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Gut Microbiome and Liver Disease

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Abstract

Gut microbiota changes are important in determining the occurrence and progression of chronic liver disease related to alcohol, non-alcoholic fatty liver disease and cirrhosis. Specifically the systemic inflammation, endotoxemia and the vasodilation that leads to complications such as spontaneous bacterial peritonitis and hepatic encephalopathy could be related to the gut milieu. Given the poor prognosis of these events, their prevention and early management are essential. Microbiota may be essential component of the gut milieu that can impact these clinical events and the study of their composition and function in a culture-independent manner could help understand the prognosis. Recent human and animal studies have shown that the relative abundance and the functional changes of microbiota in the stool, colonic mucosa and saliva have varying consequences on the presence and prognosis of chronic liver disease and cirrhosis. The impact of therapies on the microbiota is slowly being understood and will likely lead to a more targeted approach to gut microbiota modification in chronic liver disease and cirrhosis.

Importance of the Microbiome in liver disease

Nonalcoholic fatty liver disease (NAFLD) refers to the accumulation of fat in the liver (1) and occurs due to metabolic dysfunction associated with energy surplus-induced adiposopathy (2). It is the most common cause of chronic liver disease in Western countries (3) and is not confined to liver related morbidity and mortality. It has been shown that it is a multisystem disease (4) affecting extra-hepatic organs and regulatory pathways. A population based prevalence of NAFLD study (5) approximated 30 – 40% in men, 15 – 20 % in women and higher in people with type 2 diabetes mellitus (T2DM), occurring in up to 70% of the population in the study. Several of the associated pathological factors associated with NAFLD overlap with alcoholic liver disease.

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Alcohol abuse is a significant contributor to the global burden of disease leading to tissue damage, organ dysfunction and develop ALD including alcoholic steatohepatitis and cirrhosis (6). Various factors have been associated with the progression of ALD including duration of alcohol abuse, obesity, pre-existing underlying liver disease such as hepatic C infection (7) (8) (9).

Cirrhosis is defined histologically as a diffuse process in which the normal anatomical lobules are replaced by abnormal nodules separated by fibrous tissue (10) and is the end result of various types of chronic liver diseases. Cirrhosis can lead to either portal hypertension or hepatic insufficiency. Cirrhosis remains compensated for many years prior to development of decompensation and marks the onset of the following complications: jaundice, variceal hemorrhage, ascites or encephalopathy (11).

The role of the microbiome in the study of the human gut and associated diseases has been of prime importance and thoroughly established (12). Of the known microbial species, more than 2000 bacterial species are commensal organisms and only about 100 known species which are pathogenic in nature (13). A mutual coevolution of the human body and microbe has occurred over time resulting in biochemical specialization of microbes to efficiently utilize energy sources from the human gut while the human body has adapted to utilize the novel metabolic processes convened by bacterial species (14). This mutual coevolution has assisted the human body in relying on important energy sources such as a butyrate and other products of microbial fermentation (15), the biochemical capabilities of the microbiota mediates beneficial roles including but not limited to vitamin synthesis, bile salt metabolism, processing of xenobiotic degradation and angiogenesis, regulation of postnatal maturation, (16) (17). The factors that direct the establishment and maintenance of human gut - microbial interactions remains largely unknown, the general paradigm is that the human gut is equipped with pattern recognition receptors (PRRs). These PRRs include the trans-membrane Toll-like receptors (TLRs), Nod-like receptors (NLRs) and are specifically recognized and bind distinctively to microbial-associated macromolecular patterns (MAMPs) such as lipopolysaccharide, flagellin, peptidoglycans, formylated peptides (18) (19). The human gut immune regulatory process plays a vital role in shaping an optimal microbiota.

