Microbiome

Emerging Concepts in Patients with Chronic Liver Disease

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KEYWORDS

• Outcomes • Fecal transplant • Cirrhosis • Hepatic encephalopathy

KEY POINTS

- The gut microbiome is a major research focus in chronic liver disease owing to alterations in gut–liver and gut–brain axes.
- Changes in microbiota structure and function across disease stages can be analyzed in differing samples using techniques that vary in depth of sequencing and cost.
- There are consistent microbiota functional changes (bile acids, endotoxin, short chain fatty acids) and composition changes as liver disease progresses and patients develop cirrhosis and complications.
- Alteration in the microbiota with therapies for hepatic encephalopathy, diet, periodontal therapy, and fecal transplant can help in selected patients with chronic liver disease.

INTRODUCTION

Cirrhosis and liver cancer account for 3.5% of all deaths worldwide, and an estimated 50 million adults are affected with chronic liver disease.[1,2] In addition to mortality, chronic liver diseases carry a significant economic impact and low quality of life.[3]

GUT MICROBIOME

It is first important to distinguish between the human microbiota and the microbiome. The microbiota is the overall collection of microbes within the body including bacteria, archaea, fungi, microbial eukaryotes, and viruses and phages.[4] In total the microbiota consists of up to 100 trillion cells.[5] The microbiome is a term for a specific collection of microbes and their genes that exist within a specific system in the body (like the gut).[6] Although the gut microbiome has been studied and linked to many diseases, this review specifically focuses on its link to chronic liver

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disease. Specifically, the gut microbiome has been shown to influence nonalcoholic steatohepatitis, nonalcoholic fatty liver disease, alcoholic hepatitis, primary sclerosing cholangitis, cirrhosis, and hepatocellular carcinoma (HCC).[6] The healthy human gut microbiome contains an abundance of bacteria with only a small minority of nonbacterial microbes.[4] Although there is considerable variation of gut microbiome composition between even healthy individuals, the majority of bacteria are members of the phyla Bacteroidetes and Firmicutes with the combined percentage of approximately 95%.[7] Other phyla present at lower levels are Actinobacteria, Fusobacteria, Verrucomicrobia, and Proteobacteria, and facultative anaerobes 6. When functioning properly, the autochthonous taxa and nonautochthonous taxa are responsible for a wide variety of functions, including production of short chain fatty acids for gut barrier integrity and colonocyte nutrients,[8] secondary bile acid synthesis,[9] and protection against pathogens.[10] Dysbiosis is the term used to describe the alteration of a patient's normal microbiome that can result in disadvantageous changes to physiologic functions. In dysbiosis, the balance in gut microdiversity changes as beneficial microbes (symbionts) decrease and harmful (pathobionts) increase.[6] When dysbiosis occurs in cirrhosis, there is a propagation of the disease and an increase in complications.[8]

Microbiome Sample Collection for Analysis

There is no perfect answer to this question owing to differences in studies that vary in depth and collection practices. Considerations include feasibility, cost, and how the subsequent analysis of the sample will be performed. Stool is the most commonly collected and accessible material. The disadvantage with stool is that it does not capture all gut microbes, especially ones that adhere well to the mucosa and small intestine microbes.[11,12] The typical protocol for stool sampling is to collect the whole stool, homogenize it as soon as possible, then flash freeze it, with an aliquot preserved in 20% glycerol in Lysogeny broth for culturing.[4] If RNA analysis is planned the sample should be placed in an RNA later solution for nucleic acid protection. Once collected the samples can be analyzed for bacterial RNA or DNA. There are a variety of microbiome analysis techniques depending on the goal of the study (**Table 1**).

Data Analysis

The choice for data comparison depends on the question that needs to be answered. Initially the raw DNA sequence data needs to be to organized into a table/chart showing how many of each species, gene, or strain is seen per sample. Analysis is then performed at the whole microbiome level and the individual taxa and genes level.[4] Whole microbiome analysis uses alpha and beta diversity. Alpha diversity shows a number of different types of microbial taxa within a group.[18] Beta diversity shows differences in diversity between groups. Individual taxa differences discriminant analysis effect size or by nonparametric tests. Tests of function are separated into direct and indirect testing. Indirect analysis shows gene expressions based on metagenomic data, whereas direct tests are functional correlates of microbial function (endotoxemia, secondary bile acid production, etc).[18] It is important to remember that different methods provide different results, even with using the sample or raw DNA.[4] Owing to this factor, there is not a large clinical role for these techniques at this time. Pathogen diagnosis should still rely on traditional cultures and assay (polymerase chain reaction vs antibody).[4] Finally, these data are linked to relevant clinical variables in order for an analysis to occur.

