The role of microbiota in hepatic encephalopathy

Jasmohan S Bajaj

Division of Gastroenterology, Hepatology, and Nutrition; Virginia Commonwealth University and McGuire VA Medical Center; Richmond, VA USA

Keywords: covert hepatic encephalopathy, minimal hepatic encephalopathy, cirrhosis, rifaximin, lactulose, correlation network, dysbiosis, probiotic, endotoxin, systemic inflammation

Hepatic encephalopathy (HE), which consists of minimal (MHE) and overt (OHE) stages, is a model for impaired gut-liver-brain axis in cirrhosis. Microbiota changes in both stages have been associated with impaired cognition, endotoxemia, and inflammation. There is dysbiosis (reduced autochthonous taxa [*Lachnospiraceae*, *Ruminococcaceae*, and *Clostridiales XIV*] and increased *Enterobacteriaceae* and *Streptococcaceae*) with disease progression. In MHE, there is an increased abundance of *Streptococcus salivarius* linked to cognition and ammonia. In OHE, stool *Alcaligenaceae* and *Porphyromonadaceae* are associated with poor cognition. Colonic mucosal microbiome in cirrhosis is significantly different compared with stool and independently related to cognition. HE treatment can affect microbial composition and function; cognitive improvement in MHE after rifaximin, a non-absorbable antibiotic, occurred without significant stool microbiota composition change but improved metabolic linkages. Similarly, there are only modest lactulose and rifaximin-associated changes on microbiota composition in OHE. HE represents an important model to study microbiome-brain interactions.

Introduction

Gut microbiota changes play a critical role in the progression of cirrhosis and its complications.¹ The gut milieu is important in the pathogenesis of the systemic inflammation, endotoxemia, and the vasodilation that leads to complications such as spontaneous bacterial peritonitis (SBP), hepatic encephalopathy (HE), and hepato-renal syndrome.² These complications are often pre-terminal events in cirrhosis, and their prevention and early management could improve the prognosis of affected patients.³ Of the myriad components of the gut milieu, it is not clear whether the microbiota alone are responsible for these complications, however the study of their composition and function in light of the metagenomic revolution is an important goal to understand cirrhosis progression.

For the purposes of this review, we will only concentrate on human studies.

One of the most important models for the study of gut-liver-brain axis is HE. HE negatively impacts patients and

their caregivers from a medical as well as a socio-economic perspective. HE is expressed as part of the spectrum of neuro-cognitive impairment in cirrhosis (SONIC) that ranges from minimal (MHE, in which there is no asterix or disorientation but patients are impaired on specialized cognitive tests) to overt HE (apparent on regular physical examination with mental status changes ranging from simple disorientation through coma).⁴ Patients with minimal HE have difficulties in cognitive performance on several domains that test for executive function (problem solving, response inhibition, and working memory), psychomotor speed, and visuo-motor coordination.⁵ These domains result in impairment of health-related quality of life, socio-economic status, and driving skills and increases progression to the clinically evident overt HE stage. Overt HE is often precipitated by gut-related conditions such as infections like SBP, an increased protein load due to acute upper gastrointestinal bleeding, constipation, and drugs such as opioids that slow the intestinal transit.⁶ Overt HE has a major impact because it is associated with decreased survival, can predict subsequent overt HE episodes, and can severely impact caregivers of the patients.⁶ The pathogenesis of SONIC (both minimal and overt HE) is not well understood but products of nitrogenous metabolism such as ammonia, indoles, and/or oxindoles, along with local and systemic inflammation and endotoxemia, have been implicated.⁷ These are exacerbated by the underlying hepatic dysfunction that impairs the urea cycle fixation of ammonia and by porto-systemic shunting of the gut-originated blood to the systemic circulation (Fig. 1).

The importance of the gut in the potential pathogenesis of HE is underlined by the gut-centric therapies for HE. These include lactulose and lactitol, prebiotic non-absorbable disaccharides, non-absorbable antibiotics such as rifaximin and neomycin, and varying combinations of probiotics and synbiotics.⁸ These therapies have been used across SONIC and most studies have demonstrated clinical and cognitive improvement depending on the study evaluated. However, therapies that do not affect gut microbiota specifically have also successfully treated HE, therefore other factors also need to be considered.⁹

Specific issues that have to be contended with when designing, interpreting and analyzing data in human HE-trials based on gut microbiota are:

- (1) Are the microbiota changes simply due to advancing liver disease?
- (2) Are there specific microbiota changes in the mucosa, in stool, or in both, in patients with HE?
- (3) What are the effects of HE treatments on microbiota?

