**Name of Journal:** *World Journal of Hepatology*

**Manuscript NO:** 74930

**Manuscript Type:** REVIEW

**Gut microbiota contribution to hepatocellular carcinoma manifestation in non-alcoholic steatohepatitis**

Liakina V *et al*. Gut microbiota in NASH-induced HCC

Valentina Liakina, Sandra Strainiene, Ieva Stundiene, Vaidota Maksimaityte, Edita Kazenaite

**Valentina Liakina, Ieva Stundiene, Vaidota Maksimaityte, Edita Kazenaite,** Centre of Hepatology, Gastroenterology and Dietetics, Clinic of Gastroenterology, Nephrourology and Surgery, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius 01513, Lithuania

**Valentina Liakina,** Department of Chemistry and Bioengineering, Faculty of Fundamental Sciences, Vilnius Gediminas Technical University (VILNIUS TECH), Vilnius 10223, Lithuania

**Sandra Strainiene,** Faculty of Medicine, Vilnius University, Vilnius 01513, Lithuania

**Sandra Strainiene,** Therapeutic and Radiological Department, Antakalnis Polyclinic, Vilnius 10207, Lithuania

**Edita Kazenaite,** Department of Pathology, Forensic Medicine and Pharmacology, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius 01513, Lithuania

**Author contributions:** Liakina V and Maksimaityte V performed the literature search, reviewed the literature,and wrote the original manuscript; Stundiene I and Strainiene S reviewed and edited the manuscript; Kazenaite E revised the manuscript for the important intellectual content; All authors have read and approved the final manuscript.

**Corresponding author: Valentina Liakina, PhD, Senior Research Fellow,** Centre of Hepatology, Gastroenterology and Dietetics, Clinic of Gastroenterology, Nephrourology and Surgery, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, 3 Universiteto Street, Vilnius 01513, Lithuania. valentina.liakina@santa.lt

**Received:** January 15, 2022

**Revised:** April 27, 2022

**Accepted:** July 11, 2022

**Published online:**

**Abstract**

Recently, the gut microbiota has been recognized as an obvious active player in addition to liver steatosis/steatohepatitis in the pathophysiological mechanisms of the development of hepatocellular carcinoma (HCC), even in the absence of cirrhosis. Evidence from clinical and experimental studies shows the association of specific changes in the gut microbiome and the direct contribution to maintaining liver inflammation and/or cancerogenesis in nonalcoholic fatty liver disease-induced HCC. The composition of the gut microbiota differs significantly in obese and lean individuals, especially in the abundance of pro-inflammatory lipopolysaccharide-producing phyla, and, after establishing steatohepatitis, it undergoes minor changes during the progression of the disease toward advanced fibrosis. Experimental studies proved that the microbiota of obese subjects can induce steatohepatitis in normally fed mice. On the contrary, the transplantation of healthy microbiota to obese mice relieves steatosis.However, further studies are needed to confirm these findings and the mechanisms involved. In this review, we have evaluated well-documented clinical and experimental research on the role of the gut microbiota in the manifestation and promotion of HCC in nonalcoholic steatohepatitis (NASH). Furthermore, a literature review of microbiota alterations and consequences of dysbiosis for the promotion of NASH-induced HCC was performed, and the advantages and limitations of the microbiota as an early marker of the diagnosis of HCC were discussed.

**Key Words:** Gut microbiota; Hepatocellular carcinoma; Non-alcoholic steatohepatitis; non-alcoholic fatty liver disease; Microbiome

Liakina V, Strainiene S, Stundiene I, Maksimaityte V, Kazenaite E. Gut microbiota contribution to hepatocellular carcinoma manifestation in non-alcoholic steatohepatitis. *World J Hepatol* 2022; In press

**Core Tip:** Although the incidence of life-threatening cases of hepatocellular carcinoma (HCC) induced by nonalcoholic steatohepatitis (NASH) has recently increased due to the dramatic increase in steatohepatitis, the pathophysiological mechanisms of the manifestation of HCC nodules have not yet been fully elucidated. There is a lack of tools to diagnose HCC at an early stage, especially considering that HCC can occur in patients with NASH even in the absence of cirrhosis. In this review, we have evaluated the current state of research on the role of the gut microbiota in promoting NASH-induced HCC and the use of the microbiota for the early diagnosis of HCC.

**INTRODUCTION**

In the different regions of the world, NAFLD affects 4%-55% of the population[1,2]. Subjects with NAFLD are constantly at risk of developing chronic liver inflammation leading to nonalcoholic steatohepatitis (NASH) and eventually progressing from liver fibrosis to cirrhosis. The latter has a higher risk of hepatocellular carcinoma (HCC) manifestation[3]. Although the risk of NAFLD progression to cirrhosis is less likely than in viral hepatitis (approximately 10% of NASH[4], and less than 1% of patients with NAFLD developed HCC within 8 years after initial diagnosis[5,6]), NASH alone can cause HCC even in the absence of cirrhosis, and this raises concerns[7–9]. Furthermore, it is estimated that HCC cases related to NASH may increase by up to 56% in the next 10 years[10].

In some cases, prolonged inflammation of the liver caused by steatosis appears to be a sufficient circumstance to cause the rise of the so-called compensatory proliferation of hepatocytes, which triggers the formation of HCC nodules[5], but the precise pathophysiological mechanism is still far from complete elucidation. To some extent, NAFLD/NASH mice models are helpful. However, translating animal studies into a human context is always difficult because only reliable mechanistic information comes from these studies[11].

In addition to liver steatosis / steatohepatitis, the gut microbiota has recently been recognized as an obvious active player in NAFLD-induced HCC. Experimental and clinical studies demonstrate a stimulating role of the intestinal microbiota in maintaining liver inflammation and an alteration of the microbiome composition toward a more pro-inflammatory state with the progression of liver disease from NAFLD to NASH at different stages of fibrosis and HCC[12,13]. It seems like this is a mutually supportive process. This has been confirmed by a study of germ-free mice transplanted with stool from genetically obese patients. Soon after the guts of these mice were colonized by the microbiota of obese subjects, a steatosis manifested in their livers despite a balanced diet[14]. On the contrary, fecal microbiota transplantation from healthy mice alleviated steatohepatitis in mice fed a high-fat diet[15].

The liver is closely related to the intestinal tract and serves as a vital metabolic center for digestion, detoxification, and clearance of microbial products[16]. Research on the gut-liver axis has greatly contributed to understanding the basic pathophysiology of liver diseases, including NAFLD of different severity and malignancy of the liver parenchyma[17,18].

In this review, we conducted a survey of the current state of research on the contribution of the gut microbiota to the manifestation and progression of HCC in patients with NASH**.**

**LITERATURE SEARCH AND ANALYSIS OF CLINICAL AND EXPERIMENTAL STUDIES SELECTED**

An electronic search of the literature on the microbiota in NASH-induced HCC was performed. Articles available in the PubMed, Medline, Cochrane, and Web of Science databases were reviewed up to November 12, 2021. The search terms used were "nonalcoholic fatty liver disease AND hepatocellular carcinoma AND microbiome", "nonalcoholic fatty liver disease AND hepatocellular carcinoma AND microbiota”, "nonalcoholic steatohepatitis AND hepatocellular carcinoma AND microbiota", "nonalcoholic steatohepatitis AND hepatocellular carcinoma AND microbiome", “nonalcoholic steatohepatitis AND liver cancer AND microbiota” and “nonalcoholic fatty liver disease AND liver cancer AND microbiota”. No time restrictions were used for publications. A total of 1,073 articles and abstracts met the initial search criteria.

The titles, abstracts, and full papers were reviewed to identify full-text articles focusing on alterations in the gut microbiota in NASH/NAFLD - HCC compared to healthy controls, as well as animal model studies discussing changes in the gut microbiota in NASH / NAFLD-induced HCC (Supplementary Figure 1).

Inclusion criteria were: well-documented full-text articles written in English, presence of the following study groups – NAFLD/NASH with/without cirrhosis, NAFLD/NASH-HCC with/without cirrhosis, control group of healthy subjects.

Exclusion criteria after abstract and full text reviews were: articles written in other languages than English, no presence of NAFLD/NASH - HCC, no evaluation of the NASH/NAFLD - HCC microbiota, no control group.

Following a comprehensive review of the current literature, we identified only six publications focusing on the gut microbiota in NASH/NAFLD induced HCC that were fully consistent with the inclusion criteria[12,13,19–22]. Three selected articles were clinical studies, in which the microbiota composition of 86 patients with HCC induced by NAFLD was analyzed among others with NAFLD of different severity (Table 1)[13,19,20]. The other three publications included animal model studies in which mice with NAFLD and HCC microbiota were analyzed (Table 2)[12,21,22]. The circumstantial analysis of the selected studies is presented below.

***Human studies***

All three identified clinical studies on NASH-induced HCC were cross-sectional. Two of them compared cirrhotic NAFLD with or without HCC with healthy controls[19,20], and one compared patients with NASH together, NASH-HCC with or without cirrhosis, and healthy controls[13]. In total, 168 patients with NAFLD and 70 controls were enrolled. The HCC had 72(55%) of 131 cirrhotic patients and 14(37.8%) of 37 without cirrhosis.