Table 1
Microbiome analysis techniques

Type	Overview	Strengths	Weaknesses	Microbes Studied	Throughput, Time, and Cost
Culture	Classical system of isolating and growing specific microbes on specific medias under aerobic conditions	The most sensitive detection method for organisms with well-characterized selective culture conditions Can use multiple sample types (stool, blood, skin) Helpful to detect the absolute abundance of viable organisms, antibiotic sensitivities/ resistances, and phenotypic classification[13]	Limited scope of which microbes can be successfully cultured Not helpful for majority of anaerobic gut microbiome	Bacteria Fungi Archaea Viruses	Low throughput One sample per media used 24–48 h $
Assay/ PCR panels Examples: qPCR and RT-PCR[14]	Target a set of known bacteria, viruses, parasites, or functional genes Samples (stool) go through nucleic acid extraction followed by complementary DNA synthesis and amplification The end result (genomic DNA vs PCR product) is then qualified and	Provides absolute abundance of each taxon per gram or milliliter of input material Has a high dynamic range	Panels are only targeted so they will miss undiscovered gut taxa	Viruses Some other selective organisms pending the panel used	Low throughput 1–24 samples[15] 1–5 h[15] $$

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Table 1
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Type	Overview	Strengths	Weaknesses	Microbes Studied	Throughput, Time, and Cost
	quantified using the panel				
Metataxonomics/ amplicon sequencing (16S rRNA gene sequencing)	Samples undergo extraction of nuclear material then PCR amplification is done using gene matched primers (usually the 16S rRNA for bacteria and archaea) This allows for amplifications of all variants bookended by the primers, hypervariable gene sequences are targeted Samples are then compared with large databases of microbial profiles and additional bioinformatic analysis is done based on clinical question	Assessment of microbiome diversity and composition at the genus level Can be used to assess functional changes Relatively cheaper than alterative techniques	Difficult to apply to viruses owing to there being no common viral gene[5] Each genus has a wide range of strains that are genomically distinct, which cannot be adequately appreciated using this method Can typically only go as far as the genus level[4] Bacteria have different numbers copies of 16S rRNA gene, influences relative abundance[4]	16S (bacteria, some archaea) 18S (eukaryotes) ITS (fungi)	High throughput 384 samples per run 48 h $$

Shotgun metagenomics	Untargeted DNA sequencing of the whole genome All DNA from a sample is broken down into fragments These fragments are then sequenced, then software attempts to combine the fragments into a view of the whole microbiome[16]	Informs composition including species and strain Gives functional insight Gives a complete list of microbial strains present in the microbiome and how abundant each strain is[5]	Considerable technical challenges All DNA will be sequenced, including human DNA (not a good option for biopsy specimens and required human DNA analysis consent)	All Organisms including host	High throughput 384 samples per run 48 h $$$
Metaproteomics (protein), metatranscriptomics (RNA)	Metaproteomics: uses mass spectrometry to sort out the wide range of proteins in a sample[5] Metatransciptiomics: sequencing of microbial RNA	Very broad: this includes all protein or RNA made by all the organisms present Can be used to assess functional changes and can read gene expression	Lacks a link to specific organisms Most bacterial transcripts only last a few minutes[17] Poor correlation between gene expression and actual proteins in the gut	RNA viruses and all organisms including host	High throughput 96–384 samples per run 48 h $$$$
Metabolomics (targeted vs nontargeted)	Study of the nonprotein small molecules including products of metabolism[5] Metabolic responses of an individual or population	Relates directly to the function of the community	Limited list of discovered targeted molecules Difficult to annotate untargeted metabolomics	All organisms including host	High throughput 96 samples per run 48 h $$$

Abbreviations: PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction.