Correspondence to: Jasmohan S Bajaj; Email: jsbajaj@vcu.edu
Submitted: 11/19/2013; Revised: 02/19/2014; Accepted: 03/27/2014;
Published Online: 04/01/2014
<http://dx.doi.org/10.4161/gmic.28684>

Figure 1. Schema of brain dysfunction due to altered microbiota in cirrhosis. Dysbiosis due to altered gut microbiota function and composition in cirrhosis is potentially related to the decreased hepatic bile acid synthesis and subsequent release into the intestine, systemic inflammation due to the underlying liver disease and due to continued exposure of certain etiological conditions such as alcohol directly on the gut. This dysbiosis results in an increase in gut-derived products such as ammonia, endotoxin, inflammatory cytokines, and bacterial DNA into the systemic circulation via the poorly functioning liver, through porto-systemic shunting or directly through the thoracic duct. In the setting of this systemic inflammatory milieu, synthetic dysfunction due to liver disease, poor ammonia clearance due to sarcopenia, and continued exposure to neurotoxins such as alcohol and hepatitis C, the brain of a patient with cirrhosis can manifest with overt or minimal hepatic encephalopathy.

- (4) Is it the relative change in microbial abundance or a change in function that results in overall improvement?
- (5) Are these bacteria just markers of poor prognosis or do they actually have pathogenetic roles to play in disease progression?

Culture-Based Studies of Microbiota in Cirrhosis and HE

Studies before the metagenomic revolution used culture to isolate the bacterial population in the feces of patients with cirrhosis. Riggio et al. studied 21 cirrhotic patients with lactitol and lactulose and found a 2 log increase in lactobacilli in both groups with smaller degrees of reduction in *Enterobacteria* and *Enterococci*.¹⁰ Tarao et al. found that lactitol, when administered for up to 4 weeks in 8 patients significantly increased the occupation ratio, defined as number of that specific bacteria divided by the total number of bacteria detected, of *Bifidobacterium* as well as the total *Lactobacillus* count.¹¹ They also found a reduced occupation ratio of *Clostridium* and *Bacteroides* but no absolute change in number. This was accompanied by improvement in mental state, blood ammonia, and asterixis. In a symbiotic vs. fiber vs. placebo study in MHE, cirrhotic patients who were randomized to symbiotic showed an increase in *Lactobacillus* with reduction in *E. coli* and *Staphylococcus*; no change in *Bifidobacterium* was observed.¹² In those randomized to placebo alone, there was again a reduction in *E. coli* and *Staphylococcus* overgrowth but an increase in *Bifidobacterium*. Therefore, culture-based studies initially defined gut microbial changes after HE therapy but the advent of culture-independent techniques has greatly increased our understanding of the overall changes in the microbiota function and composition in cirrhosis and HE.

Culture-Independent Microbial Studies of Change in the Microbiome in Cirrhosis and HE

Over the last few years, using several culture-independent techniques, the microbiota of patients with cirrhosis has been characterized in the stool and sigmoid colonic mucosa. A few studies have also correlated specific changes in the gut microbiome to cognitive function and systemic inflammation. While some studies have linked cognitive function as a continuum with results of recommended psychometric tests, others have noted differences in the microbiome in classes of patients with HE diagnosed clinically. An important caveat of these studies is that HE patients often have worse cirrhosis severity and are on therapy. As mentioned above, therapies that treat HE are largely gut-based and therefore inclusion of patients already on these medications could potentially change the composition and function. A summary of studies evaluating microbiome in cirrhosis specifically is presented in Table 1.

