompensation, and even for acute on

chronic decompensation. Studies have shown that bacterial DNA frequently translocate into the ascitic fluid in decompensated cirrhosis (without SBP) and this translocation is associated with an increased systemic inflammatory response, endotoxemia and negative outcomes^{23, 24}. With decompensation, dysbiosis worsens and results in increased systemic inflammation and endotoxemia^{8, 25}. From an infections standpoint, SBP is typically caused by GN gut derived bacteria such as *Escherichia coli*⁷. SBP infections have a high mortality of 42-67% during admission despite management of infections^{26, 27}. In a large European cohort, bacterial infections raised the risk of mortality by 4 fold with a mortality rate of 43.5% as opposed to 13.6% for those without²⁸. A similar high mortality of 23% in 30 days and association with extrahepatic organ failures²⁹ i.e. renal failure, high grade overt HE (OHE), pulmonary failure and circulatory failure was noted in a large North American cohort. Multiple studies have shown a clear association of dysbiosis to cognition and HE^{8, 30}.

Given these strong associations between bacterial infections and mortality in cirrhosis, understanding the gut-liver axis and what influences it is very relevant as it provides us with potential points of intervention during insult (pre-cirrhosis) and after cirrhosis to prevent decompensation and its associated morbidity/mortality. For example, using rifaximin a non-absorbable antibiotic, modulates the microbiome³¹ and helps in alleviating HE³² which is closely linked to gut dysbiosis in cirrhosis. Other studies utilizing probiotics have shown to alleviate systemic inflammation and endotoxemia in cirrhosis^{33, 34} with alleviation in cognitive impairment i.e. Minimal HE (MHE). Similarly, this information can be used for prognostication and prediction of mortality will be discussed later in the review.

Historical culture-associated data on microbiota in cirrhosis:

Past studies gathered culture-based data in cirrhosis mainly in the context of SIBO and infections. There have been numerous studies on bacterial infections in cirrhosis however, the first association of the gut microbiota i.e. alterations in patients with CLD was described as early as 1921 by Hoefert et al³⁵. In 1970 Floch et al noticed that there was a significantly increased viable gram-positive(GP) and GN bacterial count in stool from cirrhosis patients³⁶. Bauer et al showed that SIBO diagnosed by jejunal aspirate was widely prevalent in cirrhosis (59%), was associated with use of acid suppressive therapy and was associated with systemic endotoxemia³⁷. From an infections standpoint, bacterial translocation to lymph nodes was noted to increase with increasing Child score but occurred with *Enterobacteriaceae* in only 25% patients³⁸. Most of the early culture data supported GN bacteria as the source for SBP and urinary tract infections but GP organisms are now emerging as important causative agents for SBP and nosocomial infections in patients with cirrhosis.³⁹

Riggio et al examined the effect of lactulose and lactitol on the fecal microbiome in 21 cirrhotic patients (without HE) and found that both drugs resulted in a reduction in *Enterobacteria* and *Enterococci* but a 2 log increase in lactobacilli after 10 days of use⁴⁰. Tarao et al studied the effect of lactitol for 4 weeks on the fecal microbiota and found an increase in occupation ratio [number of specific bacteria divided by total number of bacteria detected (relative abundance)] of *Bifidobacterium*, reduced occupation ratio of *Clostridium*

and *Bacteroides* and increased total *Lactobacillus* count⁴¹. Lastly, Liu et al examined the effect of synbiotics on fecal microbiome in cirrhosis patients with MHE and noted that synbiotics increased the *Lactobacillus* count with reduction in *E. coli* and *Staphylococcus*⁴². Culture-based studies therefore demonstrate a changing bacteriology from gram-negative to gram-positive microbiota as etiologies of infections in cirrhosis. The culture-based experience also shows favorable changes in potential probiotic bacteria after synbiotics and lactitol therapy. While the standard of care to guide clinical therapy remains culture-based, there are further developments related to culture-independent techniques to understand the patho-physiology of the altered gut/liver axis.

Cirrhosis as a systemic disease with global immune dysfunction:

Cirrhosis though primarily a liver pathology, with progression and decompensation may involve other organ systems such as the central nervous system (hepatic encephalopathy), renal system (hepatorenal syndrome) and the pulmonary system (hepatopulmonary syndrome). The connection to most of these organ systems is known to be due to portal hypertension and its resultant systemic hemodynamic changes. In hepatic encephalopathy, gut microbiota derived ammonia is felt to be a major neurotoxin though the microbiota interact with the brain indirectly via inflammatory mediators⁴³.

Cirrhosis however also affects the immune system and results in an immune paralysis state that leaves the body susceptible to the influences of dysbiosis and bacterial complications (Figure 2). In cirrhosis, not only is there an immune dysfunction locally at the liver and intestines levels, but also systemically at the body's innate immune system level with upregulation of systemic inflammation⁴⁴. Specifically, there is a reduction in the synthesis of proteins of the immune system such as complement components, soluble pattern recognition receptors (LBP, sCD14 etc.) and acute phase proteins. Additionally, at the liver level the Kupffer cells, the reticuloendothelial system and the sinusoidal endothelium are damaged and result in reduced immune surveillance capacity with reduced clearance of bacteria and endotoxins that come its way via the portal vein from the intestines⁴⁵. At the level of the intestines, there is an immune dysfunction to the gastrointestinal associated lymphoid tissue/ Peyer's patches. Due to the constant exposure to bacteria and bacterial products there is local inflammation resulting in increased intestinal permeability⁴⁶ and generation of systemic inflammatory mediators. Systemically cirrhosis results in reduced immune cell function across the spectrum i.e. reduced neutrophil opsonization/phagocytic activity^{47, 48}, reduced monocytic phagocytic activity⁴⁹, defective B cell^{50, 51}, T Cell^{52, 53} and NK cell functions⁵⁴ (Figure 2).

Eventually, the upregulated systemic inflammation overwhelms the immune systems leading to the state of immune paralysis⁴⁴. The source of the upregulated inflammation is primarily the circulating immune cells as well as immune cells in the intestine. The main instigator of inflammation is the gut derived bacteria and bacterial products (pathogen associated molecular patterns) combined with liver origin damage-associated molecular patterns. Metabolomics looking at these inflammatory markers and endotoxins have shown a clear correlation (positive and negative) to the gut microbiota to support the theory that systemic inflammation is driven by the dysbiotic microbiota. A study that examined stool microbiota

and systemic inflammatory markers in cirrhosis found a significant elevation in pro-inflammatory cytokines levels between those with HE and those without HE. More importantly the study noted a strong correlation between inflammatory cytokines (IL-2, IL-4, IL-23 and IL-13) with certain microbial families (*Enterobacteriaceae*, *Veillonellaceae* and *Fusobacteriaceae*) in those with HE, but no correlations were noted for those without HE³⁰. This suggested there could be a synergy between the gut microbiota and inflammation.