Dysbiosis occurs when the compositional alterations occur away from the conventional symbiotic gut microbiota (20). The microbiota exhibits dysbiosis in the human gut and is an ecological disorder of the bacterial community. In a healthy state, the normal microbiota compete for access to the adhesive sites on the epithelial surface and they produce a combination of interspecific and intraspecific chemical signals that could suppress pathogenic invaders as well as optimize the composition of the microbiota (21) (22). This colonization resistance helps fight many infections (23) (24). The microbiota is an important component of an infection and suppression of the normal mechanisms would disturb the normal mechanisms of community regulation and lead to a dysbiotic human gut. This dysbiosis can lead to obesity (25) (26) , metabolic syndromes (27) , diabetes (28) (29), cardiovascular diseases (30), Nonalcoholic Fatty Liver Disease (NAFLD) (31), Nonalcoholic steatohepatitis (NASH) (32) Alcoholic Liver Diseases (33). The associations of the microbiota is evident at late stages of cirrhosis and exhibit intestinal bacterial overgrowth,

small bowel dysmotility, increased gut permeability, decreased immunological defenses (34). The severity of these diseases aggravates due to promotion of bacterial translocation (BT) from the gut to the systemic circulation leading to the severity of these infections (35). The absence of bile in the intestine has shown to facilitate BT and allowing enhancement of endotoxin-induced BT (36) (37). Cecum (38) seems to be the most marked site for BT where species such as *E. coli* and enterococci (39) are present in large numbers. The BT in cirrhosis is usually prevented by selective intestinal decontamination by relying on antibiotics that selectively eliminate intestinal bacteria and has been shown to be effective in preventing bacterial infections in patients with gastrointestinal hemorrhage (40), low ascites protein (41) as well as preventing spontaneous bacterial peritonitis (SBP) (42).

Advent of Next-generation DNA sequencing has allowed for in-depth sample analysis, most importantly culture – independent methods. These methods provide information on community diversity and structure (43), distinguishing them into their taxa (44). Sequencing of the 16s rRNA genes is one of the most widely accepted method of taking a community census (45). The primary data are processed and filtered based on the chosen method of data collection (46), followed by creation of tables of taxa (47) and abundance by using widely available public databases and finally represented in the form of similarity of communities, abundance curves, biodiversity plots, statistical descriptors of the bacterial structure (48) (49). Metagenomic approaches help recognize biochemical processes, metabolic conditions in non–insulin dependent diabetes, nonalcoholic steatosis, and atherosclerosis linking the changes to the microbial population (50) and this data can be interpreted by using the bioinformatics methods and also predict the metabolic involved processes (48) (49) (51). These different methods have been successfully implemented in understanding the role of microbial population in studies to understand the microbial shifts in an omeprazole therapy (52), studying the microbiota shifts from the saliva to the gut microbiota in cirrhosis (53).

The microbiota have been associated with the progression of liver disease in several conditions. These include the occurrence and propagation of pre-cirrhotic and cirrhotic liver disease. There is emerging evidence that pre-cirrhotic liver disease due to alcohol and NAFLD is modulated by the gut microbial milieu. Once patients reach the stage of cirrhosis, there is also data regarding impairment of gut-liver axis that leads to a vicious cycle of gut inflammation, systemic inflammation and worsening of liver disease complications. These complications include the development of hepatic encephalopathy (HE), gut-based infections such as spontaneous bacterial peritonitis (SBP) and the development of multi-organ failure, known as acute on chronic liver failure (ACLF).

The continuum of liver disease starts from mild injury and fibrosis to the liver, through the development of cirrhosis and then on to liver failure. This review will focus on microbial change in animal models and human studies in pre-cirrhotic as well as cirrhotic stages of liver disease.

Impact of Microbiome on Pre-cirrhotic Liver Diseases

While there are numerous reasons for liver disease and inflammation such as viral hepatitis, autoimmune diseases, NAFLD and alcohol are the two entities in which a role for the microbiota has been described in the greatest detail.

Non-alcoholic fatty liver disease (NAFLD)

NAFLD is the liver manifestation of the metabolic syndrome and encompasses a spectrum of hepatic pathology (54). NAFLD includes steatosis and nonalcoholic steatohepatitis. Accumulation of triglycerides in hepatocytes is the commonly observed phenotype in NAFLD (55). NAFLD is characterized by pathologic fat accumulation within the liver, ballooning degeneration and poses a risk of disease progression to cirrhosis and other complications of portal hypertension (56).