Microbiota Changes with Worsening Liver Disease Severity

The severity of cirrhosis is measured by two validated scoring systems, the Child-Turcotte-Pugh (CTP, which includes serum albumin, bilirubin, prothrombin time, HE, and ascites severity) and the Model for End-Stage Liver Disease (MELD, logarithmic score of bilirubin, creatinine, and INR of the prothrombin time).¹³ A higher CTP and MELD score indicates a poor prognosis. Chen et al. showed that the bacterial taxon *Lachnospiraceae* was negatively linked while *Streptococcaceae* was positively correlated with increasing CTP score.¹⁴ However, since HE severity is a component of the CTP score, further HE-focused studies of microbiome have used the MELD score. In a study by our group, the MELD score was positively linked with the taxon *Enterobacteriaceae* and negatively with *Ruminococcaceae*.¹⁵ In a larger study of 219 cirrhotic patients, the ratio of the autochthonous (commensal and potentially beneficial bacteria)¹⁶ taxa (*Ruminococcaceae*, *Lachnospiraceae*, and clostridiales cluster XIV) to the non-autochthonous ones (*Enterobacteriaceae* and *Bacteroidaceae*) called the “cirrhosis dysbiosis ratio” was also correlated with MELD score and with endotoxin.¹⁷ Therefore the disappearance of “good” bacteria and the increased relative abundance of potentially pathogenic ones could shift the microbiota into dysbiosis and negative consequences such as HE. The relative composition of bacteria remained similar in cirrhotic patients whose liver disease remains stable over several months.¹⁷ Therefore, since most studies have included HE patients who have higher MELD scores than those without HE, it is important to further analyze these results in the context of overall disease severity in a longitudinal design.

Changes in Patients with MHE and Overt HE

Compared with patients with cirrhosis without cognitive dysfunction, there are significant differences in the stool microbiota of patients with MHE. Zhang et al. found worse dysbiosis in all cirrhotic patients compared with healthy controls but also found that patients with MHE had specific changes such as an overabundance of *Streptococcus salivarius*.¹⁸ They hypothesized that this could increase ammonia production due to its urease activity, and indeed, on their correlation network they found it was associated with blood ammonia and cognitive testing. In contrast, our group did not find a significant difference between overt HE and no-overt HE patients on the stool microbiome, although both groups independently had dysbiosis compared with healthy controls.¹⁵ This was striking because patients with overt HE are significantly different from a phenotypic perspective with worse cognition, liver disease severity, and higher endotoxemia compared with those without overt HE. This lack of difference in microbiota composition in overt HE compared with no-overt HE stool was

Table 1. Human studies of microbiome using culture-independent techniques in Cirrhosis

Study	Population Groups	Tissue tested	Level	Findings	Relationship with cognition
Chen et al. ¹⁴	Cirrhosis/control	Stool	Family	Higher <i>Streptococcaceae</i> , <i>Veillonellaceae</i> and <i>Enterobacteriaceae</i> and lower <i>Lachnospiraceae</i> in cirrhotic group	Not specifically studied
Bajaj et al. 2012 ¹⁵	Overt HE/no-overt HE/control	Stool	Family	Higher <i>Enterobacteriaceae</i> , <i>Alcaligenaceae</i> , and <i>Fusobacteriaceae</i> and lower <i>Ruminococcaceae</i> and <i>Lachnospiraceae</i> in cirrhosis	Higher <i>Veillonellaceae</i> in HE compared with no HE cirrhotics, <i>Alcaligenaceae</i> and <i>Porphyromonadaceae</i> were correlated with cognition
Bajaj et al. 2012 ²⁹	Overt HE/no overt HE/control	Sigmoid mucosa and stool	Genus	Higher <i>Dorea</i> , <i>Subdoligranulum</i> , <i>Incertae Sedis XIV</i> , <i>Blautia</i> , <i>Roseburia</i> , <i>Faecalibacterium</i> and lower <i>Enterococcus</i> , <i>Burkholderia</i> , <i>Proteus</i> in cirrhosis	<i>Enterococcus</i> , <i>Megasphaera</i> , and <i>Burkholderia</i> linked to poor cognition and higher inflammation while the opposite was seen for <i>Enterococcus</i> , <i>Megasphaera</i> , and <i>Burkholderia</i> . <i>Alcaligenaceae</i> and <i>Porphyromonadaceae</i> were associated with poor cognition
Bajaj et al. 2012 ³	Overt HE after lactulose withdrawal	Stool	Family and genus	Lower <i>Fecalibacterium</i> after lactulose withdrawal	<i>Prevotella</i> associated with good cognition and lower inflammation
Zhang et al. 2013 ¹⁸	MHE/no-MHE/control	Stool	Family and species	Higher <i>Streptococcaceae</i> and <i>Veillonellaceae</i> in cirrhotics, <i>Streptococcus salivarius</i> higher in MHE	Correlation of cognitive function and ammonia with <i>Veillonella parvula</i> and <i>Streptococcus salivarius</i>
Lu et al. 2011 ⁴²	Hepatitis B from pre-cirrhotic to decompensated cirrhotic patients	Stool	Family, genus, and species (LH-PCR only)	Lower lactic acid bacteria, <i>Bifidobacteria</i> and <i>Clostridiales XIV</i> in advancing HBV with increase in <i>Enterobacteriaceae</i> in cirrhotic patients	Not specifically studied
Bajaj et al. 2013 ²⁵	MHE cirrhosis trial before/after rifaximin therapy	Stool	Family	Modest reduction of <i>Veillonellaceae</i> after rifaximin but major changes were in metabolite associations	Improved cognition associated with change in bacterial correlations, especially <i>Enterobacteriaceae</i>
Bajaj et al. 2014 ¹⁷	Cross-sectional and longitudinal study of cirrhotic patients through the spectrum of disease	Stool	Family	Cirrhosis dysbiosis ratio worsens with advancing cirrhosis, remains stable in unchanged disease, is associated with 30-d mortality, organ failure and ACLF in infected patients	In patients who develop their first overt HE episode, there is increase in dysbiosis despite lactulose therapy
Bajaj et al. 2014 ⁴³	Randomized phase I, placebo-controlled trial of lactobacillus GG vs. placebo in MHE	Stool	Family	In the LGG group, Cirrhosis dysbiosis ratio improved (reduced <i>Enterobacteriaceae</i> and increased autochthonous taxa) with significant changes in metabolite-microbiome correlations	Being a safety phase I study, was not powered to detect cognitive change; there was no difference in cognitive testing between LGG and placebo groups