Hence, we see that in cirrhosis there is a heightened susceptibility to bacterial infections secondary to a global immune dysfunction, there is an upregulation of systemic inflammation driven primarily by a dysbiotic gut microbiota and these factors lead to worse outcomes (Figures 3)

Microbial functional changes related to dysbiosis:

As we have mentioned previously dysbiosis is not just a compositional change in the microbiota but more importantly a functional change. The biggest notable change is in BA physiology that is altered due to cirrhosis and further altered by the dysbiotic gut microbiota. Bile components apart from sloughed intestinal cells and the food we eat are sources of nutrition to the colonic intestinal microbiome, where the majority of the intestinal bacteria reside, though from a phyla perspective the diversity is low and restricted mainly to Bacteroidetes and Firmicutes⁵⁵. BA are normally produced by 2 pathways- the classic or “neutral” and the “acidic” pathways. The Classic pathway is regulated by (CYP7A1) whereas the acidic pathway is regulated by (CYP27A1). Primary BAs i.e cholic acid(CA) and chenodeoxycholic acid (CDCA) are produced in the liver by these pathways. In cirrhosis there is a downregulation of the traditional pathway (i.e downregulation of CYP7A1 by pro-inflammatory mediators) and the acidic pathway produces more CDCA⁵⁶. In the colon, CA and CDCA are 7 α -dehydroxylated exclusively by a bacteria from the genus *Clostridium* to form deoxycholic acid (DCA) and lithocholic acid (LCA), respectively⁶, and help maintain a high fecal secondary to primary BA ratio. In cirrhosis, with less substrate for conversion of primary BAs to secondary BAs the *clostridium* responsible for this are potentially reduced and more pathogenic families i.e *Enterobacteriaceae* take over during dysbiosis. Kakiyama et al examined the fecal and serum BA profiles in cirrhosis patients and correlated that to the stool microbiome samples. They noted that fecal CDCA was significantly positively correlated with *Enterobacteriaceae* and negatively with *Bacterioidaceae* abundance. On the other hand, no correlation between CA and the microbiome was noted. Additionally, Autochthonous genera positively correlated with secondary BAs and the fecal secondary to primary BA ratios⁵⁷. Hence, it is possible that with cirrhosis and a reduction in BA pool there is a reduction in the autochthonous taxa responsible for conversion of Primary BA's to secondary BA's leading to a vicious cycle, representing a function shift in the microbiome.

Metabolomic analysis had allowed us to understand the metabolic profile of the microbiome better. Hippurate, a by-product from the metabolism of dietary polyphenols, had been noted to be reduced in cirrhosis patients⁵⁸. In the same study the researchers found that a 14-day course of once daily 40 mg/day omeprazole/proton pump inhibitors (PPI) could change the functionality of the microbiome in compensated cirrhotic patients compared to controls.

Bacteroidaceae which correlated positively with Hippurate, lactate and dimethylamine (DMA) pre-PPI, changed to negative post PPI use. These metabolomic changes were more marked in the cirrhotic patient group compared to healthy controls after PPI therapy. Chen et al noticed marked depletions in the functional genes for amino acids, lipids and nucleotide/isoprenoid metabolism in cirrhotic patients⁵⁹. Qin et al found the predicted functionality of functional genes in cirrhotic patients was for Gamma-aminobutyric acid synthesis, heme synthesis and for ammonia production⁹. Another study compared cirrhotic patients to controls and noted that cirrhotic patients had a higher expression on genes for vitamins, cofactors and oxidant metabolism compared to controls who had a higher expression of genes for carbohydrate and amino-acid metabolism⁶⁰. The study by Boursier et al looked at this aspect of the microbiome at an earlier stage in NASH i.e F 2 compared to F0/1 fibrosis and found that F 2 enriched for carbohydrate and lipid metabolism¹⁷.

Therefore, dysbiosis can affect liver disease progression by potentiating systemic and local inflammation and also generating metabolites that can impact host physiology. The metabolic potential of the microbiota, in addition to their composition, needs to be explored further. The results of some of these studies and other that studied the microbiome in cirrhosis in humans are summarized in Table 1.

Microbiota as a prognostication tool:

Microbial dysbiosis in cirrhosis tends to follow a pattern with a fall in the autochthonous taxa and a rise in the pathogenic taxa (primarily Proteobacteria). It might be reasonable to use this pattern of microbial dysbiosis as biomarkers for diagnosis and prognostication in cirrhosis. However, creating such an index involves firstly identifying keystone and indicator organisms. A keystone organism is one that has a disproportionately large effect on its environment relative to its abundance i.e. its adequate presence equals good health of the overall ecosystem. For example, in the marine ecosystem, the krill are the organisms on which the entire ecosystem depends on it and the adequate presence of these krill reflect the health of the overall ecosystem. The short-chain fatty acid producing taxa *Ruminococcaceae* and *Lachnospiraceae* could be considered such organisms in humans. On the other hand, indicator organisms are those whose presence in the ecosystem indicates disruption. For example, coliforms such as *Escherichia coli* numbers are used to detect fecal contamination in drinking water as a routine. Therefore a biosensor should ideally have both indicator and keystone organisms.

One such measure of the degree of dysbiosis in cirrhosis is the cirrhosis/dysbiosis ratio (CDR)⁸. The CDR is the ratio of *Lachnospiraceae* + *Ruminococcaceae* + Clostridiales Incertae Sedis XIV + *Veillonellaceae* to *Enterobacteriaceae* + *Bacteroidaceae*. The CDR improved on the concept of using a Phylum based ratio of Firmicutes/Bacteroidetes since there are multiple potentially pathogenic taxa such as *Enterococcaceae* and *Staphylococceae* in the Firmicutes phylum as well. The CDR at baseline was higher in cirrhotic patients compared to controls (2.05 vs 0.74)⁸. On longitudinal follow up of cirrhotic patients the CDR showed significant changes as the patients went from compensated to the decompensated stage. CDR fell in patients with infections (0.34 vs 0.78) and hepatic encephalopathy (0.42 vs 1.2) indicating a rise in the denominator group

organisms i.e pathogenic shift of the microbiome; these patients also had a higher mortality. Interestingly, among different etiologies, alcoholic cirrhosis tended to have a lower CDR compared to other etiologies indicating a shift towards more pathogenic bacteria in this etiology of cirrhosis⁸.

Stool microbiota has a prognostic value for 90-day readmissions in cirrhosis. Stool *Bacteroidaceae* and Clostridiales XIV are predictive of 90-day hospitalizations and this is independent of MELD score, history of HE and PPI use. Those hospitalized tend to have a higher *Enterococcaceae* and *Enterobacteriaceae* but lower *Bacteroidaceae*, Clostridiales XIV, *Lachnospiraceae*, *Ruminococcaceae*⁶¹. Salivary microbiota dysbiosis in cirrhosis provides another avenue for prognostication. The salivary dysbiosis ratio which is the ratio of *Lachnospiraceae* + *Ruminococcaceae* + Clostridiales Incertae Sedis XIV + *Veillonellaceae* to *Streptococcaceae* was validated and has an independent predictive value for 90-day mortality in cirrhosis compared to healthy controls⁶⁰.