The α-diversity and bacterial richness were analyzed. Behary *et al*[19] confirmed dysbiosis in the NAFLD-HCC and NAFLD-cirrhosis groups compared to healthy controls. Patients in these following groups had reduced α-diversity (a measure of microbiome diversity applicable to a single sample) and the Chao-1 richness index. However, no other differences were observed in other alpha-diversity measures (Shannon’s diversity index, Evenness index). A study by Sydor *et al*[13] showed that the rarity index increased in patients with NASH-HCC with cirrhosis compared to the control group. In the third study by Ponziani *et al*[20], α-diversity was reduced in the NAFLD-HCC group compared to healthy controls. However, diversity changes were not specified when comparing NAFLD-HCC with cirrhosis and NAFLD-HCC without cirrhosis.

There is a consistent amount of evidence that the gut-liver axis plays an important role in the progression of liver diseases[17,18]. In a study by Komiyama *et al*[23], the most common phyla of the gut microbiota (*Bacteroidetes, Firmicutes*, and *Proteobacteria*) were also dominant in HCC, suggesting that an increased abundance of these phyla is also found in subjects with HCC induced by NAFLD.

Ponziani *et al*[20] demonstrated an increased quantity of *Bacteroides* and *Lactobacillus* in cirrhotic patients with or without HCC. Furthermore, with deficiency of *Bifidobacterium* and *Blautia,* HCC patients had an even higher abundance of *Bacteroides* and R*uminococccaceae, Enterococcus, Phascolarctobacterium,* and *Oscillospira* than the NAFLD-non-HCC with cirrhosis patient group. A study by Behary *et al*[19] also showed a significant enrichment of *Bacteroides xylanisolvens* and *Ruminococcus gnavus* in both the NAFLD-HCC and NAFLD-cirrhosis groups compared to healthy controls. *Bacteroides caecimuris* and *Veillonella parvula* were specifically enriched in the NAFLD-HCC group compared to the control and NAFLD-cirrhosis groups[19]. However, Sydor *et al*[13] demonstrated a reduction in the abundance of *Bacteroidetes* along with Gram-positive *Actinobacteria* and *Bifidobacterium* and an increased abundance of *Proteobacteria* and *Lactobacillus* in patients with NASH-HCC.

In a previous study, the *Bacteroides* genera were also enriched in HCC *vs* patients with cirrhosis, suggesting that the enrichment of *Bacteroides* in the gut microbiota may be associated with the diagnosis of liver cancer[24].

***Animal studies***

We identified 3 animal studies (mice) investigating changes in the gut microbiome in NAFLD-induced HCC, summarized in Table 2[12,21,22]. To induce HCC, mice were fed a high-fat diet (high-fat/high-cholesterol (HFHC) and high-fat/low-cholesterol (HFLC). In one study, additional intraperitoneal injections of CCl4 were administered once a week to induce HCC[21].

Animal studies demonstrated the same results regarding α-diversity in the gut microbiome in HCC induced by NAFLD. In all studies, α-diversity was reduced in HCC mice compared to the control group. A study by Zhang *et al*[22] also showed that mice fed the HFHC diet had lower bacterial diversity than mice fed the HFLC diet. HFHC-fed mice also had a higher association with the development of HCC.

***Increased LPS across the intestinal barrier in mice with NAFLD-induced HCC***

Some studies in humans observed increased serum lipopolysaccharide (LPS) levels in HCC patients[25,26]. It indicated an increase in permeability of the intestinal epithelial barrier[23].

Thus, it was no surprise that higher serum LPS levels were observed in three reviewed animal studies[12,21,22]. Mice fed a high-fat streptozocin diet (STZ) and developed HCC had a higher abundance of *Bacteroides* and *Desulfovibrio* in their gut microbiome[12]. Since most *Bacteroides* and *Desulfovibrio* species are producers of LPS, higher LPS concentrations were found in HCC mice' blood. In a study by Carter *et al*[21], NASH-induced HCC mice had increased gut permeability, which also resulted in elevated serum LPS.

Recent studies showed that circulating LPS was significantly elevated in patients with colorectal cancer compared to healthy controls. Furthermore, the authors concluded that serum LPS can cause chronic inflammation and activate the coagulation system, leading to cancerogenesis[27]. New studies show that elevated levels of circulating LPS may be highly associated with many chronic liver diseases, including liver fibrosis and HCC[28,29].

**NASH-INDUCED HCC PATHOGENESIS ASSOCIATIONS WITH GUT MICROBIOTA**

The accumulation of lipid droplets alone does not cause liver damage or inflammation. Hepatosteatosis (a.k.a. "bland steatosis") requires a necro-inflammatory mechanism characterized by ballooning hepatocytes, liver injury, and fibrosis[5]. The inflammation of the liver could be triggered by provocative factors, such as oxidative stress, stress of the endoplasmic reticulum, and/or the presence of infectious or commensal organisms[30]. This so-called two-hit hypothesis was first formulated by Day and James[31].

The specific mechanism that links the gut microbiota with the progression of NAFLD is still unclear. However, bacterial overgrowth, translocation of microorganisms, increased endotoxin absorption, and enterohepatic secondary bile acids may be possible explanations[32].

***Leaky gut***

Patients with exacerbated liver function have increased intestinal permeability and impaired mucosa due to the alternation of the tight epithelial junction[25,33]. This leads to the leakage of chemicals derived from the microbiota into the bloodstream of the portal vein. The more severe and long-lasting the liver disease, the higher the levels of different potentially pro-inflammatory and pro-oncogenic microbial products that might be detected in the blood of patients~~[~~25]. It should be noted that this state is often worse in the NASH population due to a high-fat/high-carbohydrate diet that maintains the pro-inflammatory alteration of the intestinal microbiota[34]. Improvement in liver function tests following dietary correction in clinical trials in patients with NASH / obesity is evidence of reduced parenchymal inflammation[35]. Mice experiments also confirmed the importance of diet for the healthy shape of the gut microbiota[15].

***Bacterial overgrowth***

There is a link between bacteria overgrowth and NAFLD/NASH. Approximately 50%-80% of patients with NAFLD/NASH have small intestine bacterial overgrowth (SIBO)[7]. SIBO, together with alteration of the intestinal microbial community, has been detected in NAFLD-induced chronic liver inflammation conditions of different stages[16].

In several clinical studies, an abundance of the *Veillonella* genus was found in the duodenum and colon of cirrhotic patients, along with the reduction of the genus *Akkermansia* and *Prevotella*[16,36]. Loomba *et al*[37] observed an increased quantity of *Bacteroides vulgatus* and *Escherichia coli* (*E. coli*) in patients with advanced NAFLD-induced fibrosis. *E. coli* was also predominant in patients with SIBO-affected NAFLD[38].

More studies are needed to show the prevalence of SIBO in patients with NASH-induced HCC.

***Dysbiosis***

Dysbiosis of the gut microbiota has been associated with a higher risk of certain cancers and has been shown to affect the body's reaction to various cancer treatments[39,40]. Furthermore, a reduction in the diversity of the intestinal microbiome has been reported in inflammatory bowel diseases, colorectal cancer, and gastric cancer[41–43]. The diversity of the gut microbiota is now considered an important environmental characteristic of NAFLD, since it can impact host metabolic processes, such as the extraction of energy from food. Through mechanisms such as altered hunger signaling, enhanced energy extraction from the diet, and altered regulation of gene expression involved in de novo lipogenesis or oxidation, the gut microbiota has the ability to increase intrahepatic fat[44].

It should be noted that researchers observed a larger difference in the abundance of bacteria at the levels of phylum, family, and genus levels between healthy and obese subjects, while relatively fewer differences were observed between obese and the NASH microbiome[45]. The only abundance of *Proteobacteria*, *Enterobacteria*, and *Escherichia* differed between obese and NASH[46]. Ezzaidi *et al*32] found that patients with NASH have a lower abundance of *Faecalibacterium* and *Anaerosporobacter,* but a higher abundance of *Parabacteroides* and *Allisonella*. They also noted that the reduction in *Firmicutes* and the increase in *Bacteroidetes* were associated with an improvement in steatosis. However, *Bacteriodetes* are known as LPS-producing bacteria, which is why they are pro-inflammatory[32].

An elevated abundance of *Bacteroides* *vulgatus* and *E. coli* has been discovered in NAFLD patients with advanced fibrosis[37]. Fecal *Bacteroides* and *Ruminococcus* were independently related to NASH and fibrosis (stage 2 or above), while *Prevotella* decreased under the same circumstances[36].

The role of the microbiome in NAFLD-HCC is mainly unknown. The clinical studies summarized in Table 1 of this review agree on the decrease in the diversity of bacteria in patients with NASH-HCC, but demonstrate a discrepancy in the abundance of various representatives of the gut microbiota. Only changes at the phyla level toward LPS producers have been confirmed in all studies.