confirmed again in a larger sample size, but interestingly, there was a significant difference in the sigmoid colonic mucosal microbiome between these patients.¹⁷ As expected the microbial composition at the mucosal surface was significantly different from the stool in cirrhotics with overt HE, without overt HE and controls. Interestingly a similar pattern of dysbiosis that was apparent in the stool between controls and cirrhotics was now seen between cirrhotics with and/or without overt HE in the mucosal surface. Colonic mucosal autochthonous genera (*Lachnospiraceae* *Roseburia*, *Lachnospiraceae* *Dorea*, and *Ruminococcaceae* *Fecalibacterium*) were linked with good cognitive performance while others (*Burkholderiaceae*

Other, *Veillonellaceae* *Megasphaera*, *Rikenellaceae* *Alistipes*, *Streptococcaceae* *Streptococcus*, *Alcaligenaceae* *Sutterella*, *Porphyromonadaceae* *Parabacteroides*) were associated with poor cognitive performance in patients with and without overt HE. This differentiation brings into focus the critical importance of the tissue being analyzed. It is likely that several important processes in the pathogenesis of HE occur at the mucosal interface rather than the lumen, including translocation and interaction of gut microbiota with the immune cells.¹⁹ Therefore the approach to cognitive dysfunction in cirrhosis and microbiota should focus on GI tract mucosal microbiota.

Effect of HE Treatment on Microbiota and Brain Function

MHE therapy

Currently there is no standard of care for the treatment of MHE. Therefore studying patients with MHE can give us an insight into microbiota-brain interactions without the confounding factor of therapies such as lactulose or rifaximin. However, the same drugs that are approved for overt HE have also been used to treat MHE.²⁰ Non-microbial studies using lactulose, probiotics, and rifaximin in MHE patients have shown varying degrees of cognitive improvement, reduction in endotoxemia, decreased brain swelling, and enhanced connectivity on multi-modal MRI.²¹⁻²⁴ A systematic study of MHE therapy with rifaximin was performed by our group in which we found a significant improvement in cognitive performance.²⁵ This was accompanied by an enhanced working memory performance via facilitation of the functioning of subcortical structures, greater inhibitory control by modulation of fronto-parietal activation, and improved white matter integrity in MHE after rifaximin on multi-modal brain MR imaging. We also found improved neural network connectivity between frontal and parietal structures and, in particular, facilitation of the left parietal operculum.²⁶ However, despite these improvements and a reduction in endotoxemia after rifaximin, there was only a modest effect on relative bacterial abundance. This, however, was in line with prior studies in settings independent of cirrhosis in which a significant change in bacterial virulence and behavior rather than change in number with rifaximin were noted.²⁷ Correlation network analysis in MHE showed changes in the overall complexity and in interactions between the metabolites, inflammatory cytokines and the microbiome after rifaximin therapy. There were also shifts in linkages of *Enterobacteriaceae* that changed from pathogenic correlations at baseline to beneficial ones after rifaximin. Interestingly, correlations centered on autochthonous taxa remained beneficial both before and after rifaximin. A phase I, randomized, double-blind, placebo-controlled trial conducted under the investigational new drug (IND) program using the probiotic *Lactobacillus GG* (LGG) in MHE patients improved endotoxemia and TNF α with significant improvement in dysbiosis (Cirrhosis dysbiosis ratio, reduced *Enterobacteriaceae*, increased *Lachnospiraceae*, and Clostridiales XIV) only in the LGG-randomized group. Being a safety study, this was not powered to detect cognitive changes, which were not observed between groups. Similar to the rifaximin MHE trial, however, there were significant changes in functionality related to pathways of amino-acid, vitamin, and bile acid metabolism in the LGG compared to the placebo group⁴³). Therefore in addition to just the microbiota composition, functional alterations of microbiota are important to analyze.