In terms of clinically relevant outcomes predicted by the microbiota in cirrhosis, apart from hospitalizations prediction, multiple studies found that the intestinal microbiota are associated with an altered gut-liver-brain axis which results in HE^{8, 30} HE itself is associated with a high mortality of up to 36%⁶² and the mortality probability of ACLF with OHE is higher than compared to ACLF without OHE⁶³. A previous single center study showed that patients with low CDR associated with lower autochthonous taxa [*Lachnospiraceae* (3 vs 5%) and *Veillonellaceae* (2 vs 4%)] and higher relative abundance of Gram negative bacteria had higher endotoxin levels and 30-day mortality⁸. In a study of the microbiome specifically for ACLF, Chen et al noted that ACLF associated with lower stool abundance of *Bacteroidaceae*, *Ruminococcaceae*, and *Lachnospiraceae* but higher abundance of *Pasteurellaceae*, *Streptococcaceae*, and *Enterococcaceae*. ACLF and HE patients had a lower abundance of *Lachnospiraceae* and importantly they noted that relative abundance of *Pasteurellaceae* was an independent predictor of mortality⁶⁴.

More recently the use of the DNA and RNA profile of the microbiota has been applied for morbidity prediction⁶⁵. The reasoning for comparing these two strategies is because the RNA profile may be more reflective of the metabolic function of the species while the DNA profile reflects a combination of living and dead bacteria. The study on 145 patients revealed that DNA and RNA profiles changed complementarily when patients with/without infections, decompensation, renal failure, rifaximin use and pre/post omeprazole were studied. Using multivariable logistic regression modelling in the RNA model, only MELD score and *Enterococcus* were significant. For the DNA model, MELD score and *Roseburia* were significant. AUC of prediction of hospitalization with RNA profile was 0.71 with clinical criteria alone but 0.77 for MELD + *Enterococcus*. The AUC of prediction with DNA profile was 0.70 with clinical criteria alone and 0.79 for MELD + *Roseburia*.

The above experiences demonstrate that microbiota composition can add to our clinical prognostication of several relevant clinical outcomes in addition to our current biomarkers. Further development of these profiles is needed for translation into practice.

Diet and Microbiota:

An often-underappreciated component of the gut-liver-brain axis is the diet which can be a major modulator of the gut microbiome. Dietary changes can be associated with systemic inflammation and endotoxemia, presumably derived from the gut in those populations who consume more meat⁶⁶ and the so called “Western diet” of refined products⁶⁷. In a study examining the influence of a Western vs Middle eastern diet on the microbiota and outcomes in cirrhosis patients, researchers compared two international cohorts (USA-157, 50 decompensated and Turkey-139, 43 decompensated) of compensated and decompensated cirrhosis patients to controls (USA-48, Turkey-46). They noted significantly more diversity in the Turkish cohort, with stability between cirrhosis and controls as opposed to the US cohort who showed much less diversity (Shannon index) that varied with disease stage. Even in the decompensated stage, compared to the USA cohort, the Turkish cohort had a higher relative abundance of beneficial taxa (*Ruminococcaceae* and other *Clostridiales*) and lower relative abundance of *Enterococcaceae*. There was a significantly lower risk of 90-day hospitalization in Turkish compared to American cirrhotic patients (p=0.016 all-cause and p=0.02 liver-related). The most common etiology for readmissions in the US cohort was infections related and for the Turkish cohort it was acute kidney injury/ascites. On Cox and binary logistic regression, microbial diversity (OR 0.78) was protective against 90-day hospitalizations, along with coffee/tea (OR 0.38), vegetable (OR 0.17) and cereal intake (OR 0.38). The take home message from this study is that dietary habits have a major impact on microbiota and the impact of a Middle Eastern diet in cirrhotic patients usually consuming a Western diet needs to be studied.

Effect of treatments on microbiota:

Several current approved treatment options may modulate microbiota in different ways. We use HE as the paradigm condition to explain the effect of treatments on gut microbiota.

Lactulose, a non-absorbable disaccharide, is essentially a laxative since the effect on the microbiota composition is negligible. In healthy controls lactulose administration did not change *Bifidobacterium* or other species⁶⁸. In patients with a history of OHE after lactulose withdrawal, only *Faecalibacterium* spp. (pre-6 % to 1 % post- withdrawal, p=0.026) decreased with a trend towards *Veillonella* decrease (2 % to 0 %, p=0.06). The rest of the stool microbiota stayed same to the 14 day pre- on lactulose abundance³⁰.

Rifaximin which is a non-absorbable antibiotic would be expected to have a much stronger influence on the gut microbiota composition. Rifaximin does affect the gut microbiota but more on a functional than the composition level. In terms of compositional change when the colonic mucosa of patients with OHE vs No OHE was studied, with the patients being on rifaximin/lactulose or lactulose only, an increase in the relative abundance of *Propionibacterium* with decrease in *Roseburia*, *Veillonellaceae*, *Alistipes* and *Blautia* were noted²⁵. The same investigators examined rifaximin given for 8 weeks for cirrhosis patients with MHE and noted a significant increase in family *Eubacteriaceae* and concomitant reduction in family *Veillonellaceae*. *Veillonellaceae* associates with increased systemic inflammation in cirrhosis and via this effect could further the progression of cirrhosis and

HE. The study showed that rifaximin did not change the overall composition and abundance but did change the microbial associations on correlations analysis⁶⁹. From a functional standpoint the same study noted that rifaximin use associated with an increase in fatty acid and carbohydrate metabolism intermediates⁶⁹. Given the proposed prominent role of BA's in dysbiosis, rifaximin has been noted to also suppress fecal DCA levels thereby altering the fecal secondary/primary BA ratio (which we know reduces in cirrhosis) in compensated cirrhosis only⁵⁷, and possibly aiding in endotoxemia reduction.

Probiotics i.e. mixtures of beneficial autochthonous taxa have been examined in cirrhosis with MHE for improving cognition via change in the microbiome. The most prominent study utilizing lactobacillus GG (LGG) capsules daily for 8 weeks compared to placebo showed that at the end of the study period there was a significant decrease in pathogenic taxa associated with worse cognition i.e. *Enterobacteriaceae* and *Porphyromonadaceae* and an increase in the autochthonous taxa *Lachnospiraceae* and Clostridiales XIV in the intervention group. The CDR consequently increased in the LGG group. BA did not change significantly³³. Functionally, post LGG the group tended to have a reduction in potentially ammoniagenic amino acids.

Liver transplant (LT) related modulation of the gut microbiome has been documented recently. A prior study on stool microbiota and cognition (Psychometric Hepatic Encephalopathy score [PHES]) in 45 cirrhosis patients who underwent LT 6±3 months after sample collections. Patients were followed and resampled 7±2 months after LT. The results were remarkable where a significant increase in microbial diversity (based on Chaol index) from 468.9 ± 263.1 to 694.9 ± 240.6 was noted post-LT. No change at the phylum level was noted post- LT. However, there was a significant decrease in potentially pathogenic genera belonging to *Enterobacteriaceae* (*Escherichia*, *Shigella*, *Salmonella*) with an increase in *Ruminococcaceae* and *Lachnospiraceae* (autochthonous taxa) in the post-LT period⁷⁰. The changes in the microbiota correlated with improved cognition in the post-LT period. In a more recent study, examining the functional aspect of the microbiota post-LT, the same investigators enrolled 40 patients and followed them till 7±3 months post-LT. They found similar compositional changes in the fecal microbiota post-LT. In the metabolomics part of the study they examined stool for BA's with serum and urine metabolomics. Serum endotoxemia reduced post-LT. They noted that post-LT there was an increase in the ratio of secondary to primary fecal BA's suggesting an increased bile flow from the liver with increased conversion by the microbiota. There was restoration of SCFA metabolism profile i.e reduction in SCFA and restoration towards long chain fatty acid profile. Urinary phenylacetylglutamine level increased post-LT indicating a return of ammonia metabolism in the microbiota. Interestingly urinary trimethylamine oxide (TMAO) levels increased post-LT indicating resumption of production via a restored gut microbiota and liver⁷¹.