LIVER DISEASES AND THE MICROBIOME

The gut, the intestinal microbiota, and the liver are uniquely matched to have a bidirectional relationship. The liver receives 75% of its blood supply via the portal vein from the intestines, and the liver releases bile acids into the biliary tract to the intestine.[19] Major mechanisms in which the intestinal microbiota effects the liver include bile acid metabolism, intestinal permeability, chronic inflammation, immune system activation, short chain fatty acids, choline, and ethanol.[20] The etiology of the dysbiosis associated with chronic liver disease remains unknown, but there are some working theories proposed. The first is that in chronic liver disease there is a decreased production of bile acids and thus less reaches the duodenum. This is important owing to the antimicrobial properties of bile acids. Bile acids have a detergent action, making them toxic to bacteria.[21] Bile acids also have an effect on the intestinal mucosa, influencing the production of peptides critical for bacterial control.[22,23] These changes allow for an environment suspectable to the development of small bacterial intestinal overgrowth. This factor leads to an increased quantity of bacteria, functional bacteria changes, and an increased intestinal permeability.[24] Cirrhosis microbiome composition has shown a wide amount of study to study variability. In a typical dysbiosis pattern, potentially pathogenic bacteria (Enterobacteriaceae Veillonellaceae, and Streptococcaceae) increase and beneficial bacteria (Proteobacteria and Fusobacteria) decrease.[25] The cirrhosis dysbiosis ratio tool was designed to estimate dysbiosis in cirrhotics.[8] This study showed a worsening in cirrhosis dysbiosis ratio in the setting of disease progression. There has been significant work done to increase the understanding of the gut microbiome in relation to specific etiologies of liver disease (**Table 2**).

CIRRHOSIS COMPLICATIONS AND HOW MICROBES MAY BE RELATED

Hepatic Encephalopathy

The gut microbiota most likely has a strong link to the pathophysiology of hepatic encephalopathy (HE), specifically endotoxemia.[53] Intestinal microbiota studies have shown a decrease in *Lachnospiraceae* and *Ruminococcacae* and an increase *in Enterobacteriaceae*, *Streptococcaceae*, and *Porphyromonadaceae* associated with HE. Specifically, *Lachnospiraceae* and *Ruminococcaceae* negatively correlated, whereas *Enterobactericeae* positively correlated with ammonia-associated astrocyte swelling.[54] White matter changes on brain MRI were positively associated with *Porphyromonadaceae.*[54] Another study showed a positive correlation with cognitive impairment with *Alcaligenaceae* and *Porphyromonadaceae*, versus *Prevotella*, which was linked to improvement in cognition and decreased inflammation.[53] Studies have shown that evaluation of the intestinal microbiota can help to predict overt HE development in cirrhotic inpatients.[55] Specifically, this patient population has higher endoxemia, lower cirrhosis dysbiosis ratios, and increased levels of *Enterobacteriaceae*.[55] This study initially looked at changes on admission for cirrhotic patients, whereas another study also showed that patients with overt HE have distinct changes in their microbiota during hospital stays, and these changes have the ability to predict HE recurrence.[56] There is an increased percentage of urease active bacteria in patients with cirrhosis, specifically *Streptococcaceae*.[57] These changes are thought to lead to increased ammonia production and contribute to the development of HE.[58,59]

Hepatocellular Carcinoma

There has been growing evidence that dysbiosis and intestinal microbiota changes impact the development of HCC by increasing steatosis, oxidative stress, and inflammation.[60] Multiple studies have shown that there are intestinal microbiota changes

Table 2
Specific liver diseases and the gut microbiome

	Findings	Take Away Points
Alcohol-related liver disease	Studies have looked at the entire spectrum of disease up to alcoholic hepatitis[26] Chronic use of alcohol results in increased intestinal permeability, thus initial gut microbial changes are provoked by the use of alcohol itself[27] Progression through the spectrum correlates in proportion with bacterial and fungal composition Alcohol consumption itself provokes microbiome changes leading eventually to dysbiosis[28] (stool)[29] There is a proportional increase in secondary bacterial products like secondary bile acids,[30] biopsy As liver disease worsens, the correlating dysbiosis shows an unfavorable increase in Enterobacteriaceae and Enterococcaceae, both of which increase the risk of gut translocation[31] (biopsy),[32] (stool) *Bifidobacterium*, *Enterobacterium*, and Lactobacillus are all decreased in ALD[33] (stool),[25] (stool),[34] (stool),[35] while cirrhotic patients with ALD show the typical trend of lower levels of Bacteroidetes and Firmicutes phyla[30] (biopsy),[36] (biopsy)[37] Alcoholism predisposes people to small intestinal bacterial overgrowth which leads in increased risk for spontaneous bacterial peritonitis and worse severity of alcoholic cirrhosis[38] (breath test),[39] (breath test) Fortunately, studies have shown these negative changes can be reversed with alcohol cessation[40] (stool)	Possible pathway exists in which alcohol itself leads to an initial dysbiosis through increased gut permeability Once this dysbiosis is established, it affects gut permeability further, allowing for this altered microbiome to enter the portal circulation along with endotoxins Once in the portal circulation, this could trigger hepatic inflammation contributing to progression of liver fibrosis If patients stop drinking, many of these microbiome changes are reversible