Overt HE therapy

The first-line treatment for overt HE in the US is lactulose, a prebiotic with laxative properties, while rifaximin is FDA-approved only for recurrent overt HE or in patients who are intolerant of lactulose.⁸ Lactulose therapy does not significantly change microbial composition in healthy individuals using

culture-independent techniques.²⁸ Our group evaluated change in microbiota after overt HE therapy was initiated and found that the relative reduction in autochthonous taxa continued despite lactulose therapy.¹⁷ However a systems biology analysis of systematic lactulose withdrawal showed that selected patients have overt HE recurrence once lactulose is withdrawn.³ Patients who had a recurrence of overt HE after lactulose withdrawal had worse cognition and systemic inflammation at the outset. In the entire group, after lactulose withdrawal, there was a modest microbial change (a relative decrease in the abundance of the autochthonous *Fecalibacterium* spp.), metabolite changes consistent with a shift in the microbial conversion of choline, and worsening neuro-inflammation on brain MR spectroscopy.

As mentioned above, due to prescribing practices in the US, patients who are on rifaximin are usually more advanced in their disease process. Therefore these patients tend to have worse cognition, higher systemic inflammation and endotoxemia than those not on rifaximin.²⁹ In cross-sectional overt HE studies of patients on rifaximin, similar to MHE studies, the stool microbiota did not change dramatically; however the mucosal microbiota reflected a greater dysbiosis in patients that required rifaximin (lower Clostridiales XIV *Blautia*, *Lachnospiraceae* *Roseburia*, *Veillonellaceae* other, *Rikenellaceae* *Alistipes*, and higher *Propionibacteriaceae* *Propionibacterium*).²⁹ Simply evaluating the relative abundance demonstrates that it is likely that the underlying disease process has a greater influence on the microbiome compared with the super-added medications. However, the effect of these medications on the functionality of the bacteria also needs to be considered in further trials.

Mechanisms of Dysbiosis and Their Effects on Cognition in Cirrhosis

The above studies have only demonstrated an association, not causation, between cognitive performance, cirrhosis severity, and microbial change. A potential mechanism could be the decrease in bile acid production as cirrhosis advances which can modulate the presence of autochthonous taxa in the gut.³⁰⁻³² The conversion from the primary to secondary BAs is seen as a key feature of microbial functionality, which is impaired as cirrhosis progresses.^{31,32} Autochthonous taxa such as *Lachnospiraceae*, *Ruminococcaceae*, and Clostridiales Cluster XIV (consist of several genera and species such as *Fecalibacterium* and/or *Roseburia*) have the ability to generate nutritive short-chain fatty acids, strengthen the integrity of the gut barrier, and are often seen as the “healthy” microbiota.^{16,33,34} As shown in the figure, bacterial products including endotoxin, ammonia, and bacterial DNA itself could initiate and propagate systemic inflammation through lack of detoxification in the liver or through bypassing it altogether through the porto-systemic shunts.³⁵ The increase in blood ammonia is worsened by the sarcopenia in cirrhosis that reduces the detoxification capacity of the skeletal muscles.^{36,37} The concomitant effect of systemic inflammation on cognition in cirrhosis is also key in driving these changes.⁷ The effect of the inflammatory milieu and toxins on the brain in turn promotes

neuro-inflammation with the increased intra-astrocytic ammonia resulting in a higher concentration of the osmotically active glutamate and/or glutamine and compensatory reduction in myoinositol and choline.³⁸ Lastly, the etiology of the cirrhosis, including continued alcohol abuse, HCV infection can also determine cognition, microbial abundance, and endotoxemia with and without cirrhosis.^{17,39,40} Therefore the etiology of liver disease and continued exposure to agents that can modulate cognition independently should also be taken into consideration.⁴¹