In conclusion both rifaximin and LGG result in a functional reversal of dysbiosis in cirrhosis with reduction in endotoxemia and systemic inflammation which can help in prevention of decompensation in cirrhosis. Rifaximin and lactulose do not compositionally alter the microbiota but LGG results in beneficial compositional changes. LT results in a recovery in microbial diversity in the post-LT period for up to 1 year with restoration of multiple important microbial and hepatic functions but remains worse than controls. Figure 3

explains the complex interaction between dysbiosis, BAs, inflammation during cirrhosis and post LT.

Future Concepts:

There are several areas that require future research in this rapidly evolving field of microbiota research in liver disease. These have been summarized in table 2. Specific areas where some progress has been made but more work is needed are shown in the section below

1. Fecal Microbial Transplant (FMT):

One of the newer novel methods for restoring the gut microbiota being explored is the use of FMT in the setting of recurrent OHE. The goal here is a direct infusion of stool from a rationally derived donor (i.e. high *Lachnospiraceae*/*Ruminococcaceae*) may improve dysbiosis and mental status. A handful of small studies/single patient case reports exist⁷² for FMT in cirrhosis, but in a larger phase 1 larger study investigators infused the donor stool as a one- time enema in 10 male outpatients with recurrent OHE, comparing them to standard of care. Patients were given a 5-day course of broad-spectrum antibiotics prior to the FMT. Patients were followed up for up to 150 days post intervention, but samples were only collected at baseline, 5 days post FMT and day 20 post FMT. In the FMT group there was a significant improvement in cognition, no readmission for OHE, reduced all cause readmission and a recovery of antibiotic-associated collapse in microbial diversity to baseline levels⁷³. In analysis of the bacterial functionality pre and post FMT there were multiple significant findings. Firstly, fecal BA profile was restored post FMT i.e. secondary BAs increased and primary reduced, to baseline levels. Secondly, SCFAs that fell pre FMT were restored partly post FMT and lastly, the predicted functionality related to xenobiotic and lipid metabolism were higher after antibiotics, whereas bioenergetics, BA, Amino acids, glycans and vitamin/cofactor metabolism were higher post FMT⁷⁴. Another phase 1 trial involving FMT via oral capsules in recurrent OHE has been completed (*NCT03152188*).

The role of FMT in cirrhosis is evolving and several unanswered questions remain regarding the 5 Ds of FMT, decision (which patients are candidates), discussion (risk-benefit discussion with patients), donor (one or multiple, known donor or from a stool bank), delivery (naso-jejunal, oral capsules, enema, colonoscopy) and discharge (integration of pre-existing SBP prophylaxis and rifaximin since traditionally antibiotics are withheld post-FMT in most cases).

2. The Oral-gut-liver-brain axis:

The oral cavity microbiome also undergoes dysbiosis in cirrhosis though this is less well understood. Most of the oral organisms are commensals such as *Streptococcus* and *Veillonella*. In cirrhosis oral microbial dysbiosis can manifest with gingivitis and periodontitis⁷⁵⁻⁷⁷. Gronkjaer et al prospectively studied 110 cirrhosis patients and noted an increased incidence of periodontitis and inflammatory markers. The etiology and severity of liver disease was not a predictor of periodontitis⁷⁸. Dental infections which could be associated with oral dysbiosis are higher in cirrhosis patients and increased dental infections

associate with a shorter time to transplant⁷⁹. Salivary dysbiosis/oral dysbiosis seems to be prominent in cirrhosis and follows intestinal dysbiosis trends and associations with inflammation⁶⁰.

From an infections perspective, studies have noted an increase in systemic infections by GP and oral origin microorganisms^{39, 80-82}. The phenomenon of distal migration of oral bacteria was noted by Qin et al⁹ then reconfirmed and hypothesized to be an epiphenomenon secondary to the use of PPI therapy^{58, 83}. This process may be critical to the infectious process. One study is evaluating the role of prophylactic dental cleaning in cirrhosis patients to study the effect on systemic inflammation and dysbiosis (*NCT03030820*)

To conclude, the oral cavity is a potent source of inflammation in cirrhosis. Oral microbiota undergoes dysbiosis in cirrhosis. Cirrhosis patients with worse dental disease have worse outcomes. Oral microbial dysbiosis is similar to stool dysbiosis and predicts readmissions. By modulation of the axis via dental cleaning we can reduce a source of inflammation and endotoxemia and modify outcomes in cirrhosis.

Further studies are needed to definitively establish the oral cavity as a source of inflammation and potentially promote oral care in all cirrhotic patients and not just those who are being considered for transplant. In addition, judicious use and withdrawal of PPI use in patients in which it is not indicated needs to be studied systematically from a microbial perspective in larger studies.

3. Fungal dysbiosis in cirrhosis:

Another important topic is fungal dysbiosis or disruption of the intestinal mycobiome. In an interesting translational paper Yang et al noticed that fungal products such as β -glucan were higher in the plasma of mice fed alcohol chronically. Alcohol feeding resulted in intestinal fungal overgrowth that correlated with the β -glucan. Interestingly *Candida parapsilosis* relative abundance decreased while other species increased. These changes were mitigated with anti-fungal therapy to the mice. Yang et al further went to show that fungal β -glucan resulted in upregulation of Plasma IL-1 β . Comparing alcoholic cirrhosis to healthy controls and HBV cirrhosis (non-alcohol consuming) it was noted that alcoholism resulted in fungal dysbiosis with increase in *Candida* species irrespective of liver disease stage. Alcoholic cirrhosis patients had increased fungal dysbiosis, systemic exposure and immune response to the fungi⁸⁴. In another study comparing all etiology cirrhotic (inpatient and outpatients) to healthy controls in a cross-sectional arm and a prospective follow up arm over a 6 months period, researchers noted that fungal diversity (defined by Shannon diversity index <1%) was most affected in those cirrhotic patients who were on antibiotics and worst in the inpatients on antibiotics. The inpatient group was noted to have a higher relative abundance of *Candida* species. Interestingly during longitudinal follow no significant changes to the fungal diversity pre and post PPI use was noted. The researchers also studied microbiota dysbiosis and developed the Bacteroidetes/Ascomycota ratio which was a valid prediction tool for 90-day hospitalization. They concluded that antibiotic use was a major risk factor for fungal dysbiosis in cirrhosis⁸⁵.

More recently as part of the NACSELD study group looking at a large multicenter cohort of cirrhotic patients the investigators studied groups with fungal infections to bacterial infections and no infections. The risk factors associated with fungal infections were ICU stay (OR 3.17), bacterial infections on admission (OR 2.15), Diabetes mellitus (OR 1.79) and acute kidney injury (OR 1.74). Fungal infections were significantly associated with higher ACLF (43% vs 6% in non-infected cohort) and lower 30-day survival (66% vs 86% in bacterial infections cohort vs 93% in the uninfected cohort)⁸⁶. Hence, we see that fungal infections in cirrhosis are not uncommon, occur more often in alcoholic cirrhotic patients, those taking antibiotics and fungal infections are associated with a higher mortality. Hence, good antibiotic stewardship is necessary, and one must pay attention to culture negative infections in cirrhosis.