Various animal and human studies have characterized microbial communities in NAFLD. In a murine model (57), mice were fed on a high fat diet for 10 weeks and it was observed that their body weight increased by 34% compared to the low fat fed mice. The liver of the mice on high fat diet exhibited dramatic increase in number of lipid droplets, inflammatory cell infiltration and inducible nitric oxide synthase protein concentration. Additionally, the levels of lactobacillus species increased in mice with high fat diet in comparison to the mice on low fat diet. The amount of lactobacillus DNA in fecal samples showed a positive correlation to the severity of steatosis within mouse livers and this increase was attributed to lactobacillus having an effect on lipid metabolism through effects on bile acid metabolism contributing to the risk for fatty liver. Another murine study (58), the fecal samples were collected from mice on a high fat diet that demonstrated weight gain with increase systemic inflammation (increased serum levels of MCP-1 and TNF-α) and the fecal transfer to germ-free mice exhibited the phenotypic features of their donors. The microbial composition showed distinct differences at the phylum, genus and species levels for the recipients from the inflammatory and insulin resistant mouse to the recipients of non-inflammation and non-insulin resistant fecal donor samples with an increase in Firmicutes observed at the phylum level in the inflammatory and insulin resistant mouse demonstrating NAFLD development was contributed by the gut microbiota.

A mouse model deficient in pro-inflammatory multi-protein complexes, termed inflammasomes was used in this study (59). The mice were on a methionine choline-deficient, genetic leptin receptor deficient steatosis and high fat diet and all exhibited NAFLD phenotype through activation of pro-inflammatory pathways due to influx of TLR4 and TLR9 agonists into the portal circulation leading to hepatic tumor-necrosis factor (TNF) expression. Co-housing and transfer of the dominant microbiome from the inflammasome-deficient mice to wild type aggravated the phenotype of NAFLD and NASH, worsening hepatic steatosis and contributing to progression of NAFLD.

To investigate the effects of Resistin-like molecule β (RELMβ), a local immune response molecule on NASH, RELMβ-KO and wild-type mice were fed on chow diet or methionine-choline deficient (MCD) over a period of 8 weeks (60). The authors reported an increased proportion of *Lactobacillus* organisms in RELMβ-KO attributing these species as reducing

the NASH development by protecting against gut permeability induced by MCD diet suggesting RELMβ may contribute to increase of Kupffer cells to NASH development and considered as a novel therapeutic target for NASH. The authors demonstrated protective role of Lactobacillus casei strain in this study (61) where mice on normal chow diet (NCD), MCD diet or MCD diet plus *Lactobacillus* casei strain (LcS) for 6 weeks and *Bifidobacterium* and *Lactobacillus* were shown to be remarkably reduced by the MCD diet leading to NASH pathogenesis. The MCD diet plus LcS beneficially affected NASH, in part by the accompanying increase in *L.casei* subgroup and other lactic acid bacteria.

To study (62) the effects of Lipopolysaccharide (LPS), CL57BL/6 mice were fed with maintenance food (MF) or a high caloric diet (HCD) over a period of 6 months. The mice inoculated with LPS in the abdominal subcutaneous tissue, showed upward inflammatory cytokines and increase in Bacteroidaceae, Peptostreptococcaceae and Erysipelotrichaceae. The authors conclude that LPS increases the hepatic inflammation but the HCD diet was more crucial in progression of NAFLD.

The human studies in NAFLD have illuminated newer pathogenetic pathways —In a human study of a longitudinal metabolic study (63), the authors involved a population of 15 normal healthy adult females entailing them with an intervened choline depletion diet. The fecal microbiome was assessed for the subjects at baseline, after 10 days on a normal controlled diet, two times during a 42 day period on a choline-depleted diet and then twice during a 10 day choline repletion diet. The microbiome shifts were observed during this longitudinal study and the microbiome remained distinct for each individual but shifts in microbial community profiles were observed during the choline-depletion period. *Gammaproteobacteria* class decreased overall with restoration of choline diet. Abundance of *Gammaproteobacteria* class and at baseline inversely related with risk for developing fatty liver from choline-deficient diet. Abundance of *Erysipelotrichi* class (Firmicutes phyla) at baseline positively related with risk of developing fatty liver from choline-deficient diet. Fecal samples collected from obese children (64) with or without NAFLD were analyzed by shotgun sequencing, mass spectrometry for proteomics and NMR spectroscopy for metabolite analysis. Children with NAFLD microbiome were distinct from lean healthy children and displayed more abundant levels of *Gammaproteobacteria* and *Prevotella* with significant higher levels of ethanol. The metagenomics and proteomic analysis shows more bacterial pathways are involved in energy production and conversion and the authors suggest identification of these pathways may provide a tool to distinguish children with NAFLD.