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	Findings	Take Away Points
NAFLD and nonalcoholic steatohepatitis	Difficult area to study owing to overlap with other components of metabolic syndromes (DM, obesity)[41] As disease progresses studies have shown a proportional increase in Enterobacteraceae[42] (stool) Studies have shown endogenous bacteria have the ability to produce alcohol[43] (stool), this may contribute to fatty liver disease initiation and progression There appears to be differences in intestinal microbiota between nonalcoholic steatohepatitis and NAFLD patients NAFLD patients have decreased Bacteroidetes and Firmicutes, along with increased Lactobacillus[44] (stool) Bacteroidetes levels were found to be lower in nonalcoholic steatohepatitis patients in one study[45] (stool) and decreased Ruminococcus, Faecalibacterium prausnitzii, and Coprococcus in another[44] (stool) When comparing nonalcoholic steatohepatitis to NAFLD populations, there has been a link showing Bacteroides associated functionality by promote nonalcoholic steatohepatitis[46]	Very difficult area to study and interpret owing to the difficult nature of studying it independently of other components of obesity and metabolic syndromes May be a link between dysbiosis leading to bacterial byproducts production (ethanol and 3- phenylpropanoate) and disease progression Significant additional work needs to be done within this area

PBC	Decreased levels of Bacteroidetes species[47] (stool) Increased levels of Fusobacteria, *Haemophilus, Veillonella, Clostridium, Lactobacillus, Streptococcus, Pseudomonas, Klebsiella*, Enterobacteriaceae, and Proteobacteria species[47] (stool) Changes in the intestinal microbiota in PBC have been associated with increased liver injury indicators and proinflammatory cytokines This may indicate a role for altered intestinal microbiota in the development or progression of PBC itself[48] (stool) Have shown differences in patients being treated or not treated with UDCA After UDCA treatment, there was found to be decreased levels of *Haemophilus* spp, *Streptococcus* spp, and *Pseudomonas* spp and increased levels of *Bacteroidetes* spp, *Sutterella* spp, and *Oscillospira* spp[49]	Clear microbiome changes have been seen between PBC patients and controls Some early data suggest that intestinal microbiota changes may be linked to disease formation/progression Treatment with UDCA has been showed to alter the intestinal microbiota and reverse dysbiosis
Primary sclerosing cholangitis	Studies thus have shown a lot of inconsistency in changes to the intestinal microbiota with dysbiosis with different genus and species populations and relative changes[50] Multiple studies have shown that there is an abundance of Veillonella[51] (stool) Dysbiosis leads to bacterobilia, which leads to increased cholangiocyte inflammation and progression to fibrosis[52]	Conflicting data about the exact changes in dysbiosis in this population Overall thought is that dysbiosis leads to bacterobilia, which in turns leads to cholangiocyte inflammation and fibrosis

Abbreviations: ALD, alcoholic liver disease; DM, diabetes mellitus; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary sclerosis; UDCA, ursodeocycholic acid.

between cirrhotic patients and patients who develop HCC.[61–63] A recent study looked to microbial diversity as a possible noninvasive biomarker for HCC.[62] This study showed an increase in *Actinobacteria* and a decrease in *Verrucomicrobia*. In looking specifically at cirrhotic patients with nonalcoholic steatohepatitis with HCC, increased levels of *Bacteroides* and *Ruminococcaceae* and decreased levels of *Akkermansia* and *Bifidobacterium* were seen in comparison with cirrhotics who did not develop HCC.[61] Correlations with calprotectin concentrations and systemic inflammation were also seen in tandem with these microbiome changes.[61] When looking specifically at hepatitis B virus–related HCC, these patients have increased levels of proinflammatory bacteria, which was thought to result in reduced levels of anti-inflammatory short-chain fatty acids.[64] There remains a lot of questions in this area especially concerning gut translocation of specific bacteria and the role of toll-like receptors (especially toll-like receptor 4) in HCC pathogenesis.[65]

