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REVIEW

Gut microbial profiling as a therapeutic and diagnostic target for managing primary biliary cholangitis.

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ABSTRACT

Introduction: Microbial antigens present in the intestine has been suggested as possible triggers of primary biliary cholangitis (PBC) and it has been demonstrated that the gut microbiome is modified in PBC patients. On this basis, the modulation of the gut microbiome has been proposed as a pharmacological target for PBC management. To provide a state-of-the-art analysis of the preclinical and clinical evidence on this topic, a systematic review of literature in PubMed, Scopus, and Science Direct was conducted (inclusive dates: 2000–2020).

Area covered: In particular, several strategies for microbiome modulation have been investigated in both experimental and clinical studies, i.e. dietary interventions, and the administration of probiotics and prebiotics and drugs. Moreover, clinical evidence point to two drugs approved for PBC, i.e. ursodeoxycholic and obeticholic acids, as gut flora modulators. Accordingly, fecal microbiota transplantation is also under evaluation for PBC treatment. On the other hand, typical alterations of the microbiome have been observed in PBC patients, although their diagnostic impact remains to be better evaluated.

Expert opinion: The addition of gut microbiome manipulation to standard pharmacological treatments is one important challenge for PBC therapy in the near future. Further studies are needed to ascertain whether microbiome profiling could be considered a diagnostic strategy in PBC.

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PBC; microbiota; microbiome; microbial profiling; metagenomics; metabolomic; bile acids; UDCA; obeticholic acid

1. Introduction

Since microorganisms can influence a number of physiological aspects of the host, including the immune response, in recent years several studies have tried to unravel the role of the microbiome in the pathogenesis of autoimmune diseases [1,2]. It is well known that in chronic liver diseases, intestinal bacteria, and related inflammasome molecules can reach the liver by the portal circulation, thereby actively participating in the disease progression [3]. In this context, the occurrence of changes in bacterial translocation from the intestine to the liver, along with a reduction of the hepatic clearance capacity for inflammasome molecules acting as antigens, perpetuates a boosting of inflammation. The mechanism of this inflammatory response passes through the activation of various toll-like receptors (TLR) which leads to a massive cellular release of proinflammatory cytokines [4]. As a consequence, elevated production of reactive oxygen species (ROS) takes place, enhancing intestinal permeability and thus sustain a vicious circle of events widely known as 'leaky gut' [5]. In this case, the intestinal tight junctions undergo injuries which allow the translocation to the liver of intestinal bacteria, endotoxins, lipopolysaccharides (LPS), pathogen-associated molecular pattern (PAMP)-like bacterial DNA fragments, and inflammatory cytokines. Taken together, these factors, by the interaction with TLR receptors, sustain and perpetuate liver inflammation. Primary biliary cholangitis (PBC) is a female-dominant, chronic inflammatory cholestatic liver disease [6], whose relationship with the gut microbiome remains to be fully understood. This review aims at summarizing the most recent studies about this possible correlation and sheds light on the use of the gut microbiome as a pharmacological or diagnostic target in PBC. To reach this goal, i.e. providing a state-of-the-art analysis of the preclinical and clinical evidence on this topic, we performed a systematic review of literature in PubMed, Scopus, and Science Direct, including the period 2000–2020. However, when possible, we selected the most recent (2019–2020) relevant manuscripts, concerning both the preclinical *in-vivo* evaluations and the clinical studies Figure 1.

2. Role of gut bacteria in the pathogenesis of PBC

2.1. Bacteria as triggers of PBC

Several microbial antigens, mainly bacteria, but also viruses, parasites, and fungi have been postulated as possible triggers of PBC [7]. A linear conformational mimicry between microbial proteins and human mitochondrial antigens has been demonstrated for *Escherichia coli, Novosphingobium aromaticivorans, Salmonella Minnesota, Pseudomonas aeruginosa, Hemophilus influenzae,*

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Article highlights

- There is an important link between the microbiome, immune system, and bile acids, which can be exploited for the management of autoimmune cholestatic diseases.
- The microbiome can be targeted to treat PBC either by dietary or pharmacological interventions or both.
- Typical microbiome profiles have been identified in PBC patients and should be evaluated as diagnostic markers.