To study the gut dysbiosis in the spectrum of NAFLD lesions (65), 57 patients with biopsy proven NAFLD were enrolled. Patients with significant F≥2 fibrosis were reported to have a significant amount of *Bacteriodes, Ruminococcus* and a decrease in *Prevotella*. The authors concluded that *Bacteriodes* is independently associated with NASH and *Ruminococcus* with significant fibrosis and suggest the microbiota analysis in NAFLD studies along with the metabolite information play a critical role to predict NAFLD classes and severity.

A 6 month study (66) of 16 NASH patients and 22 controls, NASH patients had lower fecal abundance of *Faecalibacterium* and *Anaerosporobacter* but higher abundance of

Parabacteroides and *Allisonella*. A partial least-square discriminant analysis produced a model of 10 genera discrimination between NASH patients and controls.

In a cross-sectional study to differentiate subjects based on their IM (67), 11 subjects with biopsy-proven simple steatosis, 22 NASH and 17 living liver donors as healthy controls were recruited. A quantitative real-time PCR measurement of bacterial counts showed that patients with NASH had a lower percentage of *Bacteroidetes/Prevotella* and higher fecal *C. coccoides* compared with SS. The authors concluded that there is an inverse and diet-/BMI-independent association between the percentage of *Bacteroidetes/Prevotella* and the presence of NASH.

To compare the colonic microbiome and volatile organic compounds (VOC), the authors (68) recruited 30 NAFLD patients and 30 healthy controls. An over-representation of Lactobacillus and Firmicutes was seen in NAFLD patients and a significant increase in fecal ester compounds. The authors concluded that compositional shifts in the microbiome of obese NAFLD patients is associated with obese NAFLD patients.

Zhu et al studied (32), 63 children's stool microbiome using 16s rRNA gene sequencing and assessed for cross-sectional differences between 22 children with NASH found on biopsy, 25 obese children with no clinical suspicion of NASH and 16 healthy normal weight control children. An abundance of Firmicutes and Bacteroidetes was observed in patients with NASH and those with obesity compared to controls. Increased serum ethanol levels were seen in children with NASH and the authors postulated that *Escherichia coli* might have elevated the blood ethanol levels. The authors also hypothesized that increased ethanol production by the microbiota could lead to chronic, low-level exposure to this hepatotoxin thus putting the individuals at risk for steatohepatitis.

A study (69) involving 61 children diagnosed with NAFL, NASH or obesity and 54 healthy children, NAFLD patients showed an increase of Bradyrhizobium, Anaerococcus, Peptoniphilus, Propionibacterium acnes, Dorea, Ruminococcus and reduced proportions of *Oscillospira* and *Rikenellaceae* compared to healthy subjects. A multivariate analysis of the microbiome and VOCs found that a combination of Oscillospira, Rickenellaceae, Parabacteroides, Bacteroides fragilis, Sutterella, Lachnospiraceae, 4-methyl-2-pentanone, 1-butanol, and 2-butanone could discriminate NAFLD patients from the healthy subjects.

Summary for NAFLD—The interplay of metabolic syndrome, diabetes and liver disease in NAFLD patients impacts the microbiota in complementary ways. Changes across the spectrum of NAFLD from simple steatosis through cirrhosis have been described in adults and children.

Alcoholic Liver Disease

Excessive use of alcohol over a prolonged period of time often results in alcoholic liver disease and alcoholic liver disease includes steatosis, steatohepatitis, acute alcoholic steatohepatitis, alcoholic fibrosis and cirrhosis. The interaction between the microbiome and the host liver is of special interest in alcoholic liver diseases where alcohol has shown to change the composition of the microbiome and affect the intestinal integrity and barrier

function (70) (71). Subjects under excessive consumption of alcohol and alcoholic liver cirrhosis have displayed higher levels of bacterial products in their blood than healthy humans (72) (73).