The gut microbiota produces a wide range of bioactive chemicals, including those from food substances [LPS, short-chain fatty acids (SCFA), deoxycholic acid (DCA)], resulting in a complex transgenomic metabolism between the microbiota and the host that significantly affects physiological and pathological states[47]. Through the gut-liver axis, intestinal microbial dysbiosis is linked to hepatic inflammation and HCC[32].

Dysbiosis of the intestinal microbiota appears to be a novel component that promotes the development of NALFD-induced HCC. The manifestation of HCC has been associated with increased *Bacteroides* and *Ruminococcaceae,* but lower *Bifidobacterium* in patients with NAFLD**[**20]**.**

The increase in *Bacteroides* and *Ruminococcaceae* in the HCC population is associated with higher levels of calprotectin and systemic inflammation[16,19,20,48,49]. In general, researchers agree that the gut bacteria of obese subjects promote HCC. However, the patterns of bacterial abundance were not consistent between studies. For example, some studies claimed an increase in *Bacteroidetes* in advanced NASH[19,20,37],, while other studies showed that patients with NASH possessed a lower abundance of *Bacterioidetes[*13].

**MECHANISMS OF MICROBIOTA CONTRIBUTION TO PERSISTENT LIVER INFLAMMATION AND HEPATOCARCINOGENESIS**

Since liver disease may be accompanied by SIBO and altered gut permeability, a correlation of the increased level of bacterial products in the portal blood can be expected with the severity of the disease. Due to the altered intestinal barrier, bacterial products derived from gut microbes (microbial-associated molecular patterns (MAMPs): LPS, peptidoglycan, and bacterial unmethylated cytosine–phosphate–guanine dinucleotides (CpG) DNA, DCA, and lipoteichoic acid (LTA), ethanol, acetone, butanoic acid, and many other molecules) can enter the liver and activate toll-like receptors (TLRs) in Kupffer cells, liver stellate cells, and hepatocytes, leading to an inflammatory response that promotes NASH[7,16,32]. In humans, TLR-2, TLR-4, and TLR-9 are known to be involved in the pathogenesis of NASH[50].

According to recent experimental and clinical studies, the intestinal microbiome can contribute to all histological components of NAFLD: liver steatosis, inflammation, and fibrosis[48]. As HCC in patients with NASH can occur in the absence of cirrhosis[8,9,51,52], chronic inflammation of the liver is the most important circumstance for its manifestation[53].

Several studies of NASH-induced HCC reported the correlation of *Bacteroides* and *Ruminococcaceae* expansion with systemic inflammation[19,20,48,49]. It is well known that after pro-inflammatory stimulation by nutrients metabolites or/and bacterial molecules that enter the liver, Kupffer cells, liver stellate cells, and infiltrating macrophages produce a variety of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL) -6, and IL-8, to establish the immune response. Increased levels of these cytokines have been detected in patients with NASH[54,55].

These cytokines contribute to the development of NASH and HCC by activating nuclear factor kappa-B (NF-κB) and STAT3 in initiated hepatocytes[30]. However, it is not yet clear how pro-inflammatory events trigger the development of HCC and how malignant hepatocytes escape the immune attack. Evidence from the experimental study elucidated a suppressive impact of immunoglobulin A+ plasma cells on cytotoxic T lymphocytes by expression of programmed death ligand 1 (PD-L1) that leads to the exhaustion of CD8 + T lymphocytes[56]. PD-L1 inhibitors appeared to be highly effective for HCC treatment[57]. The inflammatory cytokine profile and TNF-α activated NF-κB signaling, as well as the exhaustion of CD8+ T lymphocytes, are characteristic of HCC of non-NASH etiology[5].

***LPS producing bacteria can induce liver inflammation and promote carcinogenesis***

LPSs are active components of bacterial endotoxins released by Gram-negative bacteria after their death. LPS-specific TLR-4s are expressed by monocytes, mast cells, B cells, and the intestinal epithelium[1]. After release from the wall of the bacteria cell, LPS forms a complex with the lipopolysaccharide binding protein, CD14, and TRL4 and enters circulating blood due to increased intestinal permeability[58].

Hepatocytes, Kupffer cells, and liver stellate cells also express LPS-specific TLR-4. After activation of TRL-4 by LPS in Kupffer cells, an intracellular inflammatory cascade is triggered, inducing the production of pro-inflammatory cytokines (TNF-α, IL-6)[59,60].

TLR-4 activation also leads to overexpression of hepatomitogen epiregulin, which promotes mitosis of hepatocytes and, therefore, hepatocarcinogenesis. At the same time, LPS-activated liver stellate cells gain a pro-inflammatory state and start to secrete collagen, inducing liver fibrogenesis and vascular endothelial growth factor, which participates in hepatocarcinogenesis by promoting neoangiogenesis[47,61].

Furthermore, caspase-3 cleavage, responsible for cell apoptosis, appears in hepatocytes through the NF-κB-mediated mechanism[47]. All of the mentioned events lead to the survival of malicious hepatocytes and the formation of HCC nodules. In patients with liver cancer, the activated LPS-TLR-4 pathway is associated with increased invasiveness of tumor cells induced by NF-κB-mediated epithelial-mesenchymal transition and, consequently, metastasis and poor prognosis[62,63].

***Other pro-inflammatory and pro-oncogenic impacts of the microbiota in NASH-induced HCC***

Alongside TLR-4, Kupffer and hepatic stellate cells possess TLRs with specificity to other MAMPs. TLR-2 can be activated by components of Gram-positive bacterial cell walls, such as peptidoglycan and lipoteichoic acid. Through mitogen-activated protein kinases (MAPKs) induced by MyD88/MAL and NF-κB-mediated transcriptional programs, they promote liver tumorigenesis[16,64]. TLR-2, activated by lipoteichoic acid, along with secondary bile acid deoxycholate, promotes DNA damage, cell senescence, and apoptosis, and incites obesity-associated tumorigenesis through a pro-inflammatory and immunosuppressive pro-tumorigenic environment involving prostaglandin E2[65,66]. NASH progression and NASH-induced HCC have been prevented in an experimental model by treating mice with sequestrant bile acids[67].

TLR-9 is an intracellular receptor that detects bacterial and viral DNA. It recognizes DNA containing unmethylated CpG motifs, which are common in bacteria[64,68]. The TLR-9 signaling pathway induces IL-1b production by Kupffer cells, leading to steatosis, inflammation, and fibrosis. IL-1b promotes lipid accumulation and cell death in hepatocytes[69,70].

***Modifying bile acid metabolism and other small metabolites contribute to the development of HCC induced by NASH***

Metabolites produced by the gut microbiota have received much attention in the scientific community, and they are helping us to understand the metabolic changes that contribute to the development of NAFLD and NAFLD-HCC. Liposomes (SCFA), glucose, amino acids, and bile acids are now being investigated to improve our understanding of the pathophysiology of NAFLD-HCC[32,71].

Bile acids and their metabolites play an important role in the regulation of hepatic glucose, cholesterol, and triglyceride balance, and their changes can cause NAFLD by affecting lipid and energy metabolism[7]. In addition, bile acids can directly affect the intestinal microbiome by altering bacterial membranes[72].

The colon microbiota, particularly Gram-positive bacteria belonging to *Clostridium* clusters, convert primary bile acids, which were not resorbed in the small intestine, into secondary bile acids, deoxycholate and lithocholate, which are then transported back to the liver with portal blood[73]. Dysbiosis promotes the increase of levels of such secondary bile acids in the liver. Consequently, a senescence hepatic stellate cell phenotype appears, which is characterized by the overproduction of various pro-inflammatory and tumorigenic factors that promote the development of HCC[7,16]. Sydor *et al*[13] have determined the direct correlation of blood levels of conjugated bile acids with the severity of NAFLD, although independent of the occurrence of HCC. Enterohepatic DCA also promotes the development of HCC in mice[74].

On the other hand, liver inflammation has been shown to cause intrahepatic retention of bile acids, directly promoting the development of HCC[67].

By activating TGR5 (Takeda G protein receptor 5), secondary bile acids may participate in the regulation of insulin sensitivity[16,75]. Activation of FXR (Farnesoid X receptor) by the gut microbiota may also influence bile acid metabolism during the onset and progression of hepatic steatosis[16,76].

Other small bacterial metabolites generated by the gut microbiota are also attractive objects to study metabolic alterations that may play a role in the progression of NAFLD and NAFLD-HCC[32,77].

Branched chain amino acids (leucine, isoleucine, valine, and phenylalanine) and bile acids (glycocholic acid, taurocholic acid, glycochenodeoxycholate) were found to be strongly associated with progression of steatosis to NASH, NASH-cirrhosis, and HCC[78], while glutathione was inversely associated[79].

SCFAs (formate, acetate, propionate, and butyrate) can enter the portal vein and promote lipid build-up and glucogenesis in the liver and possibly promote inflammation and oncogenesis[19,80]. The feces of patients with NAFLD-induced HCC were enriched in those SCFs[19]. Although other researchers propagate the anti-inflammatory effects of aromatic amino acid metabolites, especially butyrate[81,82].