This box summarizes the key points contained in the article.

Yersinia enterocolitica, Streptococcus intermedius, Lactobacillus delbrueckii, Paracoccus denitrificans, Mycoplam, Mycobacterium gordonae, Borrelia burgdorferi, Trypanosoma, and *Ascaridia galli* [8]. Interestingly, the infection of genetically susceptible mouse strains with *Novosphingobium aromaticivorans* induced anti- mitochondrial Pyruvate Dehydrogenase Complex E2 (PDC E2) responses and liver lesions resembling PBC in humans [9]. Moreover, it has been found that IgG3 antibodies directed against b-galactosidase of *Lactobacillus delbrueckii* cross-react with the same major mitochondrial autoepitope and are characteristic of PBC [10].

Escherichia coli have been shown to be involved in recurrent urinary tract infections which frequently develop in patients with PBC [11–13]. Moreover, the frequent polyclonal IgM response in PBC has been suggested to be linked to chronic infections [14,15].

2.2. A link between microbial product and immune system

In general, an increased intestinal permeability has been observed in cholestatic liver disease, especially in primary sclerosing cholangitis (PSC) and PBC. In PBC an altered permeability of both stomach and small intestine was demonstrated by sucrose and lactulose/mannitol tests, respectively [16]. Although no structural changes in the intestinal mucosa have been reported in these patients, an IgA secretion defect in the intestinal epithelium was observed [17]. Elevated intestinal permeability plays a key role in the pathophysiology of cholangiopathies by increasing cytokines and lipopolysaccharide (LPS) which can disrupt tight junctions in cholangiocytes and induce cellular senescence [18,19]. Moreover, LPS, which is located in the outer membrane of G-neg bacteria, elicits a strong host immune response [20]. Toll-like receptor 4 (TLR4) which is activated by LPS has been found to be expressed in biliary epithelial cells (BECs) and periportal hepatocytes from PBC livers, suggesting the possible involvement of bacterial pathogens and TLR4 signaling in the inflammatory processes of PBC [21]. Moreover, polymorphisms in TLR-4 which have been identified in PBC may lead to accumulation of LPS in BECs [22].

2.3. Impact of gut microbiome on the bile acids

The gut microbiome is considered a 'metabolic organ' that not only facilitates harvesting of nutrients but also produces numerous metabolites that regulate host metabolism in terms not only of energetic expenditure but also of microbially induced signals which contribute to different disease phenotypes [23]. Primary bile acids (BAs) are synthesized by the liver from cholesterol into secondary BAs and metabolized in the intestine by the gut microbiota. BA synthesis is finely regulated by a number of factors, including the gut microbiome. In fact, the gut microbiome can suppress BA synthesis via activation of the farnesoid X receptor (FXR)-dependent FGF15