In an intragastric Tsukamoto-French mouse model (74), the mouse were fed on either isocaloric diet or alcohol for 3 weeks. It was observed that mice fed on an ethanol diet had a lower proportion of bacterial genes in the biosynthesis of saturated fatty acids. There was also a higher concentration of SCFA seen in the ethanol fed mice. A decrease in lactobacillus was observed at the order, family and genus levels for the ethanol fed mice when compared to control mice. This chronic ethanol administration of alcohol reduced the capacity of the intestinal bacteria to synthesize saturated LCFA in mice. When the homeostasis was restored with dietary supplementation of saturated LCFA, the ethanol-induced liver damage was reduced, dysbiosis was prevented by increasing intestinal levels of probiotic lactobacilli. The authors predicted that the lactobacilli appeared to produce factors that promote intestinal barrier function.

Experimental alcohol induced liver disease was studied (75) in a Tsukamoto-French method in wild type and Muc2 $^{-/-}$ mice to investigate the role intestinal mucus layer. The wild type mice showed higher alcohol induced liver injury and steatosis than in the Muc2 $^{-/-}$ mice. The authors concluded that Muc2 $^{-/-}$ mice were protected from the alcohol associated microbiome changes due to the presence of Muc2 and higher expression levels of antimicrobial proteins regenerating islet-derived 3 beta and gamma.

In another study (76), germ-free and conventional mice were humanized using human intestinal microbiota (IM) transplants from alcoholic patients with or without alcoholic hepatitis (AH). The IM composition of the patients showed remarkable differences: patients with severe AH (sAH) showed large amounts of *Bifidobacteria* and *Streptococci* and a tendency for less *Atopobium* than patients with no AH (noAH) showing that particular groups of gut bacteria associate with sAH. The germ free mice were transplanted with IM from 2 patients with alcohol consumption: one with noAH and the other with sAH and were fed on a Lieber-DeCarli diet containing 3% ethanol for 5 weeks. The transplant was successful in showing the disparity between the patients with noAH and sAH with only 30 Operational Taxonomic Units (OTUs) shared by their microbiomes. At the genus level, 23 genera differed significantly between sAH mice and noAH mice with *Bacteroides* displaying significantly abundance in sAH mice. *Bilophila,* which may be able to trigger colitis (77) and *Clostridium* cluster XIVa showed significant abundance numbers in sAH mice than in noAH mice which are found to induce proinflammatory cytokine responses (78). Several abundant OTUs observed in sAH corresponded to bacterial species associated with inflammatory diseases or insulin resistance. Higher intestinal permeability, liver inflammation was more severe with more infiltrating $CD45^+$ lymphocytes with a higher percentage of $CD3^+$, $CD4^+$, $CD8^+$ and NKT cells observed in mice that received IM transplants from sAH patient compared to noAH mice. A marked reduction of Muc2 expression which predominantly forms the mucin layer in the intestinal mucus layer was seen in sAH mice which could be contributors to the dysbiosis in the IM. The authors mimicked the clinical conditions and generated a fecal transplant into conventional mice and the results observed were reproducible as seen in the germ free mice.

Kirpich et al (79) evaluated the effects of dietary fat, metabolic activity and ethanol on the gut microbiota in the progression of Alcoholic Liver disease (ALD). Compared with ethanol and a saturated fat diet (medium chain triglycerides enriched), an unsaturated fat diet (corn oil enriched) Mice on unsaturated fat and ethanol diet exhibited a reduction in *Bacteroidetes* and an increase in *Proteobacteria* and *Actinobacteria*, exacerbated ethanol-induced endotoxemia, liver steatosis, and injury compared with a mice on saturated fat diet and ethanol. The authors conclude that the dietary lipids play an important role in ALD pathogenesis.

Human studies in pre-cirrhotic alcohol use have shown that gut leakiness and bacterial composition changes after short-term alcohol cessation (80). This shows dysbiosis in stool as well as colonic mucosal microbiota. In addition, there are rampant changes in the bacterial function, specifically bile acid conversion from primary to secondary in these patients. This could possibly worsen the liver injury from alcohol use.