Conclusion

Studies on the gut-liver-brain axis in cirrhosis have demonstrated that the gut microbial composition and function can impact cognition. Specifically, an imbalance between beneficial, autochthonous taxa and potentially pathogenic ones in the stool and colonic mucosal microbiota is linked with systemic inflammation, endotoxemia, and cognitive impairment

across the spectrum of HE. The microbial profile in cirrhotic patients remains similar over time if the disease is stable however, dysbiosis sets in and worsens with progression of cirrhosis and HE development despite initiation of therapy. Therapies for HE treatment may play a role in functional modulation of the microbiota since changes in the composition are modest in recent studies. Since current research is correlational, further translational studies on the mechanistic pathways linking these three vital organ systems are needed to target specific microbial constituents in order to develop focused gut-based therapies.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

This work was supported by RO1AA020203 from the National Institute on Alcohol Abuse and Alcoholism and grant RO1DK087913 from the National Institute of Diabetes and Digestive and Kidney Diseases. The author would like to thank Mikyung Kang for graphic assistance with figures.

References

- Quigley EM, Stanton C, Murphy EF. The gut microbiota and the liver. *Pathophysiological and clinical implications*. *J Hepatol* 2013; 58:1020-7; PMID:23183530; <http://dx.doi.org/10.1016/j.jhep.2012.11.023>
- Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008; 28:26-42; PMID:18293275; <http://dx.doi.org/10.1055/s-2008-1040319>
- Bajaj JS, Gillevet PM, Patel NR, Ahluwalia V, Ridlon JM, Kettenmann B, Schubert CM, Sikaroodi M, Heuman DM, Crossey MM, et al. A longitudinal systems biology analysis of lactulose withdrawal in hepatic encephalopathy. *Metab Brain Dis* 2012; 27:205-15; PMID:22527995; <http://dx.doi.org/10.1007/s11011-012-9303-0>
- Bajaj JS, Wade JB, Sanyal AJ. Spectrum of neurocognitive impairment in cirrhosis: Implications for the assessment of hepatic encephalopathy. *Hepatology* 2009; 50:2014-21; PMID:19787808; <http://dx.doi.org/10.1002/hep.23216>
- Ortiz M, Jacas C, Córdoba J. Minimal hepatic encephalopathy: diagnosis, clinical significance and recommendations. *J Hepatol* 2005; 42(Suppl):S45-53; PMID:15777572; <http://dx.doi.org/10.1016/j.jhep.2004.11.028>
- Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy—definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congress of Gastroenterology, Vienna, 1998. *Hepatology* 2002; 35:716-21; PMID:11870389; <http://dx.doi.org/10.1053/jhep.2002.31250>
- Shawcross DL, Wright G, Olde Damink SW, Jalan R. Role of ammonia and inflammation in minimal hepatic encephalopathy. *Metab Brain Dis* 2007; 22:125-38; PMID:17260161; <http://dx.doi.org/10.1007/s11011-006-9042-1>
- Bajaj JS. Review article: the modern management of hepatic encephalopathy. *Aliment Pharmacol Ther* 2010; 31:537-47; PMID:20002027; <http://dx.doi.org/10.1111/j.1365-2036.2009.04211.x>
- Romero-Gómez M, Jover M, Del Campo JA, Royo JL, Hoyas E, Galán JJ, Montoliu C, Baccaro E, Guevara M, Córdoba J, et al. Variations in the promoter region of the glutaminase gene and the development of hepatic encephalopathy in patients with cirrhosis: a cohort study. *Ann Intern Med* 2010; 153:281-8; PMID:20820037; <http://dx.doi.org/10.7326/0003-4819-153-5-201009070-00002>
- Riggio O, Varriale M, Testore GP, Di Rosa R, Di Rosa E, Merli M, Romiti A, Candiani C, Capocaccia L. Effect of lactitol and lactulose administration on the fecal flora in cirrhotic patients. *J Clin Gastroenterol* 1990; 12:433-6; PMID:2398251; <http://dx.doi.org/10.1097/00004836-199008000-00016>