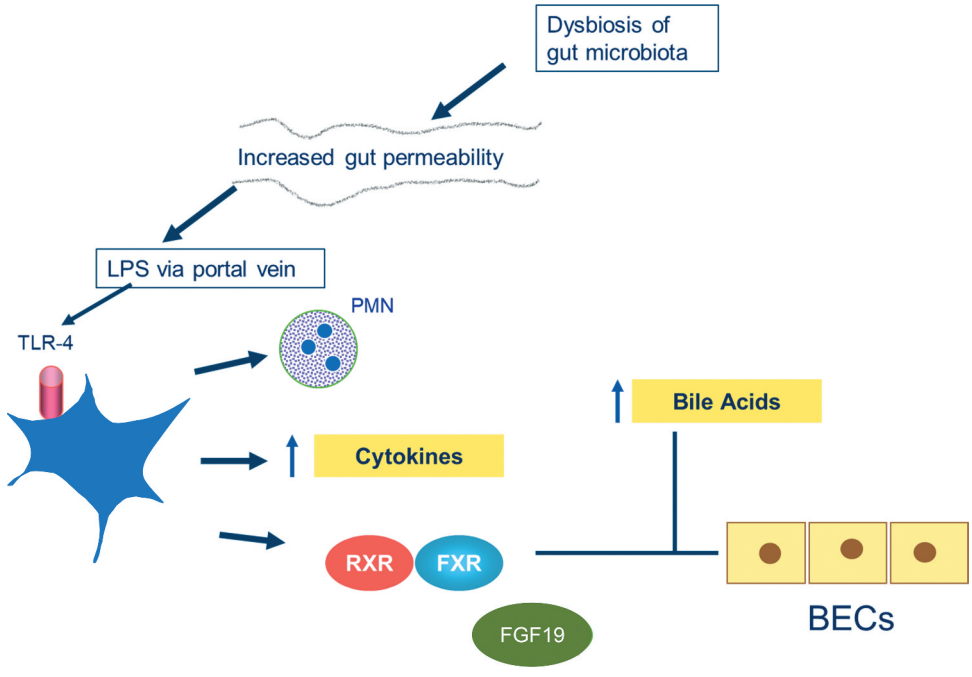

Figure 1. Effects of microbial product and immune system in cholestasis. Intestinal dysbiosis increases intestinal permeability. LPS elicits a strong host immune response activating TLR-4 expressed in Kupffer cells. RXR, FXR and FGF19 regulate the bile acid synthesis. The increased hydrophobicity of the bile acid pool contributes to the injury of the BECs. LPS = lipopolysaccharide; TLR-4 = toll-like receptor 4; RXR = retinoid X receptor; FXR = farnesoid X receptor; FGF19 = fibroblast growth factor 19; BECs = biliary epithelial cells.

induction and inhibit BA import from the ileum. In an in-vivo experimental study, Sayin et al. demonstrated that gut microbiota also leads to a more hydrophobic BA pool which may result from both the production of secondary BAs and reduction of the FXR antagonist tauro-b-muricholic acid [24]. In this way, the microbiome is protective against (BAs) toxicity, by the activation of the FXR target gene. On the other hand, the increase of hydrophobicity of the BA pool induced by the microbiome is potentially toxic, particularly in the liver. Therefore, as the gut microbiota appears to be a major determinant of the BA pool by hydrophobicity, it may contribute to cholestatic liver injury [25]. It is noteworthy that in the experimental model cholestasis fails to promote liver injury in the absence of the microbiome in vivo [26]. On the other hand, the activation of intestinal macrophages, via inflammasome, promotes intestinal permeability and influences the microbiome composition throughout the leakage of bacterial products to the liver leading to cell death and inflammation [26].

3. Gut microbial profile in PBC

3.1. Altered gut bacteria profile in PBC

The role of gut microbiota is increasingly being recognized as critically important in cholangiopathies including PBC [27]. The paradigm of altered gut bacteria profile is represented by PSC; indeed, an altered microbiota composition has been observed in PSC, independently of IBD [28–30] (Table 1). Two articles specifically analyzed the alteration of gut bacteria profiles in PBC [31,32] (Table 1). Lv et al. [31] analyzed 42 patients with early-stage PBC founding that potentially beneficial species as *Bacteroides egerthii* and *Ruminococcus* were depleted, but the gut was enriched in some bacterial pathogens such as g-*Proteobacteria, Enterobacteriaceae, Neisseriaceae, Spirochetaceae, Veillonella, Streptococcus, Klebsiella, Actinobacillus pleuropneumoniae, Anaeroglobus geminatus, Enterobacter asburiae, Hemophilus parainfluenzae, Megasphaera micronuciformis,* and *Paraprevotella clara*. Remarkably, several gut bacterial taxa exhibited potential interactions with PBC through their associations with altered metabolism, immunity, and gut function. Tang et al. [32] performed a comparative analysis of the gut microbiome in 60 UDCA treatment-naïve patients with PBC and 80 matched controls and performed thereafter a validation study on independent 19 treatment-naïve patients and 34 controls. Finally, a prospective study was carried out in a subgroup of 37 patients with PBC at baseline and after 6 months of UDCA treatment. In both patients and controls, fecal samples were collected and microbiomes were analyzed by 16S ribosomal RNA sequencing. A microbial profile characterized by 12 genera was associated with PBC with a particular abundance of *Enterobacteriaceae*. The microbial dysbiosis was partially ameliorated by UDCA treatment. These data suggest the potential role of the gut microbiota as a novel diagnostic biomarker and also a therapeutic target in PBC.