To study the effects of colonic bacteria on alcohol consumption, a study (81) evaluated the alterations in colonic microbiome in 48 alcoholics with and without ALD and compared this to 18 healthy subjects. The alcoholics demonstrated dysbiosis and displayed lower median abundances of *Bacteroidetes* and higher ones of *Proteobacteria* . These bacterial taxa correlated with high levels of serum endotoxin in a subset of the samples.

In another study (82), the authors relate alcohol dependence to depression and anxiety discussing this relationship in a subset of alcohol dependent population with increased intestinal permeability association and dysbiosis. This subset population displayed alterations in the metabolomic profiles, persistent systemic inflammation, along with increased symptoms of depression, anxiety and craving at the end of alcohol withdrawal linking the reinforcement to the processes occurring at the level of the gut with relation to the presence of dysbiosis.

Summary for ALD—The multi-factorial changes with alcohol misuse span the gut directly in addition to causing liver disease, both of which can impact the microbiota. Microbiota changes have been described in the setting of binge drinking, chronic alcohol consumption, alcoholic liver disease and cirrhosis and in alcoholic hepatitis.

Impact of Microbiome on Cirrhosis

The gut microbiota plays a key role in cirrhosis due to BT leading to complications including hepatic encephalopathy (HE) and spontaneous bacterial peritonitis (SBP) (34) (83) (84) (85). The bacterial infections which occur due to this bacterial translocation facilitate an altered intestinal immunity and bacterial overgrowth. These infections contribute to multi-organ failure, ACLF and death in cirrhosis (86) (87) (88).

The bacterial infections seen in cirrhotic patients occurs mostly due to intestinal bacterial overgrowth (11), immune dysfunction and this is multifactorial (11) (89) mainly contributed by a decreased bactericidal activity by phagocytic cells (90) (91) (92). Enteric gram-negative bacteria (mostly *Escherichia coli*) are most commonly isolated in SBP (93). BT causes local/systemic immune defense mechanisms failure and play a significant role in progression to

cirrhosis with the bacterial products leading to activation of monocytes, lymphocytes and increased serum levels of tumor necrosis factor (TNF-α) an inflammatory cytokine with consequent activation of nitric oxide (NO). Activation of NO and increased serum levels contributes to systemic vasodilation, increased cardiac output, decreased mean arterial pressure and is responsible for variceal growth, ascites and hepatorenal syndrome (94) (95).

Bacterial infections are commonly seen in decompensated cirrhotic patients and SBP is the most common type of infection. SBP is mostly induced by aerobic Gram negative enteric organisms, mainly *E. coli* and *Klebsiealla pneumoniae*(96) (97) (98) . An increased levels of endotoxin has been shown in patients with advanced stage of cirrhosis (99) and this presence confirms the role of multiple inflammatory cytokines such as TNF-α (100). The activation of the cytokine cascade in SBP has been directly related to complications in patients and death (101) (102). In humans with cirrhosis admitted with infections such as SBP, there was inherent pre-existing gut dysbiosis that was significantly worse in MELD-matched uninfected patients and healthy controls (73). This shows that infections may be related to altered gut microbiota in cirrhotic patients.

Hepatic encephalopathy (HE) is a very common neurocognitive impairment in cirrhosis and ranges from minimal HE (no asterixis or disorientation) to overt HE (mental status changes range from simple disorientation through coma) (103). The gut microbiota is altered in cirrhotic patients with HE with a higher relative abundance of potentially pathogenic bacteria and a reduction of commensal bacteria (104). This was also found in the colonic mucosal microbiome composition which was associated with systemic inflammation and cognitive function (105) .

A study (106) carried out in 69 outpatients with minimal HE showed a significant fecal overgrowth of potentially pathogenic *Escherichia coli* (*E. coli*) and *Staphylococcus spp.* in the gut microbiota. In another minimal HE study (107), a higher amount of *Streptococceae* and *Veillonellaceae* in cirrhotics with and without minimal HE. *Streptococcus salivarius* abundance was shown to be significantly higher in patients with minimal HE and this change also showed a correlation with serum ammonia.