Spontaneous Bacterial Peritonitis

It is logical to assume that dysbiosis would be linked to spontaneous bacterial peritonitis in the context of all the known data concerning increased gut permeability and translocation. In patients with ascites, their serum microbiome showed higher levels of lipopolysaccharide binding protein (a biomarker for translocation). This finding was associated with a higher abundance of Clostridiales and an unknown genus belonging to the Cyanobacteria phylum.[66] These patients may have a more significant deterioration of their intestinal barrier integrity and increase rates of translocation, placing them more at risk for development of spontaneous bacterial peritonitis. In cirrhotics, there is an increase in the gram-negative taxa, specifically components of Enterobacteriaceae (the major causative organisms in the pathogenesis of spontaneous bacterial peritonitis).[67]

TREATMENTS BASED ON THE MICROBIOTA

Numerous strategies have been developed to modulate the gut microbiome. They can be delineated by lifestyle modifications versus clinical interventions. Lifestyle modifications include nutritional intervention and modification, caloric restriction, and exercise. Clinical interventions include fecal microbiota transfer, antibiotics, prebiotics, probiotics, pharmabiotics, laxatives, and bile acid/fibroblast growth factor analogues.[68]

Antibiotics

Any antibiotic that is oral or undergoes biliary excretion and enterohepatic circulation has the capability to impact the gut microbiota.[68] The obvious concern is for elimination of beneficial phyla and the expansion of harmful phyla, contributing to dysbiosis. This process can lead to antibiotic resistance, *Clostridium difficile* infection, small bowel bacterial overgrowth, and fungal overgrowth. Antibiotics have also been shown to both positively and negatively impact microbiota factors including inflammation, metabolism, and tumorigenesis.[69–71]

Owing to the harmful microbiome effects of broad-spectrum antibiotics there has been a push for more narrow-spectrum treatments which treat the target pathogen but allow the commensals unharmed.[72] Quorum sensing inhibition[73] and antitoxin drugs[74] offer promise, but there have been no significant studies looking at the use of these drugs in chronic liver disease. For this limited review, we only focus on trials in which agents that influence gut microbiota with analyses of gut microbiota composition before and after therapy (**Table 3**). Several trials that only studied microbial interventions without testing for microbiota composition were not included.

Table 3
Microbiota altering therapeutic trial in chronic liver disease

Patient Population	Study	Intervention	Microbiota Analysis After the Intervention	Conclusions
Probiotics				
Mild alcohol-induced liver injury and subgroup of mild alcoholic hepatitis	Kirpich et al,[75] 2008	5 d of *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 vs standard therapy alone (abstinence plus vitamins)	Alcoholic patients had significantly increased numbers of both bifidobacteria and lactobacilli	In the mild alcoholic hepatitis subgroup therapy associated with reduction in ALT, AST, GGT, LDH and total bilirubin Therapy showed restoration of the bowel flora and greater improvement in alcohol-induced liver injury
Cirrhosis and MHE	Bajaj et al,[76] 2014	*Lactobacillus* GG vs placebo in 30 patients with cirrhosis and MHE, followed for 8 wk	Improvement in dysbiosis (reduced Enterobacteriaceae and increased *Clostridiales incertae* Sedis XIV and *Lachnospiraceae*) and bacterial composition and function No improvement in cognition Safely tolerated	*Lactobacillus* GG is safe and can improve dysbiosis and microbial functionality on metabolomics

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Patient Population	Study	Intervention	Microbiota Analysis After the Intervention	Conclusions
HBV-induced cirrhosis with MHE	Xia et al,[77] 2018	*Clostridium butyricum* and *Bifidobacterium* infantis in MHE (n = 30) vs no treatment (n = 37) for 3 mo	*Clostridium* and Bifidobacterium increased while Enterococcus and Enterobacteriaceae decreased Cognition improved Decrease in venous ammonia Improvement in intestinal mucosal barrier	MHE in patients with HBV-induced cirrhosis improved after probiotics
Outpatients with ci rrhosis and cognitive dysfunction	Roman et al,[78] 2019	One-half of patients had fecal microbiome analysis (n = 9 probiotic group, n = 8 placebo group)	No significant changes seen at a phylum, genus, or species level	Improved cognitive function, risk of falls, and inflammatory response