Interestingly, biliary microbiota was assessed in bile taken aseptically from the gallbladder of 15 patients with PBC at the time of liver transplantation. In 75% of the cases Gram-positive cocci were identified, whereas only 5% of the patients with cholecystolithiasis were observed [33]. This result raises the possibility of a pathogenic role of Gram-positive bacteria throughout the molecular mimicry mechanism with PDC-E2 antigens of AMA. Alternatively, Gram-positive bacteria may trigger inflammation of biliary epithelial cells (BECs). (Table 1). As far as PSC is concerned, bile samples collected from 80 patients with PSC during endoscopic retrograde cholangiography (ERC) examination revealed that the most common phyla found were *Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, and Actinobacteria* [34]. (Table 1). Looking at specific bacteria taxa, the genus positively correlated with disease progression.

Table 1. Microbial profile in PBC and PSC.

	PBC [31–33]	PSC [28–30,34]
Gut microbiota	*γ-Proteobacteria*	*Enterococcus*
	Enterobacteriaceae	*Fusobacterium*
	Neisseriaceae	*Lactobacillus*
	Spirochetaceae	*Morganella*
	Veillonella	*Lactobacillus*
	Streptococcus	*Veillonella*
	Klebsiella	*γ-Proteobacteria*
	Actinobacillus pleuropneumoniae	*Proctobacteria*
	Anaeroglobus geminatus	*Bacteroidaceae*
	Enterobacter asburiae	
	Hemophilus parainfluenzae	
	Megasphera micronuciformis	
	Paraprevotella clara	
Biliary microbiota	*Staphylococcus*	*Streptococcus*
	Enterococcus	*Prevotella*
	Streptococcus	*Fusobacterium*
	Lactobacillus	*Veillonella*
	Helicobacter	*Hemophylus*
	Propionibacterium	
	Corynebacterium	
	Agrobacterium	
	Flavobacterium	
	Clostridium	
	Micrococcus	

4. Targeting microbiome for treating PBC

Nutritional and pharmacological interventions have been and are currently investigated with the aim of treating PBC by targeting the microbiome (Table 2).

4.1. Diet

Nutritional programs in the prevention and treatment of liver injury have been proposed for NAFLD only [35]. Regarding PBC there is only one study ongoing (NCT01603199) focusing on micronutrients specifically vitamin D for osteoporosis prevention. This diet is also balanced with fiber intake for increasing nitrogen products together with an adequate protein intake (1–1.5 g/Kg) in order to prevent the endogenous catabolism in patients with end-stage liver disease.

4.2. Probiotics

Probiotics are very interesting products with a specific background focusing on hepatic encephalopathy (HE). A recent meta-analysis including 9 RCT studies indicated that probiotics were associated with improvement of minimal HE, prophylaxis of overt HE, and reduction of physical and psychosocial sickness impact profile score and severe adverse events [36]. The only study utilizing probiotics in PBC is still ongoing (NCT03521297)

Table 2. Summary of the clinical evidence of targeting microbiome for cholestatic liver disease treatment.