Intestinal bacteria can convert dietary choline to trimethylamine (TMA), which is then further metabolized in the liver to trimethylamine-N-oxide (TMAO). Contrary to the useful choline metabolite, phosphatidylcholine, TMAO promotes the accumulation of triglycerides leading to hepatic steatosis and, thus, contributes to inflammation[7].

The difference between bland and NASH steatosis is the accumulation of free non-sterified cholesterol in the latter[5]. Free cholesterol and its oxidized derivatives are cytotoxic and can cause liver damage[5,83].

NAFLD patients had higher serum alcohol concentrations than healthy controls and obese subjects, indicating the possible impact of ethanol-producing bacteria on the pathogenesis of NASH[7].

How the aforementioned bacterial metabolites contribute to the manifestation of HCC in subjects with NASH must be elucidated.

***Modifying antitumor immunity***

The multilayer immune components of the colon wall, together with the genetic diversity of the colon microbiota, create an ideal environment for intestinal microbe-human immunological interactions[84]**.** The gut microbiota and its metabolites alter host gene pathways implicated in immunological and metabolic diseases[85].

In addition to promoting inflammation, the gut microbiota can possibly affect antitumor immunity. *A. muciniphila* and *Ruminococcaceae spp*. were found to be enriched in the gut of HCC patients who respond to anti-PD-1 immune checkpoint inhibitor compared to nonresponders[86]. The gut microbiota of patients with unresectable HCC differs: those with progressive HCC were characterized by the abundance of fecal *Prevotella*, while those with a good response to immune checkpoint inhibitors were distinguished in the amount of *Veillonella, Lachnospiraceae, Lachnoclostridium, Lactobacillales, Streptococcaceae,* and *Ruminococcaceae*[87].

In several clinical studies of using an anti-CTLA-4 treatment for cancers of other etiology, the promoting effect for response to treatment by several species of the gut microbiota was also reported. However, the possible mechanism of such an impact is not very clear[84]. Furthermore, molecules born of the microbiota, including genomic material, the so-called bacterial signature, have been found in the liver parenchyma and the HCC nodules themselves[16]. These molecules could certainly play an active role in modulating the immune response in favor of more severe inflammation and hepatocarcinogenesis. A direct association of intrahepatic *Gamma-proteobacteria* abundance with liver disease progression from non-NAFLD to NAFLD and NASH of different severity was reported[88]. And finally, bile acids themselves possess immunomodulatory properties. Therefore, their modulation by the gut microbiota directly impacts host immunity.

**LIMITATIONS AND FUTURE PERSPECTIVES**

Most healthy individuals demonstrate relative stability of their gut microbiota with the transient effect of diet and the slightly longer effect of antibiotics[89–91]. For example, shared housing promotes the preservation of the same microbiota profiles[92]. On the contrary, discrepancies in the data on the composition of the gut microbiota are observed in clinical studies, including those of NASH-induced HCC. Due to the small number of subjects enrolled, the absence of control groups, different sample collection techniques, and distinctive sequencing methods, the results of clinical studies are difficult to compare, and there are always doubts about their reproducibility.

Estimated differences between the composition of the gut microbiota of a healthy population, NAFLD, NASH, and those with NASH-induced HCC, even at the phyla level, can be considered as evidence of the participation of the microbiota in the pathogenesis of HCC, especially with a shift towards LPS-producing phyla. However, the collected data is not sufficient to draw reasonable conclusions so far.

Moreover, even in the generally pro-inflammatory LPS-producing phyla, there is a huge difference between the properties of bacteria depending on the species. Furthermore, bacterial strains belonging to the same species can also vary greatly in properties. Since affordable measures, such as a balanced diet and aerobic exercises, gradually shift the microbiota toward a healthy shape, it can be presumed that substantial changes are likely to occur at the species/strain level. Possibly, the research of some representative of the gut microbiota at the species/strain level in subjects with NASH-induced HCC in comparison with those without HCC will provide us with more definitive hepatocarcinogenesis provokers in the NASH population, or at least a noninvasive marker of early HCC will be confirmed. One such candidate – *Veillonella parvula* – has already been discovered. However, it is too early to draw conclusions about whether it was an incidental finding or a reliable HCC marker[93]**.**

The microbiota as a potential noninvasive marker for the diagnosis of HCC, especially in the early stages, is intensively studied and might be promising since researchers determine some peculiarities distinguishing the microbiota composition in cirrhotic patients with HCC patients[48,49]. A more attentive study of comparing the gut microbiota of non-cirrhotic NAFLD-HCC patients with cirrhotic ones may prove useful in clarifying the most provocative representatives of liver oncogenicity. HCC of different stages can also be characterized using a dysbiosis index[49]. Although the cohorts of patients in such studies are too small to expect reproducibility of the results.

Experimental studies of the gut microbiota are characterized by another limiting aspect, different methodological approaches. These problems were perfectly elucidated in the Ponziani *et al*[94] review. However, the authors state that despite existing limitations, research on the impact of the gut microbiota on liver diseases has diagnostic, preventive and therapeutic potential, especially in patients with early stage HCC[94].

The therapeutic potential of the microbiota is currently intensively studied. In multiple clinical trials, fecal microbiota transplantation is applied with the expectation of reducing the progression of various etiology liver diseases, including NAFLD of different stages and NASH-induced HCC. Unfortunately, the published results are not promising so far[95]. More clinical trials are needed to better understand the efficacy of intestinal microbiota transplantation in NASH liver and HCC. Prebiotic and probiotic therapy appears to be more promising for the prevention and/or treatment of HCC, although it is necessary to determine its long-lasting effect[96,97].

The other members of the gut microbiome community, including fungi, viruses, and bacteriophages, are also worthy of consideration by researchers as possible participants in the pathogenesis of liver diseases, including NASH and HCC. They can also potentially contribute to the relief of liver disease. For example, Duan *et al*[98] presented experimental research on the beneficial effect on reducing liver disease of bacteriophages targeting *Enterococcus faecalis* that produces toxin cytolysin. Due to more affordable and powerful sequencing technologies, in addition to bacterial components, enteric fungal and viral species will certainly become objects of future research not only in connection with NASH-induced HCC, but also in elucidating the pathophysiological mechanisms of liver diseases of other etiologies[32]. Furthermore, a healthy lifestyle is an affordable approach that can be an effective measure in modulating the microbiota to a healthier shape, reducing obesity, and prophylaxis of NASH and NASH-induced HCC[2,99].

**CONCLUSION**

Current research claims that in the long run, steatohepatitis and the gut microbiota establish mutually maintaining pathological circuit that trigger liver inflammation. This can result in the manifestation of HCC and the growth of malignant nodules, even in the absence of obvious cirrhosis. However, a definite picture of that circuit treads remains blurred.

**REFERENCES**

1 **Borrelli A**, Bonelli P, Tuccillo FM, Goldfine ID, Evans JL, Buonaguro FM, Mancini A. Role of gut microbiota and oxidative stress in the progression of non-alcoholic fatty liver disease to hepatocarcinoma: Current and innovative therapeutic approaches. *Redox Biol* 2018; **15**: 467-479 [PMID: 29413959 DOI: 10.1016/j.redox.2018.01.009]

2 **Anstee QM**, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 411-428 [PMID: 31028350 DOI: 10.1038/s41575-019-0145-7]

3 **Argo CK**, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J Hepatol* 2009; **51**: 371-379 [PMID: 19501928 DOI: 10.1016/j.jhep.2009.03.019]

4 **Yatsuji S**, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J Gastroenterol Hepatol* 2009; **24**: 248-254 [PMID: 19032450 DOI: 10.1111/j.1440-1746.2008.05640.x]

5 **Febbraio MA**, Reibe S, Shalapour S, Ooi GJ, Watt MJ, Karin M. Preclinical Models for Studying NASH-Driven HCC: How Useful Are They? *Cell Metab* 2019; **29**: 18-26 [PMID: 30449681 DOI: 10.1016/j.cmet.2018.10.012]

6 **Marengo A**, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Annu Rev Med* 2016; **67**: 103-117 [PMID: 26473416 DOI: 10.1146/annurev-med-090514-013832]

7 **Chu H**, Williams B, Schnabl B. Gut microbiota, fatty liver disease, and hepatocellular carcinoma. *Liver Res* 2018; **2**: 43-51 [PMID: 30416839 DOI: 10.1016/j.livres.2017.11.005]

8 **Chagas AL**, Kikuchi LO, Oliveira CP, Vezozzo DC, Mello ES, Oliveira AC, Cella LC, Herman P, Bachella T, Caldwell SH, Alves VA, Carrilho FJ. Does hepatocellular carcinoma in non-alcoholic steatohepatitis exist in cirrhotic and non-cirrhotic patients? *Braz J Med Biol Res* 2009; **42**: 958-962 [PMID: 19787150 DOI: 10.1590/s0100-879x2009005000019]

9 **Ertle J**, Dechêne A, Sowa JP, Penndorf V, Herzer K, Kaiser G, Schlaak JF, Gerken G, Syn WK, Canbay A. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011; **128**: 2436-2443 [PMID: 21128245 DOI: 10.1002/ijc.25797]