Further strategies focusing on earlier microbial culture-independent techniques to diagnose fungal and resistant bacterial infections earlier than current culture-dependent techniques are needed.

4. Older patients and HE:

This topic of the elderly/ageing cirrhosis patients is becoming increasingly relevant as the population in western countries and worldwide is projected to reach 1 billion by 2030 (National institute of ageing). The process of ageing brings about multiple physiological changes to the body and affects the gut microbiota. Elderly people with no chronic liver disease conditions tend to have dysbiosis with a shift towards Bacteroidetes in those who are frail⁸⁷. When elderly cirrhotics were compared to non-cirrhotic patients and reclassified based on neurophysiological status i.e. amnesic memory impairment and amnesic/anamnestic memory impairment the latter group was noted to have a reduced relative abundance of autochthonous and oral origin families but higher abundance of *Bacteroides*. The unimpaired patients had a higher relative abundance of *Fecalibacterium* compared to impaired patients. Hence the microbes could also be used to differentiate who has memory compared to other issues.

Further studies are needed to integrate the aging gastro-intestinal tract with liver disease patho-physiology and the resilience of the gut microbiota in elderly patients with liver disease. The role of microbial products in the progression and overlap of dementia and HE as well as in the metabolism of liver-related drugs is needed.

Conclusion:

Cirrhosis is not just a liver disease but a multisystem disease. The common thread between the organ systems could be the gut microbiota and its pathological change in this multisystem disease state i.e. dysbiosis. Dysbiosis is associated with systemic inflammation, endotoxemia and poor outcomes, but is a potential modifiable factor. Modulation could be done at a compositional or a functional level. Our goals of therapy in cirrhosis in the future could be to target the gut-liver axis to reduce mortality in cirrhosis.

References:

1. Alessandria C, Ozdogan O, Guevara M, et al. MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology* 2005;41:1282–9. [PubMed: 15834937]
2. Bajaj JS, O'Leary JG, Tandon P, et al. Hepatic Encephalopathy Is Associated With Mortality in Patients With Cirrhosis Independent of Other Extrahepatic Organ Failures. *Clin Gastroenterol Hepatol* 2017;15:565–574 e4. [PubMed: 27720916]
3. Baquero F, Nombela C. The microbiome as a human organ. *Clin Microbiol Infect* 2012;18 Suppl 4:2–4.
4. Peng L, Li ZR, Green RS, et al. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr* 2009;139:1619–25. [PubMed: 19625695]
5. Rios-Covian D, Ruas-Madiedo P, Margolles A, et al. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Front Microbiol* 2016;7:185. [PubMed: 26925050]
6. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* 2006;47:241–59. [PubMed: 16299351]
7. Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008;28:26–42. [PubMed: 18293275]
8. Bajaj JS, Heuman DM, Hylemon PB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940–7. [PubMed: 24374295]
9. Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59–64. [PubMed: 25079328]
10. Begley M, Gahan CG, Hill C. The interaction between bacteria and bile. *FEMS Microbiol Rev* 2005;29:625–51. [PubMed: 16102595]
11. Vlahcevic ZR, Buhac I, Bell CC, Jr, et al. Abnormal metabolism of secondary bile acids in patients with cirrhosis. *Gut* 1970;11:420–2. [PubMed: 5428044]
12. Swann JR, Want EJ, Geier FM, et al. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc Natl Acad Sci U S A* 2011;108 Suppl 1:4523–30. [PubMed: 20837534]
13. Bode C, Bode JC. Effect of alcohol consumption on the gut. *Best Pract Res Clin Gastroenterol* 2003;17:575–92. [PubMed: 12828956]
14. Wigg AJ, Roberts-Thomson IC, Dymock RB, et al. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2001;48:206–11. [PubMed: 11156641]
15. Pande C, Kumar A, Sarin SK. Small-intestinal bacterial overgrowth in cirrhosis is related to the severity of liver disease. *Aliment Pharmacol Ther* 2009;29:1273–81. [PubMed: 19302262]
16. Bauer TM, Steinbruckner B, Brinkmann FE, et al. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. *Am J Gastroenterol* 2001;96:2962–7. [PubMed: 11693333]
17. Boursier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764–75. [PubMed: 26600078]
18. Loomba R, Seguritan V, Li W, et al. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2017;25:1054–1062 e5. [PubMed: 28467925]
19. Leclercq S, Cani PD, Neyrinck AM, et al. Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. *Brain Behav Immun* 2012;26:911–8. [PubMed: 22521198]
20. Bala S, Marcos M, Gattu A, et al. Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. *PLoS One* 2014;9:e96864. [PubMed: 24828436]
21. Mutlu EA, Gillevet PM, Rangwala H, et al. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G966–78. [PubMed: 22241860]

22. Leclercq S, Matamoros S, Cani PD, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc Natl Acad Sci U S A* 2014;111:E4485–93. [PubMed: 25288760]
23. Frances R, Zapater P, Gonzalez-Navajas JM, et al. Bacterial DNA in patients with cirrhosis and noninfected ascites mimics the soluble immune response established in patients with spontaneous bacterial peritonitis. *Hepatology* 2008;47:978–85. [PubMed: 18306221]
24. Zapater P, Frances R, Gonzalez-Navajas JM, et al. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis. *Hepatology* 2008;48:1924–31. [PubMed: 19003911]
25. Bajaj JS, Hylemon PB, Ridlon JM, 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* 2012;303:G675–85. [PubMed: 22821944]
26. Tandon P, Garcia-Tsao G. Renal dysfunction is the most important independent predictor of mortality in cirrhotic patients with spontaneous bacterial peritonitis. *Clin Gastroenterol Hepatol* 2011;9:260–5. [PubMed: 21145427]
27. Angeli P, Guarda S, Fasolato S, et al. Switch therapy with ciprofloxacin vs. intravenous ceftazidime in the treatment of spontaneous bacterial peritonitis in patients with cirrhosis: similar efficacy at lower cost. *Aliment Pharmacol Ther* 2006;23:75–84. [PubMed: 16393283]
28. Arvaniti V, D'Amico G, Fede G, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010;139:1246–56, 1256 e1-5. [PubMed: 20558165]
29. Bajaj JS, O'Leary JG, Reddy KR, et al. Survival in infection-related acute-on-chronic liver failure is defined by extrahepatic organ failures. *Hepatology* 2014;60:250–6. [PubMed: 24677131]
30. Bajaj JS, Ridlon JM, Hylemon PB, et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G168–75. [PubMed: 21940902]
31. Bajaj JS. Review article: potential mechanisms of action of rifaximin in the management of hepatic encephalopathy and other complications of cirrhosis. *Aliment Pharmacol Ther* 2016;43 Suppl 1:11–26. [PubMed: 26618922]
32. Bass NM, Mullen KD, Sanyal A, et al. Rifaximin treatment in hepatic encephalopathy. *N Engl J Med* 2010;362:1071–81. [PubMed: 20335583]
33. Bajaj JS, Heuman DM, Hylemon PB, et al. Randomised clinical trial: Lactobacillus GG modulates gut microbiome, metabolome and endotoxemia in patients with cirrhosis. *Aliment Pharmacol Ther* 2014;39:1113–25. [PubMed: 24628464]
34. Dhiman RK, Rana B, Agrawal S, et al. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* 2014;147:1327–37 e3. [PubMed: 25450083]
35. Hoefert B. Über Bakterienbefunde im Duodenalsaft von Gesunden und Kranken. 1921.
36. Floch MH, Katz J, Conn HO. Qualitative and quantitative relationships of the fecal flora in cirrhotic patients with portal systemic encephalopathy and following portacaval anastomosis. *Gastroenterology* 1970;59:70–5. [PubMed: 4913353]
37. Bauer TM, Schwacha H, Steinbrückner B, et al. Small intestinal bacterial overgrowth in human cirrhosis is associated with systemic endotoxemia. *Am J Gastroenterol* 2002;97:2364–70. [PubMed: 12358257]
38. Cirera I, Bauer TM, Navasa M, et al. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001;34:32–7. [PubMed: 11211904]
39. Fernandez J, Navasa M, Gomez J, et al. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002;35:140–8. [PubMed: 11786970]
40. Riggio O, Varriale M, Testore GP, et al. Effect of lactitol and lactulose administration on the fecal flora in cirrhotic patients. *J Clin Gastroenterol* 1990;12:433–6. [PubMed: 2398251]
41. Tarao K, Tamai S, Ito Y, et al. [Effects of lactitol on fecal bacterial flora in patients with liver cirrhosis and hepatic encephalopathy]. *Nihon Shokakibyo Gakkai Zasshi* 1995;92:1037–50. [PubMed: 7643458]