Synbiotics				
NAFLD	Scorletti et al,[81] 2020	Synbiotic agents (fructo-oligosaccharides, 4 g twice per day, plus Bifidobacterium animalis subspecies lactis BB-12; n = 55) or placebo (n = 49) for 10–14 mo	Synbiotic patients had higher proportions of Bifidobacterium and Faecalibacterium species, and reductions in *Oscillibacter* and *Alistipes* species Changes in the composition of fecal microbiota were not associated with liver fat or markers of fibrosis	Treatment altered the microbiome but did not decrease liver fat content or markers of liver fibrosis
Adult outpatients with nonalcoholic steatohepatitis	Manzhalii et al,[82] 2017	Experiment group (n = 38) vs control (n = 37) Low-fat diet plus LBSF synbiotic for 12 wk (*L casei*, *L rhamnosus*, *L bulgaris*, *B longum*, and *S thermophilus* with fructooligo-saccharides)	A shift toward a more normal microbiome in the treatment group with increases in Bifdobacteria, lactobacillus, *E coli*, etc	Treatment showed improvement in liver inflammation without adverse events

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Patient Population	Study	Intervention	Microbiota Analysis After the Intervention	Conclusions
Diet				
Outpatients with cirrhosis (compensated and decompensated)	Bajaj et al,[83] 2018	United States patients (n = 157), Turkish patients (n = 139) Compared differing dietary habits on gut microbiota and clinical outcomes	The Turkish cohort had a significantly higher microbial diversity No change between controls and cirrhotics in the Turkish group In contrast, microbial diversity changed in the US-based cohort and was the lowest in decompensated patients	A diet rich in fermented milk, vegetables, cereals, coffee, and tea is associated with a higher microbial diversity Microbial diversity was associated with an independently lower risk of 90-d hospitalizations
Outpatient cirrhosis (compensated and decompensated)	Bajaj et al,[84] 2020	Compared American and Mexican diet cohorts to assess hospitalization and MHE (n = 275)	On regression, Prevotellaceae, Ruminococcaceae, and Lachnospiraceae lowered hospitalization Risk independent of MELD and ascites MHE rate was similar MELD, decompensation increased, whereas the cirrhosis dysbiosis ratio and Prevotellaceae decreased the risk of MHE	Changes in diet and microbiota, especially related to animal fat and protein intake and Prevotellaceae, are associated with MHE and hospitalizations in Mexican patients with cirrhosis compared with an American cohort

Periodontal therapy				
Cirrhotics with chronic gingivitis and/or mild or moderate period-ontitis	Bajaj et al,[86] 2018	N = 30 cirrhosis and N = 20 noncirrhotic controls, 30 d of periodontal therapy	Treatment resulted in favorable changes with higher relative abundance of autochthonous taxa (Ruminococcaceae and Lachnospiraceae) and reduction in potentially pathogenic (Enterobacteriaceae) and oral-origin taxa (Porphyromonadaceae and Streptococcaceae)	Systematic periodontal therapy in cirrhotic outpatients improved endotoxemia, as well as systemic and local inflammation, and modulated salivary and stool microbial dysbiosis
Fecal/intestinal microbiota transplantation				
PSC patients concurrent IBD	Allegretii et al,[88] 2019	Ten patients underwent a single FMT by colonoscopy Primary outcome was safety Secondary outcome was decreased ALP levels and metabonomic dynamics assessed	Diversity and similarity to donor increased in all patients after FMT, with changes seen as early as week 1 and maintained an upward trend throughout week 24	FMT in PSC is safe In addition, increases in bacterial diversity and engraftment may correlate with an improvement in ALP among patients with PSC

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Patient Population	Study	Intervention	Microbiota Analysis After the Intervention	Conclusions
ALD	Philips et al,[89] 2018	16 patients with ALD received FMT, were compared with other treatment modalities (corticosteroids, nutrition support only, and pentoxifylline)	After FMT, Actinobacteria and Proteobacteria decreased substantially with a increase in Firmicutes Persisted at day 30 and 90 after transplantation	Healthy donor FMT for SAH improves survival compared with current therapies
Severe alcoholic hepatitis	Phillips et al,[90] 2017	Eight male patients ineligible for corticosteroids given 1 wk of daily FMT	Microbiota analysis showed no difference in phyla composition of donors and recipients at baseline Firmicutes dominated in donors and recipients at 1 y, Proteobacteria reduced, and Actinobacteria increased after FMT in recipients Certain pathogenic species were also reduced after FMT at 1 year	FMT was safe and improved liver disease severity and survival at 1 y