Intervention	Study	Observed effect	Ref.
Probiotics	Meta-analysis of 9 RCT	Improvement and prophylaxis of HE, and reduction of physical and psychosocial sickness	[36]
Probiotics, prebiotics and symbiotics	Meta-analysis of 9 RCT	Improvement of minimal HE	[37]
UDCA	Cross-sectional study of 60 UDCA-naïve patients with PBC and 80 matched healthy controls	Partial reversion of gut microbiome composition after 6 months of treatment	[34]
Rifaximin	Retrospective study including 421 non-HCC patients and 621 HCC patients	Prolongation of overall survival, reduction of SBP risk, variceal bleeding and HE	[43]
Metronidazole	RCT with 80 patients (40 UDCA + metronidazole; 40 UDCA alone) treated for 36 months	Liver function amelioration	[44]
Minocycline	Open label study with 12 PSC patients	Amelioration of liver function	[45]
Vancomycin and metronidazole	Pilot RCT with 35 patients	Amelioration of liver function, with vancomycin superior than metronidazole	[46]
FMT	RCT with 20 cirrhotic patients	Improvement of cognition and inflammation	[48]
FMT	Pilot CT with 10 patients	Amelioration of liver function	[49]

and consists of phase 2, 6 months randomized trial including 60 patients with poor response to UDCA. According to the study design, feces and serum of patients will be collected to analyze the differences in fecal microbial polymorphisms in treated patients and controls. Metabolomics will be used to study the differences in bile acids and short-chain fatty acid metabolites of the serum and feces. The estimated study completion is August 2021.

4.3. Prebiotics and symbiotics

Analogously to probiotics, prebiotics and symbiotics have been tested in a randomized trial to prevent HE. A meta-analysis including nine studies showed that prebiotics, probiotics, and symbiotics were associated with significant improvement in minimal HE [37].

4.4. Ursodeoxycholic acid

Ursodeoxycholic acid (UDCA) is the standard therapy for PBC and improves survival by delaying progression toward cirrhosis and decompensation in approximately 60–70% of the patients [38]. The use of UDCA was found to partially reverse the composition of the gut microbiome after 6 months of treatment [32]. In particular, *Haemophilus, Streptococcus,* and *Pseudomonas* species decreased after UDCA treatment, whereas *Bacteroidetes, Sutterella,* and *Oscillospira* species increased in PBC patients post-UDCA treatment [32]. Interestingly, *Veillonella* which was found to be significantly increased compared to controls was significantly more prevalent in treated patients who had an inadequate response to UDCA. An observational Chinese study including 60 patients with PBC and treated with UDCA is still ongoing (NCT03590886). The aim is to compare intestinal flora diversity in different PBC patients with UDCA responses, and further study the differences in bile acid metabolism and short-chain fatty acid metabolism in feces and serum in PBC.

4.5. Obeticholic acid

Obeticholic acid (OCA), a potent FXR specific agonist, licensed as second-line therapy for PBC, can alter the gut microbial structure in an animal model of nonalcoholic fatty liver disease obtained by the administration of a diet rich in fat [39]. Moreover, Ubeda et al. explored the effect of OCA on bacterial translocation, and changes in intestinal microbiome in a rat model of nonalcoholic cirrhosis and ascites [40]. OCA-treated cirrhotic rats showed a reduction in *Proteobacteria* (*Escherichia coli* and *Shigella*) and an increased in *Firmicutes* (*Lactobacillus*). Moreover, treated rats showed a reduction in systemic inflammation as indicated by monocytes and T lymphocytes in mesenteric lymph nodes and peripheral blood. More recently, Palmer et al. found that rats with cirrhosis and ascites, treated with OCA, had decreased abundance of *Enterococcus spp*. in both the ileum and in the colon when compared to rats treated with placebo [41]. Of note, OCA also reduces bacterial translocation in an animal model of cholestasis induced by bile duct ligation [42]. The proposed mechanism for preventing bacterial translocation is a reduction in the recruitment of both natural killer cells (NKCs) and the NKC-derived proinflammatory cytokine IFNg, leading to a restoration of intestinal epithelial integrity through up-regulation of claudin-1, occluding, and zonulin-1, and thus preventing bacterial translocation.