42. Liu Q, Duan ZP, Ha DK, et al. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004;39:1441–9. [PubMed: 15122774]
43. Shawcross D, Jalan R. The pathophysiologic basis of hepatic encephalopathy: central role for ammonia and inflammation. *Cell Mol Life Sci* 2005;62:2295–304. [PubMed: 16158192]
44. Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol* 2014;61:1385–96. [PubMed: 25135860]
45. Jenne CN, Kubes P. Immune surveillance by the liver. *Nat Immunol* 2013;14:996–1006. [PubMed: 24048121]
46. Du Plessis J, Vanheel H, Janssen CE, et al. Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* 2013;58:1125–32. [PubMed: 23402745]
47. Rajkovic IA, Williams R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity in alcoholic cirrhosis and hepatitis. *Hepatology* 1986;6:252–62. [PubMed: 3007318]
48. Shawcross DL, Wright GA, Stadlbauer V, et al. Ammonia impairs neutrophil phagocytic function in liver disease. *Hepatology* 2008;48:1202–12. [PubMed: 18697192]
49. Seidler S, Zimmermann HW, Weiskirchen R, et al. Elevated circulating soluble interleukin-2 receptor in patients with chronic liver diseases is associated with non-classical monocytes. *BMC Gastroenterol* 2012;12:38. [PubMed: 22530792]
50. Laso FJ, Madruga JI, Lopez A, et al. Distribution of peripheral blood lymphoid subsets in alcoholic liver cirrhosis: influence of ethanol intake. *Alcohol Clin Exp Res* 1996;20:1564–8. [PubMed: 8986204]
51. Doi H, Iyer TK, Carpenter E, et al. Dysfunctional B-cell activation in cirrhosis resulting from hepatitis C infection associated with disappearance of CD27-positive B-cell population. *Hepatology* 2012;55:709–19. [PubMed: 21932384]
52. Lario M, Munoz L, Ubeda M, et al. Defective thymopoiesis and poor peripheral homeostatic replenishment of T-helper cells cause T-cell lymphopenia in cirrhosis. *J Hepatol* 2013;59:723–30. [PubMed: 23742913]
53. Albillos A, Hera Ad Ade L, Reyes E, et al. Tumour necrosis factor- α expression by activated monocytes and altered T-cell homeostasis in ascitic alcoholic cirrhosis: amelioration with norfloxacin. *J Hepatol* 2004;40:624–31. [PubMed: 15030978]
54. Laso FJ, Madruga JI, Giron JA, et al. Decreased natural killer cytotoxic activity in chronic alcoholism is associated with alcohol liver disease but not active ethanol consumption. *Hepatology* 1997;25:1096–100. [PubMed: 9141423]
55. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;464:59–65. [PubMed: 20203603]
56. Axelson M, Sjoval J. Potential bile acid precursors in plasma--possible indicators of biosynthetic pathways to cholic and chenodeoxycholic acids in man. *J Steroid Biochem* 1990;36:631–40. [PubMed: 2214780]
57. Kakiyama G, Pandak WM, Gillevet PM, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* 2013;58:949–55. [PubMed: 23333527]
58. Bajaj JS, Cox IJ, Betrapally NS, et al. Systems biology analysis of omeprazole therapy in cirrhosis demonstrates significant shifts in gut microbiota composition and function. *Am J Physiol Gastrointest Liver Physiol* 2014;307:G951–7. [PubMed: 25258407]
59. Chen Y, Qin N, Guo J, et al. Functional gene arrays-based analysis of fecal microbiomes in patients with liver cirrhosis. *BMC Genomics* 2014;15:753. [PubMed: 25179593]
60. Bajaj JS, Betrapally NS, Hylemon PB, et al. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* 2015;62:1260–71. [PubMed: 25820757]
61. Bajaj JS, Betrapally NS, Hylemon PB, et al. Gut Microbiota Alterations can predict Hospitalizations in Cirrhosis Independent of Diabetes Mellitus *Sci Rep* 2015;5:18559. [PubMed: 26692421]
62. Jepsen P, Ott P, Andersen PK, et al. Clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. *Hepatology* 2010;51:1675–82. [PubMed: 20186844]