An increased *Alcaligenaceae* abundance was significantly associated with poor cognitive performance in pathogen free mice study (108). *Alcaligenaceae* are Proteobacteria that degrade urea to produce ammonia, which may explain his association with poor cognitive function whereas *Enterobacteriaceae* were associated with worsening inflammation. This was shown in human HE studies, where a higher relative abundance of *Enterobacteriaceae, Alcaligeneceae*, and *Fusobacteriaceae* and lower abundances of *Ruminococcaceae, Lachnospiraceae* was shown in cirrhosis (104) (105). Both these studies confirmed that *Alcaligeneceae* and *Porphyromonadaceae* were correlated with poor cognition. *Enterobacteriaceae* was strongly associated with worsening inflammation and MELD scores with triggering of the IL-23/IL-17 pathways indicating a repeated exposure of infectious agents in the intestine, activating the proinflammatory cytokines.

The worsening of cirrhosis dysbiosis ratio (CDR) was observed in a longitudinal cross-sectional study of cirrhotic patients (73), a lower CDR ratio indicating dysbiosis. 244

subjects at various cirrhotic stages were recruited and it was observed that CDR was highest in controls (2.05) followed by compensated (0.89), decompensated (0.66) and inpatients (0.32). The autochthonous bacteria were also reported to relatively decrease with a lower CDR particularly, *Lachnospiraceae*, *Ruminococcaceae* and *Clostridiales XIV*, with relative overgrowth of potentially pathogenic taxa; *Staphylococcaeae, Enterobacteriaceae* and *Enterococcaceae*. This reduction of autochthonous taxa affects the production of short-chain fatty acids and their reduction results from a reduction in overall bile acid production with worsening cirrhosis severity.

ACLF and outcomes can be predicted by microbiota: Microbial profile can predict the occurrence of organ failures and death in hospitalized cirrhotic patients (73) (74). Moreover gut microbial changes can predict the occurrence of hospitalizations in outpatient cirrhotic patients independent of cirrhosis severity, prior hepatic encephalopathy and other clinical biomarkers (109).

Interestingly, not only the gut-liver axis, but also salivary inflammation and microbiota have been found to be impaired in patients with cirrhosis (53). The microbiota in both saliva and stool were related to the systemic inflammation and presence of the salivary microbiota in the stool is likely due to an epiphenomenon of impaired bile and gastric acid output in cirrhosis. The salivary dysbiosis was found to be greater in hospitalized patients with cirrhosis and could be due to a systemic pro-inflammatory milieu over a 90 day period.

Summary for cirrhosis

The impact of microbiota in cirrhosis progression and as a target for therapies is being investigated in recent human studies. Changes in gut-liver-brain axis, specifically targeting hepatic encephalopathy and infections are particularly interesting in this field.

Microbiota-related treatments in Liver Disease

There is much interest in manipulating the gut microbiota in order to modify the effects of the pathogenic bacteria in the broad spectrum of pre-cirrhotic liver diseases.

Probiotics, prebiotics and synbiotics are regularly used to improve the health of the IM. Probiotics are live bacteria that add or replace the beneficial bacteria normally present in the human gut. Animal studies (110) including both wild-type mice and high fat diet-induced obese mice were given either placebo or a probiotic consisting of *Bifidobacterium pseudocatenulatum* for 7 weeks. Metabolic parameters such as changes in insulin resistance, were measured between the mouse groups and samples were tested to study the preventive effects of steatosis with pre and post probiotic intervention. The authors found that probiotic supplementation was able to improve the metabolic and immunological dysfunction and resulted in a decreased insulin resistance, decreased hepatic steatosis, reduction in serum inflammatory markers compared with the high fat diet fed mice.

In a longitudinal microbiome study (66), 20 adult patients with biopsy-proven NASH were randomly given a probiotic supplements for 6 months. In the probiotic group, hepatic fat

content decreased and an increase in Bacteroidetes with a decrease in Firmicutes was shown among the overall group of NASH patients.

Treatments in Cirrhosis

The prevention of bacterial infections has been focused on use of prophylactic antibiotics which target the most common pathogenic microorganisms in cirrhosis, Enterobacteriaceae and non-enterococcal streptococci.