10 **Huang DQ**, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 223-238 [PMID: 33349658 DOI: 10.1038/s41575-020-00381-6]

11 **Schmidt TSB**, Raes J, Bork P. The Human Gut Microbiome: From Association to Modulation. *Cell* 2018; **172**: 1198-1215 [PMID: 29522742 DOI: 10.1016/j.cell.2018.02.044]

12 **Xie G**, Wang X, Liu P, Wei R, Chen W, Rajani C, Hernandez BY, Alegado R, Dong B, Li D, Jia W. Distinctly altered gut microbiota in the progression of liver disease. *Oncotarget* 2016; **7**: 19355-19366 [PMID: 27036035 DOI: 10.18632/oncotarget.8466]

13 **Sydor S**, Best J, Messerschmidt I, Manka P, Vilchez-Vargas R, Brodesser S, Lucas C, Wegehaupt A, Wenning C, Aßmuth S, Hohenester S, Link A, Faber KN, Moshage H, Cubero FJ, Friedman SL, Gerken G, Trauner M, Canbay A, Bechmann LP. Altered Microbiota Diversity and Bile Acid Signaling in Cirrhotic and Noncirrhotic NASH-HCC. *Clin Transl Gastroenterol* 2020; **11**: e00131 [PMID: 32352707 DOI: 10.14309/ctg.0000000000000131]

14 **Wang R**, Li H, Yang X, Xue X, Deng L, Shen J, Zhang M, Zhao L, Zhang C. Genetically Obese Human Gut Microbiota Induces Liver Steatosis in Germ-Free Mice Fed on Normal Diet. *Front Microbiol* 2018; **9**: 1602 [PMID: 30079055 DOI: 10.3389/fmicb.2018.01602]

15 **Zhou D**, Pan Q, Shen F, Cao HX, Ding WJ, Chen YW, Fan JG. Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci Rep* 2017; **7**: 1529 [PMID: 28484247 DOI: 10.1038/s41598-017-01751-y]

16 **Giraud J**, Saleh M. Host-Microbiota Interactions in Liver Inflammation and Cancer. *Cancers (Basel)* 2021; **13** [PMID: 34503151 DOI: 10.3390/cancers13174342]

17 **Tripathi A**, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 397-411 [PMID: 29748586 DOI: 10.1038/s41575-018-0011-z]

18 **Ohtani N**, Kawada N. Role of the Gut-Liver Axis in Liver Inflammation, Fibrosis, and Cancer: A Special Focus on the Gut Microbiota Relationship. *Hepatol Commun* 2019; **3**: 456-470 [PMID: 30976737 DOI: 10.1002/hep4.1331]

19 **Behary J**, Amorim N, Jiang XT, Raposo A, Gong L, McGovern E, Ibrahim R, Chu F, Stephens C, Jebeili H, Fragomeli V, Koay YC, Jackson M, O'Sullivan J, Weltman M, McCaughan G, El-Omar E, Zekry A. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat Commun* 2021; **12**: 187 [PMID: 33420074 DOI: 10.1038/s41467-020-20422-7]

20 **Ponziani FR**, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, Sanguinetti M, Morelli D, Paroni Sterbini F, Petito V, Reddel S, Calvani R, Camisaschi C, Picca A, Tuccitto A, Gasbarrini A, Pompili M, Mazzaferro V. Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. *Hepatology* 2019; **69**: 107-120 [PMID: 29665135 DOI: 10.1002/hep.30036]

21 **Carter JK**, Bhattacharya D, Borgerding JN, Fiel MI, Faith JJ, Friedman SL. Modeling dysbiosis of human NASH in mice: Loss of gut microbiome diversity and overgrowth of Erysipelotrichales. *PLoS One* 2021; **16**: e0244763 [PMID: 33395434 DOI: 10.1371/journal.pone.0244763]

22 **Zhang X**, Coker OO, Chu ES, Fu K, Lau HCH, Wang YX, Chan AWH, Wei H, Yang X, Sung JJY, Yu J. Dietary cholesterol drives fatty liver-associated liver cancer by modulating gut microbiota and metabolites. *Gut* 2021; **70**: 761-774 [PMID: 32694178 DOI: 10.1136/gutjnl-2019-319664]

23 **Komiyama S**, Yamada T, Takemura N, Kokudo N, Hase K, Kawamura YI. Profiling of tumour-associated microbiota in human hepatocellular carcinoma. *Sci Rep* 2021; **11**: 10589 [PMID: 34012007 DOI: 10.1038/s41598-021-89963-1]

24 **Ren Z**, Li A, Jiang J, Zhou L, Yu Z, Lu H, Xie H, Chen X, Shao L, Zhang R, Xu S, Zhang H, Cui G, Chen X, Sun R, Wen H, Lerut JP, Kan Q, Li L, Zheng S. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut* 2019; **68**: 1014-1023 [PMID: 30045880 DOI: 10.1136/gutjnl-2017-315084]

25 **Lin RS**, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, Hsu WC, Huang CC, Wang SS, Lo KJ. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995; **22**: 165-172 [PMID: 7790704 DOI: 10.1016/0168-8278(95)80424-2]

26 **Zheng R**, Wang G, Pang Z, Ran N, Gu Y, Guan X, Yuan Y, Zuo X, Pan H, Zheng J, Wang F. Liver cirrhosis contributes to the disorder of gut microbiota in patients with hepatocellular carcinoma. *Cancer Med* 2020; **9**: 4232-4250 [PMID: 32281295 DOI: 10.1002/cam4.3045]

27 **de Waal GM**, de Villiers WJS, Pretorius E. The Link Between Bacterial Inflammagens, Leaky Gut Syndrome and Colorectal Cancer. *Curr Med Chem* 2021; **28**: 8534-8548 [PMID: 33605849 DOI: 10.2174/0929867328666210219142737]

28 **Harmey JH**, Bucana CD, Lu W, Byrne AM, McDonnell S, Lynch C, Bouchier-Hayes D, Dong Z. Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. *Int J Cancer* 2002; **101**: 415-422 [PMID: 12216068 DOI: 10.1002/ijc.10632]

29 **Liu WT**, Jing YY, Gao L, Li R, Yang X, Pan XR, Yang Y, Meng Y, Hou XJ, Zhao QD, Han ZP, Wei LX. Lipopolysaccharide induces the differentiation of hepatic progenitor cells into myofibroblasts constitutes the hepatocarcinogenesis-associated microenvironment. *Cell Death Differ* 2020; **27**: 85-101 [PMID: 31065105 DOI: 10.1038/s41418-019-0340-7]

30 **Font-Burgada J**, Sun B, Karin M. Obesity and Cancer: The Oil that Feeds the Flame. *Cell Metab* 2016; **23**: 48-62 [PMID: 26771116 DOI: 10.1016/j.cmet.2015.12.015]

31 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/s0016-5085(98)70599-2]

32 **Ezzaidi N**, Zhang X, Coker OO, Yu J. New insights and therapeutic implication of gut microbiota in non-alcoholic fatty liver disease and its associated liver cancer. *Cancer Lett* 2019; **459**: 186-191 [PMID: 31185249 DOI: 10.1016/j.canlet.2019.114425]

33 **Rainer F**, Horvath A, Sandahl TD, Leber B, Schmerboeck B, Blesl A, Groselj-Strele A, Stauber RE, Fickert P, Stiegler P, Møller HJ, Grønbaek H, Stadlbauer V. Soluble CD163 and soluble mannose receptor predict survival and decompensation in patients with liver cirrhosis, and correlate with gut permeability and bacterial translocation. *Aliment Pharmacol Ther* 2018; **47**: 657-664 [PMID: 29266346 DOI: 10.1111/apt.14474]

34 **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850 DOI: 10.2337/db06-1491]

35 **Singh RK**, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T, Liao W. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017; **15**: 73 [PMID: 28388917 DOI: 10.1186/s12967-017-1175-y]

36 **Boursier J**, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Hunault G, Oberti F, Calès P, Diehl AM. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016; **63**: 764-775 [PMID: 26600078 DOI: 10.1002/hep.28356]

37 **Loomba R**, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2017; **25**: 1054-1062.e5 [PMID: 28467925 DOI: 10.1016/j.cmet.2017.04.001]

38 **Kapil S**, Duseja A, Sharma BK, Singla B, Chakraborti A, Das A, Ray P, Dhiman RK, Chawla Y. Small intestinal bacterial overgrowth and toll-like receptor signaling in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2016; **31**: 213-221 [PMID: 26212089 DOI: 10.1111/jgh.13058]

39 **McQuade JL**, Daniel CR, Helmink BA, Wargo JA. Modulating the microbiome to improve therapeutic response in cancer. *Lancet Oncol* 2019; **20**: e77-e91 [PMID: 30712808 DOI: 10.1016/S1470-2045(18)30952-5]

40 **Helmink BA**, Khan MAW, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. *Nat Med* 2019; **25**: 377-388 [PMID: 30842679 DOI: 10.1038/s41591-019-0377-7]