4.6. Antibiotics

Antibiotics represent the typical drugs for the manipulation of the gut microbiome. In general, there are two types of antibiotics: a) absorbable and b) nonabsorbable. Absorbable antibiotics include rifaximin, paromomycin, and neomycin. Currently, they are employed in cirrhotic patients for spontaneous bacterial peritonitis (SBP) prophylaxis. As regard rifaximin, a large retrospective cohort demonstrated that in patients without hepatocellular carcinoma, rifaximin treatment was significantly associated with prolonged overall survival and reduced risk of SBP, variceal bleeding, and recurrent hepatic encephalopathy [43]. No, specific study has been conducted with either absorbable or nonabsorbable antibiotics in PBC. In contrast, in PSC two RCTs and an open-label study have been performed. Eighty patients were randomized to 36 months of UDCA (15 mg/Kg/day) plus metronidazole or UDCA alone [44]. A significant reduction in ALP, Mayo risk score and histologic stage and grade were reported in the group with metronidazole plus UDCA. An open-label study has been performed on 16 patients with PSC treated for 12 months with minocycline; a significant decrease in ALP was noticed,

but 25% of the patients withdrew from the study due to intolerance to minocycline [45]. Finally, a double-blind, randomized, pilot study was conducted in 35 patients randomized into 4 groups: low-dose vancomycin (125 mg 4 times daily), high-dose vancomycin (250 mg 4 times daily), low-dose metronidazole (250 mg 3 times daily), high-dose metronidazole (500 mg 3 times daily). Low-dose and high-dose vancomycin groups were superior to metronidazole and achieved a significant reduction in ALP [46].

4.7. Intestinal microbiota transplantation

Intestinal microbiota transplantation has been proposed to treat liver disease and cirrhosis [45]. Small studies including PSC, alcoholic hepatitis, and hepatitis B in the pre-cirrhotic stage have been published. In a prospective trial (NCT0315188) 20 cirrhotic patients were randomized into groups to receive fecal microbial transplant (FMT) capsules from a donor enriched in *Lachnospiraceae* and *Ruminococcaceae* or placebo. FMT capsules improved inflammation and cognition in cirrhotics. Interestingly, less secondary bile acids were detected in subjects who developed poor outcomes [47,48]. In a small trial, 10 PSC patients underwent IMT; 3 of them experienced a 50% reduction in alkaline phosphatase [49].

5. Microbiota profiling as diagnostic biomarker

5.1. Metabolomics

The conventional method for the identification of microbiome is the 16S rRNA gene sequencing assay. 16S rRNA consists of conserved and variable regions. While the conserved region makes universal amplification feasible, sequencing the variable regions allows discrimination between specific different micro-organisms [50]. Metagenomics (also known as environmental genomics) has been used since its discovery by Handelsman in 1998 [51]. Metagenomics of the intestinal tract were first described by Breitbart in 2006 to characterize gut microbiota from patients with inflammatory bowel disease (IBD) [52].

Besides serum and fecal sample microbiota has also been assayed in bile from patients with PBC. Gallbladder bile samples from 15 patients with PBC were tested with 16S rRNA profiling and bacterial sequences were found in 75% of the patients [53]. *Streptococcus aureus* was the most frequently detected microorganism (5/15 PBC patients, 33%; 40% of all PBC clones). And *Enterococcus faecium, Lactobacillus plantarum, Helicobacter pylori, Streptococcus pneumoniae* were the other commonly found bacteria.

Interestingly, duodenal mucosal microbiota was analyzed in 30 patients with cirrhosis and 28 healthy controls using the 16 rRNA gene pyrosequencing method. Overexpression of *Veilonella, Megasphaera, Dialister, Atopobium,* and *Prevotella* was found in cirrhotic patients compared to healthy subjects. When considering the etiology of cirrhosis, two operational taxonomic units (OTUs), OUT-23 (*Neisseria*) and OUT-36 (*Gemella*) were found discriminative between HBV-related cirrhosis and PBC. [54]

6. Conclusion

An increasing number of studies on the impact of microbiome alterations in PBC has been published over the last 10 years. In general, metabolomic analysis has provided evidence that a difference exists between PBC and other cholestatic liver diseases, particularly PSC. From the pathogenic point of view, gut bacteria can trigger the disease, by playing a pivotal role in the immune response and the synthesis of AMA trough a mimicry mechanism. There is an important link between the microbiome, bile acids, and their synthesis regulated by two important nuclear factors, i.e. FXR, and FGF15. Therefore, gut microbiome manipulation of IMT is one important challenge in the near future with the obvious implications of combination therapy with standard agents and eventually new drugs. Further studies are needed to test whether microbiome profiling could be utilized as a diagnostic biomarker in PBC.