- Tarao K, Tamai S, Ito Y, Okawa S, Hayashi M. [Effects of lactitol on fecal bacterial flora in patients with liver cirrhosis and hepatic encephalopathy]. *Nihon Shokakibyo Gakkai Zasshi* 1995; 92:1037-50; PMID:7643458
- Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; 39:1441-9; PMID:15122774; <http://dx.doi.org/10.1002/hep.20194>
- Kamath PS, Kim WR; Advanced Liver Disease Study Group. The model for end-stage liver disease (MELD). *Hepatology* 2007; 45:797-805; PMID:17326206; <http://dx.doi.org/10.1002/hep.21563>
- Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, Wang Y, Zhu B, Li L. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011; 54:562-72; PMID:21574172; <http://dx.doi.org/10.1002/hep.24423>
- Bajaj JS, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, Sikaroodi M, Gillevet PM. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012; 302:G168-75; PMID:21940902; <http://dx.doi.org/10.1152/ajpgi.00190.2011>
- Nava GM, Stappenbeck TS. Diversity of the autochthonous colonic microbiota. *Gut Microbes* 2011; 2:99-104; PMID:21694499; <http://dx.doi.org/10.4161/gmic.2.2.15416>
- Bajaj JS, Hylemon PB, Heuman DM, et al. The Cirrhosis Dysbiosis Ratio defines Changes in the Gut Microbiome Associated with Cirrhosis and its Complications. *J Hepatol* 2014; <http://dx.doi.org/10.1016/j.jhep.2013.12.019>
- Zhang Z, Zhai H, Geng J, Yu R, Ren H, Fan H, Shi P. Large-scale survey of gut microbiota associated with MHE Via 16S rRNA-based pyrosequencing. *Am J Gastroenterol* 2013; 108:1601-11; PMID:23877352; <http://dx.doi.org/10.1038/ajg.2013.221>
- Teltschik Z, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, Bevens CL, Stange EF, Wehkamp J. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* 2012; 55:1154-63; PMID:22095436; <http://dx.doi.org/10.1002/hep.24789>
- Kappus MR, Bajaj JS. Covert hepatic encephalopathy: not as minimal as you might think. *Clin Gastroenterol Hepatol* 2012; 10:1208-19; PMID:22728384; <http://dx.doi.org/10.1016/j.cgh.2012.05.026>
- Ahluwalia V, Wade JB, Heuman DM, Hammeke TA, Sanyal AJ, Sterling RK, Stravitz RT, Luketic V, Siddiqui MS, Puri P, et al. Enhancement of functional connectivity, working memory and inhibitory control on multi-modal brain MR imaging with Rifaximin in Cirrhosis: Implications for the gut-liver-brain axis. *Metab Brain Dis* 2014; PMID:24590688; <http://dx.doi.org/10.1007/s11011-014-9507-6>
- Bajaj JS, Sacian K, Christensen KM, Hafeezullah M, Varma RR, Franco J, Pleuss JA, Krakower G, Hoffmann RG, Binion DG. Probiotic yogurt for the treatment of minimal hepatic encephalopathy. *Am J Gastroenterol* 2008; 103:1707-15; PMID:18691193; <http://dx.doi.org/10.1111/j.1572-0241.2008.01861.x>
- Mittal VV, Sharma BC, Sharma P, Sarin SK. A randomized controlled trial comparing lactulose, probiotics, and L-ornithine L-aspartate in treatment of minimal hepatic encephalopathy. *Eur J Gastroenterol Hepatol* 2011; 23:725-32; PMID:21646910; <http://dx.doi.org/10.1097/MEG.0b013e32834696f5>
- Jain L, Sharma BC, Srivastava S, Puri SK, Sharma P, Sarin S. Serum endotoxin, inflammatory mediators, and magnetic resonance spectroscopy before and after treatment in patients with minimal hepatic encephalopathy. *J Gastroenterol Hepatol* 2013; 28:1187-93; PMID:23425082; <http://dx.doi.org/10.1111/jgh.12160>
- Bajaj JS, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, Fuchs M, Ridlon JM, Daita K, Monteith P, et al. Modulation of the microbiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One* 2013; 8:e60042; PMID:23565181; <http://dx.doi.org/10.1371/journal.pone.0060042>