Chronic hepatitis B	Ren et al,[91] 2017	Patients who remained persistently positive for HBeAg after > 3 y of ongoing ETV- or TDF-based antiviral therapy (FMT = 5, control = 13) End point was effect of FMT on HBV antigen titers	Monthly FMT treatment decrease HBeAg titers and 2/5 patients achieved HBeAg clearance No change in HBV surface antigen	There is a potential role for modulating gut microbiota in chronic hepatitis B treatment
Patients with cirrhosis with recurrent HE	Bajaj et al,[92] 2017	SOC (n = 10) vs FMT (n = 10) Primary outcome was safety Secondary were serious adverse events, cognition, microbiota and metabolomic changes	Eight SOC patients had 11 SAEs vs 2 FMT patients had SAEs Five SOC and no FMT patients developed further HE FMT increased diversity and beneficial taxa	FMT in HE patients is safe and reduced hospitalizations, improved cognition and dysbiosis in cirrhosis with recurrent HE

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Patient Population	Study	Intervention	Microbiota Analysis After the Intervention	Conclusions
Antibiotics				
Cirrhotic patient with refractory ascites	Lv et al,[93] 2020	Rifaximin and IV antibiotics	Rifaximin alone reduced the levels of *Roseburia*, *Haemophilus*, and *Prevotella* The combination of rifaximin and IV antibiotics resulted in a decrease in *Lachnospiraceae_noname*, *Subdoligranulum*, and *Dorea* and increase in *Coprobacillus* Gene expression of virulence factors was significantly reduced after treatment in both groups	Through microbiota alterations rifaximin may mitigate ascites and improve survival in cirrhotic patients with refractory ascites
HE and MHE therapies				
Cirrhosis	Bajaj et al,[94] 2013	Rifaximin	Small decrease in eillonellaceae and increase in Eubacteriaceae Reduction in network connectivity, specifically Enterobacteriaceae, Porphyromonadaceae, and Bacteroidaceae Increase in serum fatty acids	Rifaximin was associated with improvements in cognitive function and endotoxemia in MHE
Cirrhosis	Bajaj et al,[95] 2012	Lactulose N = 7 Men who were controlled on lactulose compared with their baseline after lactulose withdrawn over 30 d	Small decrease in Fecalibacterium and Veillonellaceae but no change in diversity There were metabolomics changes seen	Lactulose may a have important noncompositional effect on the gut microbiome

Cirrhosis	Bajaj et al,[8] 2014, Lactulose initiation	Lactulose N = 7 Compared before and after lactulose given for OHE Re-analyzed after 30 d of treatment	Enterobacteriacea increased and cirrhosis dysbiosis ration decreased after HE developed	Starting lactulose was not able to change the microbiome changes typically seen with cirrhosis progression
Cirrhosis	Sarangi et al,[96] 2017 Lactulose initiation in outpatients	Lactulose N = 21 Compared before and after lactulose was started on outpatient cirrhotics Looked at metagenomic changes and differences between patients who responded to lactulose	No change in any microbial output	Consistent with other studies with respect to resistance of change to the microbial and bacterial composition

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Patient Population	Study	Intervention	Microbiota Analysis After the Intervention	Conclusions
		and those who did not		
Cirrhosis	Wang et al,[97] 2019, multicenter study in MHE	Lactulose N = 67 Multicenter study Compared metagenomic changes and lactose responsiveness before and after lactulose adjusted to MHE reversal	Increased levels of Firmicutes in lactulose responders No significant changes before and after lactulose therapy	There may be a link between lactulose response and microbiome differences but this needs additional studies
Cirrhosis	Bajaj et al,[94] 2013, rifaximin before vs after in MHE	Rifaxamin N = 20 Compared before and after 8 wk of rifaximin (550 mg BID) Assessed microbiota, cognition, metabolomics, and endotoxemia, changes in brain function, and MRI	As cognition improved there was seen a transition toward more beneficial metabolite links compared with pathogenic (Enterobacteriaceae, Porphyromonadaceae and Bacteroidaceae) Although a link was seen between decreased endotoxemia and improved cognition, no significant composition changes were noted, just metabolomics	Bacteria function was improved with rifaximin

Cirrhosis (decompensated)	Kaji et al,[98] 2017	Rifaximin N = 20 Compared microbiota, endotoxema, ammonia, and cognition before and after treatment (440 mg TID)	No changes in microbiome diversity but improved cognition, endotoxin, and ammonia levels Minor reductions seen in levels of Veillonella and Streptococcus	No significant change in microbiome composition, but improved cognition and decreased endotoxin activity with treatment
Cirrhosis	Schulz et al,[99] 2019	Rifaximin (550 mg BID) with or without lactulose N = 5 MHE patients treated for 3 months Assessed cognition, duodenal, and fecal microbiota changes	MHE improved but there were no changes seen in the samples (duodenal and fecal)	There was no change in microbiome composition but there was a improvement in cognition