63. Cordoba J, Ventura-Cots M, Simon-Talero M, et al. Characteristics, risk factors, and mortality of cirrhotic patients hospitalized for hepatic encephalopathy with and without acute-on-chronic liver failure (ACLF). *J Hepatol* 2014;60:275–81. [PubMed: 24128414]
64. Chen Y, Guo J, Qian G, et al. Gut dysbiosis in acute-on-chronic liver failure and its predictive value for mortality. *J Gastroenterol Hepatol* 2015;30:1429–37. [PubMed: 25711972]
65. Bajaj JS, Thacker LR, Fagan A, et al. Gut microbial RNA and DNA analysis predicts hospitalizations in cirrhosis. *JCI Insight* 2018;3.
66. Esmailzadeh A, Kimiagar M, Mehrabi Y, et al. Dietary patterns and markers of systemic inflammation among Iranian women. *J Nutr* 2007;137:992–8. [PubMed: 17374666]
67. Bullo M, Casas-Agustench P, Amigo-Correig P, et al. Inflammation, obesity and comorbidities: the role of diet. *Public Health Nutr* 2007;10:1164–72.
68. Vanhoutte T, De Preter V, De Brandt E, et al. Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and *Saccharomyces boulardii*. *Appl Environ Microbiol* 2006;72:5990–7. [PubMed: 16957220]
69. Bajaj JS, Heuman DM, Sanyal AJ, et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One* 2013;8:e60042. [PubMed: 23565181]
70. Bajaj JS, Fagan A, Sikaroodi M, et al. Liver Transplant Modulates Gut Microbial Dysbiosis and Cognitive Function in Cirrhosis. *Liver Transpl* 2017.
71. Bajaj JS, Kakiyama G, Cox JJ, et al. Alterations in Gut Microbial Function Following Liver Transplant. *Liver Transpl* 2018.
72. Kao D, Roach B, Park H, et al. Fecal microbiota transplantation in the management of hepatic encephalopathy. *Hepatology* 2016;63:339–40. [PubMed: 26264779]
73. Bajaj JS, Kassam Z, Fagan A, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology* 2017;66:1727–1738. [PubMed: 28586116]
74. Bajaj JS, Kakiyama G, Savidge T, et al. Antibiotic-Associated Disruption of Microbiota Composition and Function in Cirrhosis is Restored by Fecal Transplant. *Hepatology* 2018.
75. Nagao Y, Kawahigashi Y, Sata M. Association of Periodontal Diseases and Liver Fibrosis in Patients With HCV and/or HBV infection. *Hepat Mon* 2014;14:e23264. [PubMed: 25737729]
76. Silva Santos PS, Fernandes KS, Gallottini MH. Assessment and management of oral health in liver transplant candidates. *J Appl Oral Sci* 2012;20:241–5. [PubMed: 22666844]
77. Gronkjaer LL. Periodontal disease and liver cirrhosis: A systematic review. *SAGE Open Med* 2015;3:2050312115601122. [PubMed: 26770799]
78. Gronkjaer LL, Holmstrup P, Schou S, et al. Presence and consequence of tooth periapical radiolucency in patients with cirrhosis. *Hepat Med* 2016;8:97–103. [PubMed: 27695370]
79. Aberg F, Helenius-Hietala J, Meurman J, et al. Association between dental infections and the clinical course of chronic liver disease. *Hepatol Res* 2014;44:349–53. [PubMed: 23607641]
80. Bert F, Noussair L, Lambert-Zechovsky N, et al. Viridans group streptococci: an underestimated cause of spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Eur J Gastroenterol Hepatol* 2005;17:929–33. [PubMed: 16093870]
81. Cholongitas E, Papatheodoridis GV, Lahanas A, et al. Increasing frequency of Gram-positive bacteria in spontaneous bacterial peritonitis. *Liver Int* 2005;25:57–61. [PubMed: 15698399]
82. Bajaj JS, O'Leary JG, Reddy KR, et al. Second infections independently increase mortality in hospitalized patients with cirrhosis: the North American consortium for the study of end-stage liver disease (NACSELD) experience. *Hepatology* 2012;56:2328–35. [PubMed: 22806618]
83. Bajaj JS, Fagan A, Gavis E, et al. Tu1512 - Proton Pump Inhibitor Initiation and Withdrawal can Modulate Gut Microbiota in Patients with Decompensated Cirrhosis. *Gastroenterology* 2018;154:S-948–S-949.
84. Yang AM, Inamine T, Hochrath K, et al. Intestinal fungi contribute to development of alcoholic liver disease. *J Clin Invest* 2017;127:2829–2841. [PubMed: 28530644]
85. Bajaj JS, Liu EJ, Kheradman R, et al. Fungal dysbiosis in cirrhosis. *Gut* 2017.

86. Bajaj JS, Rajender Reddy K, Tandon P, et al. Prediction of Fungal Infection Development and Their Impact on Survival Using the NACSELD Cohort. *Am J Gastroenterol* 2018;113:556–563. [PubMed: 29257141]
87. Claesson MJ, Cusack S, O’Sullivan O, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci U S A* 2011;108 Suppl 1:4586–91. [PubMed: 20571116]
88. Chen Y, Yang F, Lu H, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011;54:562–72. [PubMed: 21574172]
89. Zhang Z, Zhai H, Geng J, et al. Large-scale survey of gut microbiota associated with MHE Via 16S rRNA-based pyrosequencing. *Am J Gastroenterol* 2013;108:1601–11. [PubMed: 23877352]
90. Bajaj JS, Betrapally NS, Gillevet PM. Decompensated cirrhosis and microbiome interpretation. *Nature* 2015;525:E1–2. [PubMed: 26381988]
91. Ahluwalia V, Betrapally NS, Hylemon PB, et al. Impaired Gut-Liver-Brain Axis in Patients with Cirrhosis. *Sci Rep* 2016;6:26800. [PubMed: 27225869]

Figure 1:
Pathway for specimen processing and bioinformatics analysis for 16S and metagenomic analysis for microbiota

Figure 2:

Multiple levels of immune dysfunction in cirrhosis, which are complicit in allowing alteration of microbiota in the gut and elsewhere. GALT: Gastrointestinal associated lymphoid tissue, SIBO: small intestinal bacterial overgrowth, BA: bile acids, RES: reticulo-endothelial system

Figure 3: Schematic of changes of key features with progression from healthy to acute-on-chronic liver failure and resolution after liver transplant. ACLF: acute on chronic liver failure, PPI: proton pump inhibitors

Table 1:

Studies of the gut microbiome in human cirrhosis.