41 **Liang W**, Yang Y, Wang H, Wang H, Yu X, Lu Y, Shen S, Teng L. Gut microbiota shifts in patients with gastric cancer in perioperative period. *Medicine (Baltimore)* 2019; **98**: e16626 [PMID: 31464899 DOI: 10.1097/MD.0000000000016626]

42 **Heiman ML**, Greenway FL. A healthy gastrointestinal microbiome is dependent on dietary diversity. *Mol Metab* 2016; **5**: 317-320 [PMID: 27110483 DOI: 10.1016/j.molmet.2016.02.005]

43 **Kowalska-Duplaga K**, Gosiewski T, Kapusta P, Sroka-Oleksiak A, Wędrychowicz A, Pieczarkowski S, Ludwig-Słomczyńska AH, Wołkow PP, Fyderek K. Differences in the intestinal microbiome of healthy children and patients with newly diagnosed Crohn's disease. *Sci Rep* 2019; **9**: 18880 [PMID: 31827191 DOI: 10.1038/s41598-019-55290-9]

44 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004; **101**: 15718-15723 [PMID: 15505215 DOI: 10.1073/pnas.0407076101]

45 **Li F**, Sun G, Wang Z, Wu W, Guo H, Peng L, Wu L, Guo X, Yang Y. Characteristics of fecal microbiota in non-alcoholic fatty liver disease patients. *Sci China Life Sci* 2018; **61**: 770-778 [PMID: 29948900 DOI: 10.1007/s11427-017-9303-9]

46 **Kolodziejczyk AA**, Zheng D, Shibolet O, Elinav E. The role of the microbiome in NAFLD and NASH. *EMBO Mol Med* 2019; **11** [PMID: 30591521 DOI: 10.15252/emmm.201809302]

47 **Yu Q**, Wu L, Ji J, Feng J, Dai W, Li J, Wu J, Guo C. Gut Microbiota, Peroxisome Proliferator-Activated Receptors, and Hepatocellular Carcinoma. *J Hepatocell Carcinoma* 2020; **7**: 271-288 [PMID: 33150145 DOI: 10.2147/JHC.S277870]

48 **Piñero F**, Vazquez M, Baré P, Rohr C, Mendizabal M, Sciara M, Alonso C, Fay F, Silva M. A different gut microbiome linked to inflammation found in cirrhotic patients with and without hepatocellular carcinoma. *Ann Hepatol* 2019; **18**: 480-487 [PMID: 31023615 DOI: 10.1016/j.aohep.2018.10.003]

49 **Ni J**, Huang R, Zhou H, Xu X, Li Y, Cao P, Zhong K, Ge M, Chen X, Hou B, Yu M, Peng B, Li Q, Zhang P, Gao Y. Analysis of the Relationship Between the Degree of Dysbiosis in Gut Microbiota and Prognosis at Different Stages of Primary Hepatocellular Carcinoma. *Front Microbiol* 2019; **10**: 1458 [PMID: 31293562 DOI: 10.3389/fmicb.2019.01458]

50 **Valentini M**, Piermattei A, Di Sante G, Migliara G, Delogu G, Ria F. Immunomodulation by gut microbiota: role of Toll-like receptor expressed by T cells. *J Immunol Res* 2014; **2014**: 586939 [PMID: 25147831 DOI: 10.1155/2014/586939]

51 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]

52 **Desai A**, Sandhu S, Lai JP, Sandhu DS. Hepatocellular carcinoma in non-cirrhotic liver: A comprehensive review. *World J Hepatol* 2019; **11**: 1-18 [PMID: 30705715 DOI: 10.4254/wjh.v11.i1.1]

53 **Rattan P**, Minacapelli CD, Rustgi V. The Microbiome and Hepatocellular Carcinoma. *Liver Transpl* 2020; **26**: 1316-1327 [PMID: 32564483 DOI: 10.1002/lt.25828]

54 **Wang JK**, Feng ZW, Li YC, Li QY, Tao XY. Association of tumor necrosis factor-α gene promoter polymorphism at sites -308 and -238 with non-alcoholic fatty liver disease: a meta-analysis. *J Gastroenterol Hepatol* 2012; **27**: 670-676 [PMID: 22097889 DOI: 10.1111/j.1440-1746.2011.06978.x]

55 **Bahcecioglu IH**, Yalniz M, Ataseven H, Ilhan N, Ozercan IH, Seckin D, Sahin K. Levels of serum hyaluronic acid, TNF-alpha and IL-8 in patients with nonalcoholic steatohepatitis. *Hepatogastroenterology* 2005; **52**: 1549-1553 [PMID: 16201116 DOI: 10.1111/j.1742-1241.2004.00312.x]

56 **Shalapour S**, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, Vrbanac AF, Li W, Perkins A, Matsutani T, Zhong Z, Dhar D, Navas-Molina JA, Xu J, Loomba R, Downes M, Yu RT, Evans RM, Dorrestein PC, Knight R, Benner C, Anstee QM, Karin M. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature* 2017; **551**: 340-345 [PMID: 29144460 DOI: 10.1038/nature24302]

57 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]

58 **Brun P**, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, Martines D. Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G518-G525 [PMID: 17023554 DOI: 10.1152/ajpgi.00024.2006]

59 **Wright SD**, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990; **249**: 1431-1433 [PMID: 1698311 DOI: 10.1126/science.1698311]

60 **Beutler B**, Hoebe K, Du X, Ulevitch RJ. How we detect microbes and respond to them: the Toll-like receptors and their transducers. *J Leukoc Biol* 2003; **74**: 479-485 [PMID: 12960260 DOI: 10.1189/jlb.0203082]

61 **Dapito DH**, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Bataller R, Lefkowitch JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012; **21**: 504-516 [PMID: 22516259 DOI: 10.1016/j.ccr.2012.02.007]

62 **Liu WT**, Jing YY, Yu GF, Han ZP, Yu DD, Fan QM, Ye F, Li R, Gao L, Zhao QD, Wu MC, Wei LX. Toll like receptor 4 facilitates invasion and migration as a cancer stem cell marker in hepatocellular carcinoma. *Cancer Lett* 2015; **358**: 136-143 [PMID: 25511737 DOI: 10.1016/j.canlet.2014.12.019]

63 **Hsiao CC**, Chen PH, Cheng CI, Tsai MS, Chang CY, Lu SC, Hsieh MC, Lin YC, Lee PH, Kao YH. Toll-like receptor-4 is a target for suppression of proliferation and chemoresistance in HepG2 hepatoblastoma cells. *Cancer Lett* 2015; **368**: 144-152 [PMID: 26276725 DOI: 10.1016/j.canlet.2015.08.004]

64 **Kawai T**, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int Immunol* 2009; **21**: 317-337 [PMID: 19246554 DOI: 10.1093/intimm/dxp017]

65 **Loo TM**, Kamachi F, Watanabe Y, Yoshimoto S, Kanda H, Arai Y, Nakajima-Takagi Y, Iwama A, Koga T, Sugimoto Y, Ozawa T, Nakamura M, Kumagai M, Watashi K, Taketo MM, Aoki T, Narumiya S, Oshima M, Arita M, Hara E, Ohtani N. Gut Microbiota Promotes Obesity-Associated Liver Cancer through PGE2-Mediated Suppression of Antitumor Immunity. *Cancer Discov* 2017; **7**: 522-538 [PMID: 28202625 DOI: 10.1158/2159-8290.CD-16-0932]

66 **Payne CM**, Weber C, Crowley-Skillicorn C, Dvorak K, Bernstein H, Bernstein C, Holubec H, Dvorakova B, Garewal H. Deoxycholate induces mitochondrial oxidative stress and activates NF-kappaB through multiple mechanisms in HCT-116 colon epithelial cells. *Carcinogenesis* 2007; **28**: 215-222 [PMID: 16887864 DOI: 10.1093/carcin/bgl139]

67 **Xie G**, Wang X, Huang F, Zhao A, Chen W, Yan J, Zhang Y, Lei S, Ge K, Zheng X, Liu J, Su M, Liu P, Jia W. Dysregulated hepatic bile acids collaboratively promote liver carcinogenesis. *Int J Cancer* 2016; **139**: 1764-1775 [PMID: 27273788 DOI: 10.1002/ijc.30219]

68 **Kawai T**, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 2010; **11**: 373-384 [PMID: 20404851 DOI: 10.1038/ni.1863]

69 **Rivera CA**, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 2007; **47**: 571-579 [PMID: 17644211 DOI: 10.1016/j.jhep.2007.04.019]

70 **Gäbele E**, Mühlbauer M, Dorn C, Weiss TS, Froh M, Schnabl B, Wiest R, Schölmerich J, Obermeier F, Hellerbrand C. Role of TLR9 in hepatic stellate cells and experimental liver fibrosis. *Biochem Biophys Res Commun* 2008; **376**: 271-276 [PMID: 18760996 DOI: 10.1016/j.bbrc.2008.08.096]

71 **Gitto S**, Schepis F, Andreone P, Villa E. Study of the Serum Metabolomic Profile in Nonalcoholic Fatty Liver Disease: Research and Clinical Perspectives. *Metabolites* 2018; **8** [PMID: 29495258 DOI: 10.3390/metabo8010017]