Bacterial overgrowth of the small intestine is prominent in cirrhotic rats with ascites and patients with cirrhosis and is thought to be the most common site of bacterial translocation (111) (112) (113). Rifaximin is a broad spectrum bacteria that was used to reduce the overall burden of intestinal microbiota by non-selectively eliminating gut microbes (114) (115). This study (116) has also shown that rifaximin has a direct effect on bacterial function by impairing their ability to translocate. Rifaximin's activity is specific to the gut showing no absorption into the systemic circulation limiting its systemic toxicity or side effects (117). Rifaximin has also been shown to maintain remission from HE in cirrhosis (118) and a tendency towards beneficial impact on the infection and variceal bleeding rate (119).

On the other hand, the first-line treatment, lactulose showed minimal impact on bacterial composition. A longitudinal systems biology analysis on lactulose withdrawal in a hepatic encephalopathy study (132) showed that withdrawal of lactulose resulted in worsening cognition, mixed inflammatory response effect, lowered stool *Faecalibacterium* and increase in MR-measurable brain Glutamate and glutamine. However, these studies show that microbial functionality rather than composition, may play a greater role in the mechanism of action of rifaximin and lactulose in hepatic encephalopathy.

Lactobacillus spp. have been shown to have protective effects on the intestinal mucosa by lowering intestinal pH, they prevent colonization of pathogenic species, and modulate the immune response; improving the overall gut function (120). *Lactobacillus* GG is a well-studied probiotic and to determine its effects on patients with cirrhosis, this study (121) showed that use of *lactobacillus* GG in a randomized placebo-controlled double blind trial showed a reduction in endotoxemia and a reduction in gut dysbiosis with improved gut microbiome-metabolome linkages.

Prebiotics are non-digestable food ingredients which the beneficial bacteria utilize for their nutrition and help their survival in the gut. Synbiotics are a combination of probiotics and prebiotics. Administration of Lactobacilli has been shown to be effective at reducing bacterial translocation, serum alanine aminotransferase levels in a study (122) involving a rat model with acute liver injury. *Lactobacillus johnsonii* La1 administered along with antioxidants has shown to reduce bacterial translocation and endotoxemia compared to control (123). VSL#3, an eight-species probiotic cocktail (three bifidobacteria species and five lactobacilli species) has been shown to decrease bacterial translocation and improved intestinal permeability in rats with cirrhosis (124).

Vasodilatation (splanchnic and systemic) which is seen in cirrhosis mostly occurring in patients with decompensated cirrhosis. It leads to a delayed intestinal transit and can be

reversed with β-blockers. β-blockers may act as an antibacterial targeting mechanisms responsible for bacterial translocation in cirrhosis by blocking norepinephrine which helps the growth of Gram-negative rods and intestinal permeability (125) (126). Propranolol has been shown to significantly accelerate intestinal transit, reduce rates of bacterial overgrowth in the bowel and bacterial translocation (127). In a propranolol study (128) on patients with cirrhosis, propranolol reduced intestinal permeability.

Farnesoid X receptor (FXR), a nuclear receptor and transcription factor is a chief regulator of the metabolism of bile acid, lipids and carbohydrates. It is activated by bile acids such as cholic and chenodeoxycholic acid. FXR prevent bacterial translocation in cirrhosis by inducing genes involved in enteroprotection. Studies (129) in FXR-deficient rats exhibit bacterial translocation and intestinal permeability. Obeticholic acid (6-ethylchenodeoxycholic acid) an agonist of FXR, was shown in bile duct-ligated rats to reduce intestinal inflammation and reduce the translocation of bacteria to mesenteric lymph nodes. In a human study (130) with noncirrhotic, nonalcoholic steatohepatitis patients, obeticholic acid was recently shown to improve liver fibrosis.

Omeprazole, is a common drug used to treat gastric mucosal lesion. To understand the protective effect of gastric mucosal region, this study (131) studied the effects on rats. The authors suggest that omeprazole plays a protective role in the gastric mucosa by improving the gastric environment, by reducing the damage to the gastric mucosa through the influence of inflammatory cytokines. In a human study (52), the subjects given a short period of omeprazole therapy displayed a shift in the urinary NMR metabolic profile and also the stool microbiota function and composition showing positive effects of omeprazole.

Future directions

There is emerging strong translational and clinical evidence that microbiota can influence the development and progression of liver disease. Further studies focusing on specific components of the microbial functional output need to be developed along with human studies with clinically relevant endpoints. This will further increase the adoption of these techniques into practice.

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