Study	Samples/ Groups compared	Findings	Significance
Chen et al 2011 ⁸⁸	Stool/ cirrhosis (36) vs controls (24)	-Bacteroidetes reduced whereas Proteobacteria increased in cirrhosis - <i>Streptococcaceae</i> correlated positively with CTP while <i>Lachnospiraceae</i> negatively	First major study to show widespread dysbiosis in cirrhosis. First to show reduction in autochthonous taxa and increase in pathogenic ones
Bajaj et al 2012 ³⁰	Stool/Cirrhosis (25) with 17 with OHE vs controls (10)	- Increased Bacteroidetes and reduced autochthonous taxa in cirrhosis. - Increased <i>Veillonellaceae</i> in OHE compared to No OHE as only significant difference. - <i>Enterobacteriaceae</i> positively correlated with MELD and <i>Ruminococcaceae</i> negatively. - <i>Porphyromonadaceae</i> correlated with poor cognition. - <i>Enterobacteriaceae</i> and <i>Veillonellaceae</i> associated positively with inflammation and endotoxemia where as <i>Ruminococcaceae</i> negatively	-First major study to assess the correlation between dysbiosis, systemic inflammation, endotoxemia and OHE in cirrhosis. -Showed that certain families predominated during decompensation and associated with inflammation and poor cognition. -Stool microbiome between OHE and No-OHE was not very different. -Showed that lactulose had minimal effect on the composition of the stool microbiota.
Mutlu et al 2012 ²¹	Sigmoid mucosa/ Alcoholics with no liver disease (28) vs alcoholic cirrhosis (19) vs control (18)	-Median abundance of Bacteroidetes reduced and Proteobacteria increased in alcoholics. -Increased endotoxin levels in alcoholics	-Sigmoid mucosa exhibits dysbiosis in cirrhosis. -Dysbiosis and endotoxemia occurs in alcoholic liver disease irrespective of cirrhosis. -This is a long- term phenomenon not influenced by sobriety.
Bajaj et al 2012 ²⁵	Stool + sigmoid mucosa/ Cirrhosis (60) with 36 with OHE vs controls (17)	-Increased Firmicutes noted in Stool and Sigmoid mucosa. - Higher abundance in mucosa of members of <i>Enterococcus</i> , <i>Veillonella</i> , <i>Megasphaera</i> , <i>Bifidobacterium</i> , and <i>Burkholderia</i> in the HE patients. - <i>Enterococcus</i> , <i>Megasphaera</i> , and <i>Burkholderia</i> linked to poor cognition and inflammation.	-Sigmoid mucosal microbiome differs in cirrhotics between those with HE and No HE in terms of specific genera of Firmicutes. - Colonic mucosa of HE patients is associated with the proinflammatory state, endothelial activation, and poor cognition.
Zhang et al 2013 ⁸⁹	Stool/ Controls (26) vs MHE (26) vs No MHE (25) cirrhosis	- Higher abundance of <i>Streptococcus salivarius</i> in MHE. - <i>Veillonellaceae</i> and <i>Streptococcaceae</i> not different between cirrhosis with and without MHE, though different between Cirrhosis and controls. - <i>Streptococcus salivarius</i> correlated negatively with cognitive function	- <i>Streptococcus salivarius</i> a urease producing bacteria may be implicated in MHE
Qin et al 2014 ⁹	Stool/cirrhosis (98) vs controls (83)	-Reduced Bacteroidetes with increased Proteobacteria at the phylum level. - Increased <i>Veillonella</i> and <i>Streptococcus</i> species in the cirrhosis group compared to controls.	-Major study that created a general catalog for microbiota in cirrhosis. -Oral-origin bacteria found in higher relative abundance in cirrhotic patients
Bajaj et al 2014 ⁵⁸	Stool/ Compensated cirrhosis (15) vs controls (15)	- significant increase in the relative abundance of <i>Streptococcaceae</i> after PPI therapy in all groups. - No significant change in relative abundances of other major families in patients with cirrhosis before/ after PPI	-PPI therapy increases relative abundance of stool <i>Streptococcaceae</i> . - PPI therapy induces a functional microbiota change. -Hypothesized that salivary microbiota in stool is an epiphenomenon secondary to PPI use.
Bajaj et al 2014 ⁸	Stool/Cirrhosis (244, 98 decompensated) vs controls (25)	Increased pathogenic bacteria (<i>Enterobacteriaceae</i>) with no stable autochthonous taxa in OHE. - ACLF associated with increased abundance of <i>Propionibacteriaceae</i> and <i>Halomonadaceae</i> but reduced <i>Lachnospiraceae</i> and <i>Veillonellaceae</i> - CDR lowest in alcoholic cirrhosis. - CDR lower in decompensated cirrhosis with infections.	-Proposed the CDR as a tool for prediction of outcomes. - Lower CDR indicating worse dysbiosis and poorer prognosis. -Profiled dysbiosis in ACLF.

Study	Samples/ Groups compared	Findings	Significance
Bajaj et al 2014 ⁹⁰	Reanalysis of Qin et al	Several oral origin species (<i>Streptococcus oralis</i> / <i>veillonella sp.</i>) were only found in decompensated patients.	Phenomenon of distal migration only in advanced cirrhosis.
Kakiyama et al 2014 ⁵⁷	Stool/ cirrhosis (47) vs controls (14)	-Cirrhotics, had a significantly higher abundance of <i>Enterobacteriaceae</i> but lower <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> and <i>Blautia</i> (7 α - dehydroxylating bacteria). - <i>Enterobacteriaceae</i> correlated positively with CDCA and <i>Ruminococcaceae</i> with DCA. -Rifaximin resulted in significant reduction in secondary/primary bile acids and LCA/CDCA ratios	-Potentially pathogenic genera correlated with primary BAs. - Cirrhosis is associated with a decrease in total fecal BA concentration and a reduced secondary BA conversion - Change in BAs is modulated by certain key microbiota,
Chen et al 2015 ⁶⁴	Stool/cirrhosis (42) vs controls (50)	-ACLF associated with significantly lower abundance of <i>Bacteroidaceae</i> , <i>Ruminococcaceae</i> , and <i>Lachnospiraceae</i> , but higher <i>Pasteurellaceae</i> , <i>Veillonellaceae</i> , <i>Streptococcaceae</i> , and <i>Enterococcaceae</i> . - lower abundance of <i>Lachnospiraceae</i> in HE. -The relative abundance of <i>Pasteurellaceae</i> predicted mortality.	- Gut dysbiosis is associated with mortality in ACLF.
Bajaj et al 2015 ⁶⁰	Stool and salivary microbiota/cirrhosis (102, 43 HE) vs controls (32)	-Higher abundance of pathogenic taxa (<i>Enterobacteriaceae</i> , <i>Enterococcaceae</i>), with reduction of autochthonous taxa in HE, in salivary microbiome. - Significantly higher IL-6/IL-1 β , secretory IgA with lower lysozyme, and histatins 1 and 5 in cirrhosis. - Predicted functionality for salivary microbiome in cirrhosis was for endotoxin and endotoxin protein synthesis.	-Salivary dysbiosis widespread in cirrhosis and follows stool dysbiosis patterns -Salivary dysbiosis ratio predicts 90-day readmission.
Ahulwalia et al 2016 ⁹¹	Stool/cirrhosis (147) vs controls (40)	-Functional shift in microbiome towards ammoniagenic amino acid profile and predicted functionality of endotoxin and endotoxin protein synthesis in cirrhosis. - The family <i>Porphyromonadaceae</i> was linked with all aspects of DTI -Autochthonous taxa correlated negatively with MRS. -Pathogenic taxa correlated positively with increased Glx and lower ml and Cho, in controls and cirrhotic patients i.e Hyperammonemia related brain changes.	-Specific bacterial taxa (pathogenic ones more commonly) associate with rain MRI changes (astrocytic and neuronal)

OHE-overt hepatic encephalopathy; PPI-proton pump inhibitors; CTP- Child-Turcott-Pugh; MELD-model for end stage liver disease; MHE-minimal hepatic encephalopathy; CDR-cirrhosis dysbiosis ration; ACLF- acute-on-chronic liver failure; BA-bile acids; CDCA-chenodeoxycholic acid; DCA-Deoxycholic acid; LCA-lithocholic acid; DTI-Diffusion tensor imaging; MRS-Magnetic resonance spectroscopy; Glx-Glutamine/ glutamate ratio; mi-myoinositol; Cho-choline

Table 2:**Future Directions of Microbiota Research in Liver Disease**

Focus	Potential Investigations
Diagnosis of advanced fibrosis	Functional and composition of microbiota to differentiate between patients with and without advanced fibrosis
Functional characterization of microbiota	Greater integration of multiple platforms, phenotype, metabolomics, metatranscriptomics, metaproteomics and microbiota in the characterization of microbial functions in human liver disease
Non-bacterial microbiota analysis	Fungal, archaeal and viral, including bacteriophages and their interactions with bacteria in differing stages of liver disease
Sources of microbially-mediated inflammation	Define sources other than the gut, including mouth or other parts of the body that could potentiate systemic inflammation in patients with liver disease
Diet and cultural habits on microbiota composition	Effect of dietary and cultural practices on microbiota and their modulation of outcomes in liver disease
Prognostication using microbial composition and functionality	Greater integration of microbiota in clinical decision- making in human liver disease
Precision microbial therapies	Specific microbial “cocktails” directed towards differing etiologies as well as stages of liver disease.