72 **Stacey M**, Webb M. Studies on the antibacterial properties of the bile acids and some compounds derived from cholanic acid. *Proc R Soc Med* 1947; **134**: 523-537 [PMID: 20265566 DOI: 10.1098/rspb.1947.0029]

73 **Ridlon JM**, Harris SC, Bhowmik S, Kang DJ, Hylemon PB. Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes* 2016; **7**: 22-39 [PMID: 26939849 DOI: 10.1080/19490976.2015.1127483]

74 **Yoshimoto S**, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013; **499**: 97-101 [PMID: 23803760 DOI: 10.1038/nature12347]

75 **Mobraten K**, Haugbro T, Karlstrom E, Kleiveland CR, Lea T. Activation of the bile acid receptor TGR5 enhances LPS-induced inflammatory responses in a human monocytic cell line. *J Recept Signal Transduct Res* 2015; **35**: 402-409 [PMID: 25418122 DOI: 10.3109/10799893.2014.986744]

76 **Aron-Wisnewsky J**, Gaborit B, Dutour A, Clement K. Gut microbiota and non-alcoholic fatty liver disease: new insights. *Clin Microbiol Infect* 2013; **19**: 338-348 [PMID: 23452163 DOI: 10.1111/1469-0691.12140]

77 **Ding Y**, Yanagi K, Cheng C, Alaniz RC, Lee K, Jayaraman A. Interactions between gut microbiota and non-alcoholic liver disease: The role of microbiota-derived metabolites. *Pharmacol Res* 2019; **141**: 521-529 [PMID: 30660825 DOI: 10.1016/j.phrs.2019.01.029]

78 **Kalhan SC**, Guo L, Edmison J, Dasarathy S, McCullough AJ, Hanson RW, Milburn M. Plasma metabolomic profile in nonalcoholic fatty liver disease. *Metabolism* 2011; **60**: 404-413 [PMID: 20423748 DOI: 10.1016/j.metabol.2010.03.006]

79 **Han J**, Dzierlenga AL, Lu Z, Billheimer DD, Torabzadeh E, Lake AD, Li H, Novak P, Shipkova P, Aranibar N, Robertson D, Reily MD, Lehman-McKeeman LD, Cherrington NJ. Metabolomic profiling distinction of human nonalcoholic fatty liver disease progression from a common rat model. *Obesity (Silver Spring)* 2017; **25**: 1069-1076 [PMID: 28452429 DOI: 10.1002/oby.21855]

80 **Coppola S**, Avagliano C, Calignano A, Berni Canani R. The Protective Role of Butyrate against Obesity and Obesity-Related Diseases. *Molecules* 2021; **26** [PMID: 33525625 DOI: 10.3390/molecules26030682]

81 **Bansal T**, Alaniz RC, Wood TK, Jayaraman A. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc Natl Acad Sci U S A* 2010; **107**: 228-233 [PMID: 19966295 DOI: 10.1073/pnas.0906112107]

82 **Venkatesh M**, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, Qiu Z, Maher L, Redinbo MR, Phillips RS, Fleet JC, Kortagere S, Mukherjee P, Fasano A, Le Ven J, Nicholson JK, Dumas ME, Khanna KM, Mani S. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. *Immunity* 2014; **41**: 296-310 [PMID: 25065623 DOI: 10.1016/j.immuni.2014.06.014]

83 **Nakagawa H**, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, Zhong Z, Valasek MA, Seki E, Hidalgo J, Koike K, Kaufman RJ, Karin M. ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell* 2014; **26**: 331-343 [PMID: 25132496 DOI: 10.1016/j.ccr.2014.07.001]

84 **Frankel AE**, Deshmukh S, Reddy A, Lightcap J, Hayes M, McClellan S, Singh S, Rabideau B, Glover TG, Roberts B, Koh AY. Cancer Immune Checkpoint Inhibitor Therapy and the Gut Microbiota. *Integr Cancer Ther* 2019; **18**: 1534735419846379 [PMID: 31014119 DOI: 10.1177/1534735419846379]

85 **Delzenne NM**, Bindels LB. Microbiome metabolomics reveals new drivers of human liver steatosis. *Nat Med* 2018; **24**: 906-907 [PMID: 29988145 DOI: 10.1038/s41591-018-0126-3]

86 **Zheng Y**, Wang T, Tu X, Huang Y, Zhang H, Tan D, Jiang W, Cai S, Zhao P, Song R, Li P, Qin N, Fang W. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. *J Immunother Cancer* 2019; **7**: 193 [PMID: 31337439 DOI: 10.1186/s40425-019-0650-9]

87 **Lee PC**, Wu CJ, Hung YW, Lee CJ, Chao Y, Hou MC, Kuo YL, Chou SH, Huang YH. Association of gut microbiota and metabolites with tumor response to immune checkpoint inhibitors in patients with unresectable hepatocellular carcinoma. *J Clin Oncol* 2021; **39**: e16165-e16165 [DOI: 10.1200/jco.2021.39.15\_suppl.e16165]

88 **Sookoian S**, Salatino A, Castaño GO, Landa MS, Fijalkowky C, Garaycoechea M, Pirola CJ. Intrahepatic bacterial metataxonomic signature in non-alcoholic fatty liver disease. *Gut* 2020; **69**: 1483-1491 [PMID: 31900291 DOI: 10.1136/gutjnl-2019-318811]

89 **Haak BW**, Lankelma JM, Hugenholtz F, Belzer C, de Vos WM, Wiersinga WJ. Long-term impact of oral vancomycin, ciprofloxacin and metronidazole on the gut microbiota in healthy humans. *J Antimicrob Chemother* 2019; **74**: 782-786 [PMID: 30418539 DOI: 10.1093/jac/dky471]

90 **Palleja A**, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, Hansen TH, Liang S, Feng Q, Zhang C, Pyl PT, Coelho LP, Yang H, Wang J, Typas A, Nielsen MF, Nielsen HB, Bork P, Wang J, Vilsbøll T, Hansen T, Knop FK, Arumugam M, Pedersen O. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat Microbiol* 2018; **3**: 1255-1265 [PMID: 30349083 DOI: 10.1038/s41564-018-0257-9]

91 **Jin Y**, Dong H, Xia L, Yang Y, Zhu Y, Shen Y, Zheng H, Yao C, Wang Y, Lu S. The Diversity of Gut Microbiome is Associated With Favorable Responses to Anti-Programmed Death 1 Immunotherapy in Chinese Patients With NSCLC. *J Thorac Oncol* 2019; **14**: 1378-1389 [PMID: 31026576 DOI: 10.1016/j.jtho.2019.04.007]

92 **Caussy C**, Tripathi A, Humphrey G, Bassirian S, Singh S, Faulkner C, Bettencourt R, Rizo E, Richards L, Xu ZZ, Downes MR, Evans RM, Brenner DA, Sirlin CB, Knight R, Loomba R. A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease. *Nat Commun* 2019; **10**: 1406 [PMID: 30926798 DOI: 10.1038/s41467-019-09455-9]

93 **Loomba R**, Ling L, Dinh DM, DePaoli AM, Lieu HD, Harrison SA, Sanyal AJ. The Commensal Microbe Veillonella as a Marker for Response to an FGF19 Analog in NASH. *Hepatology* 2021; **73**: 126-143 [PMID: 32794259 DOI: 10.1002/hep.31523]

94 **Ponziani FR**, Nicoletti A, Gasbarrini A, Pompili M. Diagnostic and therapeutic potential of the gut microbiota in patients with early hepatocellular carcinoma. *Ther Adv Med Oncol* 2019; **11**: 1758835919848184 [PMID: 31205505 DOI: 10.1177/1758835919848184]

95 **Allegretti JR**, Kassam Z, Mullish BH, Chiang A, Carrellas M, Hurtado J, Marchesi JR, McDonald JAK, Pechlivanis A, Barker GF, Miguéns Blanco J, Garcia-Perez I, Wong WF, Gerardin Y, Silverstein M, Kennedy K, Thompson C. Effects of Fecal Microbiota Transplantation With Oral Capsules in Obese Patients. *Clin Gastroenterol Hepatol* 2020; **18**: 855-863.e2 [PMID: 31301451 DOI: 10.1016/j.cgh.2019.07.006]

96 **Wan MLY**, El-Nezami H. Targeting gut microbiota in hepatocellular carcinoma: probiotics as a novel therapy. *Hepatobiliary Surg Nutr* 2018; **7**: 11-20 [PMID: 29531939 DOI: 10.21037/hbsn.2017.12.07]

97 **Lambert JE**, Parnell JA, Eksteen B, Raman M, Bomhof MR, Rioux KP, Madsen KL, Reimer RA. Gut microbiota manipulation with prebiotics in patients with non-alcoholic fatty liver disease: a randomized controlled trial protocol. *BMC Gastroenterol* 2015; **15**: 169 [PMID: 26635079 DOI: 10.1186/s12876-015-0400-5]