26. Ahluwalia V, Wade JB, Heuman DM, Hammeke TA, Sanyal AJ, Sterling RK, Stravitz RT, Luketic V, Siddiqui MS, Puri P, et al. Enhancement of functional connectivity, working memory and inhibitory control on multi-modal brain MR imaging with Rifaximin in Cirrhosis: Implications for the gut-liver-brain axis. *Metab Brain Dis* 2014; PMID:24590688; <http://dx.doi.org/10.1007/s11011-014-9507-6>
27. Jiang ZD, Ke S, Dupont HL. Rifaximin-induced alteration of virulence of diarrhoea-producing *Escherichia coli* and *Shigella sonnei*. *Int J Antimicrob Agents* 2010; 35:278-81; PMID:20045287; <http://dx.doi.org/10.1016/j.ijantimicag.2009.11.013>
28. Vanhoutte T, De Preter V, De Brandt E, Verbeke K, Swings J, Huys G. Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and *Saccharomyces boulardii*. *Appl Environ Microbiol* 2006; 72:5990-7; PMID:16957220; <http://dx.doi.org/10.1128/AEM.00233-06>
29. Bajaj JS, Hylemon PB, Ridlon JM, Heuman DM, Daita K, White MB, Monteith P, Noble NA, Sikaroodi M, Gillevet PM. 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; PMID:22821944; <http://dx.doi.org/10.1152/ajpgi.00152.2012>
30. Vlahcevic ZR, Buhac I, Bell CC Jr., Swell L. Abnormal metabolism of secondary bile acids in patients with cirrhosis. *Gut* 1970; 11:420-2; PMID:5428044; <http://dx.doi.org/10.1136/gut.11.5.420>
31. Kakiyama G, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, Takei H, Muto A, Nittono H, Ridlon JM, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* 2013; 58:949-55; PMID:23333527; <http://dx.doi.org/10.1016/j.jhep.2013.01.003>
32. Ridlon JM, Alves JM, Hylemon PB, Bajaj JS. Cirrhosis, bile acids, and gut microbiota: Unraveling a complex relationship. *Gut Microbes* 2013; 4:4; PMID:23851335; <http://dx.doi.org/10.4161/gmic.25723>
33. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A* 2008; 105:16731-6; PMID:18936492; <http://dx.doi.org/10.1073/pnas.0804812105>
34. Zoetendal EG, Ben-Amor K, Harmsen HJ, Schut F, Akkermans AD, de Vos WM. Quantification of uncultured *Ruminococcus obeum*-like bacteria in human fecal samples by fluorescent in situ hybridization and flow cytometry using 16S rRNA-targeted probes. *Appl Environ Microbiol* 2002; 68:4225-32; PMID:12200269; <http://dx.doi.org/10.1128/AEM.68.9.4225-4232.2002>
35. Zapater P, Francés R, González-Navajas JM, de la Hoz MA, Moreu R, Pascual S, Monfort D, Montoliu S, Vila C, Escudero A, et al. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis. *Hepatology* 2008; 48:1924-31; PMID:19003911; <http://dx.doi.org/10.1002/hep.22564>
36. Montano-Loza AJ, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, Sawyer MB. Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012; 10:166-73, e1; PMID:21893129; <http://dx.doi.org/10.1016/j.cgh.2011.08.028>
37. Olde Damink SW, Jalan R, Dejong CH. Interorgan ammonia trafficking in liver disease. *Metab Brain Dis* 2009; 24:169-81; PMID:19067143; <http://dx.doi.org/10.1007/s11011-008-9122-5>
38. McPhail MJ, Taylor-Robinson SD. The role of magnetic resonance imaging and spectroscopy in hepatic encephalopathy. *Metab Brain Dis* 2010; 25:65-72; PMID:20221679; <http://dx.doi.org/10.1007/s11011-010-9171-4>
39. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, Kwasny M, Lau CK, Keshavarzian A. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012; 302:G966-78; PMID:22241860; <http://dx.doi.org/10.1152/ajpgi.00380.2011>
40. Hartmann P, Chen WC, Schnabl B. The intestinal microbiome and the leaky gut as therapeutic targets in alcoholic liver disease. *Front Physiol* 2012; 3:402; PMID:23087650; <http://dx.doi.org/10.3389/fphys.2012.00402>
41. Bajaj JS, Forton DM. Cognitive improvement after HCV eradication: Extending the benefits. *Hepatology* 2013; 58:480-2; PMID:23696270; <http://dx.doi.org/10.1002/hep.26481>
42. Lu H, Wu Z, Xu W, Yang J, Chen Y, Li L. Intestinal microbiota was assessed in cirrhotic patients with hepatitis B virus infection. Intestinal microbiota of HBV cirrhotic patients. *Microb Ecol* 2011; 61:693-703; PMID:21286703; <http://dx.doi.org/10.1007/s00248-010-9801-8>
43. Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, Puri P, Sterling RK, Luketic V, Stravitz RT, Siddiqui MS, Fuchs M, et al. Randomised clinical trial: *Lactobacillus GG* modulates gut microbiome, metabolome and endotoxemia in patients with cirrhosis. *Aliment Pharmacol Ther* 2014; 39:1113-25; PMID:24628464; <http://dx.doi.org/10.1111/apt.12695>