Abbreviations: ALD, alcoholic liver disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BID, 2 times per day; ETV, entecavir; FMT, fecal microbiota transplantation; GGT, gamma glutamyl transferase; HBeAg, hepatitis B virus e antigen; HBV, hepatitis B virus; HE, hepatic encephalopathy; IBD, inflammatory bowel disease; IV, intravenous; LDH, lactate dehydrogenase; MELD, Model for End Stage Liver Disease; MHE, minimal hepatic encephalopathy; OHE, overt hepatic encephalopathy; PSC, primary sclerosing cholangitis; SAH, subarachnoid hemorrhage; SOC, standard of care; TDF, tenofovir; TID, 3 times per day.

Probiotics

Probiotics are defined as live microorganisms that, when given in the correct dosing, confer a health benefit on the host.[75] Probiotics have been studied in a wide variety of human diseases as a way to modulate the gut microbiota. There has been a growing body of evidence for the use of probiotics in the treatment of chronic liver disease (see **Table 3**).

Prebiotics

Prebiotics consistent of nondigestive food ingredients that are fermented in the gut, the largest subgroup being prebiotic fibers, which are usually nondigestible carbohydrates. They then can modulate the microbiome in beneficial ways to the host. It has been shown that prebiotics can modify gut barrier integrity and endotoxin translocation.[79] Prebiotics have been showed to be able to stimulate bacterial production of short chain fatty acids, stimulate growth of *Bifidobacteria* and *Lactobacilli*, and provide additional pathogen protection by lowering the luminal pH.[80] Although there have been numerous studies looking at the use of prebiotics in chronic liver disease, there have been no definitive studies that meet our criteria (human, adult, pretreatment and post-treatment microbiome analysis). There are some ongoing clinical trials and promising rodent studies, however, that show encouraging treatment with prebiotics, including pectin.

Synbiotics

Synbiotics are combinations of prebiotics and probiotics, used to gain the benefit of both. A wave of new studies has decided to use this strategy in the hopes of maximizing the benefit of both interventions (see **Table 3**).

Diet

The studies looking at diet for possible microbiota therapy in chronic liver disease are relatively new and have looked at how different cultural diets impact microbial diversity.[83] There has been interest to see how animal fat and protein intake impacts the microbiota and impactions compensated and compensated cirrhotic patient[84] (see **Table 3**). As more information is gathered in this area, hopefully new dietary guidelines can be generated for cirrhotic patients.

Periodontal therapy

Periodontitis leads to destruction of tooth-supporting structures through inflammation and a dysregulation of the immune response to a dysbiotic biofilm.[85] There is concern that a prolonged inflammatory response may lead to systemic complications. This possible therapeutic target has been investigated in cirrhotic patients (see **Table 3**).

Fecal/Intestinal Microbiota Transplantation

Although there is robust literature for the use of fecal microbiota transplantation for treatment of refractory *C difficile* infection, its use in chronic liver disease is relatively new. One major difference between these 2 illness groups is that because the microbiome has been destroyed by antibiotics in refractory *C difficile* infection, normalization can often be obtained after a single inoculation and with a small dose of donor material.[90] The etiology of liver disease-associated intestinal microbiota is much more complex. It thus makes attempts at normalization more difficult and there remains a significant amount of questions surrounding what the target microbiota composition and functionality should be in chronic liver disease overall and for individual disease etiologies. It is unclear what the optimal treatment regiments are, including

the length of treatment, amount of material, and identification of treatment endpoints.[87] fecal microbiota transplantation has been studied in a wide variety of chronic liver disease patients (see **Table 3**).

Hepatic Encephalopathy and Minimal Hepatic Encephalopathy

Although lactulose and rifaximin are mainstays in the treatment of HE and minimal HE, there remains poor understanding of their underlying mechanisms in the disease process. Numerous studies have looked at better understanding HE pathophysiology and how these treatments impact the microbiome (see **Table 3**).

SUMMARY

Gut microbiota analysis and interpretation is now a major part of clinical and translational research in chronic diseases, including liver disease and cirrhosis. There are specific areas in liver disease where gut microbiota composition and functional changes can be cost effective,[100] but further work needs to be done to translate these changes into clinical practice.

DISCLOSURE

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