98 **Duan Y**, Llorente C, Lang S, Brandl K, Chu H, Jiang L, White RC, Clarke TH, Nguyen K, Torralba M, Shao Y, Liu J, Hernandez-Morales A, Lessor L, Rahman IR, Miyamoto Y, Ly M, Gao B, Sun W, Kiesel R, Hutmacher F, Lee S, Ventura-Cots M, Bosques-Padilla F, Verna EC, Abraldes JG, Brown RS Jr, Vargas V, Altamirano J, Caballería J, Shawcross DL, Ho SB, Louvet A, Lucey MR, Mathurin P, Garcia-Tsao G, Bataller R, Tu XM, Eckmann L, van der Donk WA, Young R, Lawley TD, Stärkel P, Pride D, Fouts DE, Schnabl B. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019; **575**: 505-511 [PMID: 31723265 DOI: 10.1038/s41586-019-1742-x]

99 **Panasevich MR**, Peppler WT, Oerther DB, Wright DC, Rector RS. Microbiome and NAFLD: potential influence of aerobic fitness and lifestyle modification. *Physiol Genomics* 2017; **49**: 385-399 [PMID: 28600319 DOI: 10.1152/physiolgenomics.00012.2017]

**Footnotes**

**Conflict-of-interest statement:** All authors report no relevant conflicts of interest for this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Corresponding Author's Membership in Professional Societies:** Lithuanian Society of Gastroenterology; Lithuanian Society of Immunology; European Association of the Study of the Liver.

**Peer-review started:** January 15, 2022

**First decision:** April 12, 2022

**Article in press:**

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Lithuania

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Chen YH, China; Fu M, China **S-Editor:** Ma YJ **L-Editor:** Filipodia **P-Editor:** Ma YJ

**Figure Legends**

**Table 1 Clinical studies investigating gut microbiota composition in patients with nonalcoholic fatty liver disease - induced hepatocellular carcinoma**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.** | **Participants (groups)** | **Exclusion criteria** | **Main findings** | **Other metabolites investigated** |
| Behary *et al*[19] | Patients with NAFLD-HCC-cirrhosis *n* = 32; Patients with NAFLD-cirrhosis *n* = 28; Control group (non-NAFLD) *n* = 30. | Unspecified | Subjects with NAFLD-HCC and NAFLD-cirrhosis had reduced α-diversity indices compared to non-NAFLD controls; NAFLD-HCC was characterized by expansion of *Proteobacteria* compared to a non-NAFLD group; Expansion of *Enterobacteriaceae* in NAFLD-HCC compared to NAFLD-cirrhosis and controls; NAFLD-HCC was characterized by a reduction in *Oscillospiraceae* and *Erysipelotrichaceae* compared to non-NAFLD; NAFLD-cirrhosis was characterized by an expansion of *Eubacteriaceae* compared to both NAFLD-HCC and controls; *Bacteroides caecimuris* and *Veillonella parvula,* were both significantly enriched in NAFLD-HCC, compared to NAFLD cirrhosis and controls | Pyruvate carboxylase (pycA), responsible for the production of oxaloacetate from pyruvate, was overexpressed in NAFLD-HCC compared to NAFLD-cirrhosis and non-NAFLD control; Genes related to acetate synthesis (phosphate acetyltransferase) and butyrate/acetyl phosphate synthesis (phosphate butyryltransferase) were both overexpressed in NAFLD-HCC compared to NAFLD cirrhosis and non-NAFLD controls; The feces of NAFLD-HCC subjects were enriched in acetate, butyrate and formate compared to NAFLD-cirrhosis and controls; Fecal SCFA was NAFLD-HCC specific |
| Sydor *et al*[13] | Patients with NASH-non-HCC without cirrhosis *n* = 23; Patients with NASH-non-HCC with cirrhosis *n* = 11; Patients with NASH-HCC without cirrhosis *n* = 14; Patients with NASH-HCC with cirrhosis *n* = 19; Control group *n* = 20. | Unspecified | B*acteroidetes* and, to a lesser extent, *Actinobacteria* were gradually decreased in abundance from controls to NASH-non-HCC to NASH-HCC; The abundance of *Proteobacteria* was significantly increased in NASH-HCC with cirrhosis; The abundances of *Bacteroides* and *Bifidobacterium* were decreased in NASH-non-HCC and NASH-HCC compared with controls; *Lactobacillus* showed a progressive increase in abundance from controls to NASH-HCC with cirrhosis; Abundance of *Clostridium* and *Escherichia/Shigella* remained unchanged; *Lactobacillus*-related ranks showed a progressive increase in abundance from controls to NASH-HCC with cirrhosis | Significant increase of BA associated with disease severity between healthy, NASH-non- HCC, and NASH-HCC; Individual and conjugated serum BA were associated with the abundance of *Lactobacillus* |
| Ponziani *et al*[20] | Patients with NAFLD-HCC with cirrhosis *n* = 21; Patients with NAFLD-non-HCC with cirrhosis *n* = 20; Control group *n* = 20. | Patients with CVH, AH, cholestatic disorders such as PBC or PSC, and inherited liver disorders leading to cirrhosis such as hemochromatosis, Wilson's disease, and alpha-1 antitrypsin deficiency; Patients who were taking drugs such as antibiotics, probiotics, prebiotics, PPIs, and laxatives during the last 6 mo; affected by diseases potentially influencing the gut microbiota composition; Patients with a history of cancer. | α-diversity was less diverse in patients with cirrhosis compared to controls; Cirrhosis patients showed enriched *Proteobacteria*, *Bacteroidetes* and *Cyanobacteria* compared to healthy controls; The gut microbiota of the HCC group was enriched with *Bacteroides*, *Ruminococcaceae*, *Enterococcus*, *Phascolarctobacterium*, and *Oscillospira* compared to patients with cirrhosis but without HCC and controls; Reduced abundance of *Verrucomicrobiaceae*, *Bifidobacteriaceae*, *Akkermansia*, *Bifidobacterium*, *Dialister, Collinsella*, and *Adlercreutzia* were seen in NAFLD-HCC compared with NAFLD-non-HCC. | Intestinal permeability was increased in all patients with liver cirrhosis, who had higher levels of plasma ZO1 and LPS compared to controls |

AH: autoimmune hepatitis; BA: bile acids; CVH: chronic viral hepatitis; HCC: hepatocellular carcinoma; LPS: lipopolysaccharides; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; PBC: primary biliary cholangitis; PPI: proton pump inhibitors; PSC: primary sclerosing cholangitis; SCFA: short-chain fatty acid.

**Table 2 Animal models investigating gut microbiota composition in nonalcoholic fatty liver disease induced hepatocellular carcinoma**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Experimental animal** | **Participants ( groups)** | **Main findings** |
| Xie *et al*[12] | Mice | Mice with STZ-HFD induced NASH-HCC; Control group | STZ-HFD group exhibited lower α-diversity than controls; The most abundant species in both control group and STZ-HFD group were primarily from the *Bacteroides* genus; The most decreased in abundance in the STZ-HFD group were *Parasutterella spp., Bacteroides acidofaciens, Odoribacter spp., Barnesiella spp., Moryella spp., Paraprevotella spp., Lactobacillus intestinalis, and Akkermansia spp*; *Atopobium spp.*, *Bacteroides acidifaciens*, *Bacteroides spp.*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Clostridium cocleatum*, *Clostridium xylanolyticum*, and *Desulfovibrio spp.* were significantly positively correlated with LPS in plasma, liver and feces; As most *Bacteroides* and *Desulfovibrio* were LPS-producers, LPS concentration was significantly increased in the STZ-HFD group. |
| Carter *et al*[21] | Mice | Western diet only (high fat and fructose diet, no CCl4 injection); CCl4 only (CCl4 injection intraperitoneal once a week and normal diet); NASH-HCC (Western diet and CCl4 injection intraperitoneally once a week); Control group (normal diet, no CCl4 injection); | NASH mice display impaired intestinal barrier function, leading to increased leakage of bacterial byproducts such as LPS into the circulation; NASH mice had reduced alpha diversity; Expansion of *Erysipelotrichales* was only observed in NASH mice |
| Zhang *et al*[22] | Mice | HFHC-fed mice (NAFLD-HCC group); HFHC-fed mice; Normal diet-fed mice (control group). | The microbiota composition changed during NAFLD-HCC formation: *Mucispirillum, Desulfovibrio, Anaerotuncus* were sequentially increased; Gut bacterial metabolites alteration like TCA and IPA were increased in NAFLD-HCC mice; Lower bacterial diversity and increased bacterial richness were observed in HFHC-fed mice with HCC than HFLC diet-fed mice with only steatosis; LPS concentration was elevated in HFHC-fed mice compared to HFLC-fed mice. |

HCC: hepatocellular carcinoma; HFHC: high-fat/high-cholesterol; HFLC: High-fat/low-cholesterol; IPA: indole-3-propionic acid; LPS: Lipopolysaccharides; NAFLD: non-alcoholic fatty liver disease; STZ-HFD: streptozocin-high-fat diet; TCA: Trichloroacetic acid.