7. Expert opinion

Which is the role of the intestinal gut microbiota (IGM) in the diagnosis of PBC? Nowadays metagenomics for IGM does not have a distinct role for diagnostic purposes in PBC. This is not the case of PSC, the other autoimmune cholangiopathy. In spite of the close affinity between PSC and PBC, these two diseases do have profound differences in pathogenesis and clinical management. Historical studies in PSC have identified a strong association between PSC and IBD, and a peculiar condition in the natural history of PSC is represented by the recurrent bacterial cholangitis. Thus, there is a very impressive and increasing knowledge on this issue [55,56]. As consequence, IGM modulation has been evaluated as a therapeutic approach first in PSC, and later in PBC. However, several issues should be considered regarding the manipulation of IGM in PBC.

First of all, PBC, in contrast to PSC, might have in its natural history recurrent urinary infections. Thus, the employment of antibiotics could be a challenge in the management of PBC. In this case, a specific approach against selected bacteria should be indicated.

As far as PBC pharmacological management is concerned, standard therapy with UDCA is beneficial for 60–70% of the patients with PBC but is controversial for PSC. Indeed, one trial with a moderate dose UDCA (17–23 mg/kg/day) has not shown a clear benefit to survival [57], and higher doses (25–30 mg/kg/day) may be harmful [58]. In PSC a benefit of UDCA (with moderate dose) at least for the amelioration of liver enzymes has been observed [59]. Furthermore, a Japanese observational study presented at AASLD meeting 2020 showed that UDCA treatment performed in 278 patients was associated with an improvement in liver transplantation-free survival and was likely to reduce the incidence of biliary tract cancer [60]. However, despite these important results, a large-scale cohort with an international collaboration is required to produce more convincing evidences for long-term therapy with UDCA in PSC. The reasons for less efficacy of UDCA in PSC compared to PBC are due to a profound difference between the two conditions. PBC has a long natural

history of cholestasis in which the target of immune-mediated damage is the intrahepatic bile duct. It is rather impossible that UDCA in PSC might have a benefit on the abnormalities of the biliary tree; moreover, there are a number of considerations regarding the limited effect of UDCA in a variety of clinical conditions (the association with inflammatory bowel disease, the overlap with autoimmune hepatitis, the small duct variant, the cystic dilatation variant). A second-line therapy with OCA may be beneficial in approximately 50% of the nonresponders to first-line therapy. OCA stimulates FGF15 in mice and FGF19 in humans. In a recent meta-analysis including eight studies (three in nonalcoholic steatohepatitis; 3 in PBC; 1 in PSC and 1 in gallstones) for a total of 2.854 patients treated with OCA, the rise in FGF19 in 5 and 50 mg were 75 ± 13.2 ng/L and 169 ± 7.72, respectively, [61]. In a murine model of intestinal inflammation, Gadaleta et al. found that FGF15 modulates intestinal microbiota in presence of FXR [62]. This finding strengthens the link between the regulation of bile acid homeostasis and inhibition of intestinal inflammation highlighting the potential role of FGF15/19 in chronic cholestasis. Moreover, FGF19 acts directly on the liver to suppress the expression of CYP7A1, the key enzyme that catalyzes the first and rate-limiting step in the pathway of bile acid synthesis [63]. On the basis of these considerations, we can speculate that specific gut microbiome profiles may lead to specific bile acid composition with different impact on both FXR and FGF19. Furthermore, an engineered analog of FGF19 (NGM282) has been evaluated in a 28-day multicentre, randomized, double-blind phase 2 trial in 45 patients with PBC [64]. At the end of the study, alkaline phosphatase was significantly reduced with NGM282 at both 0.3 mg and 3 mg vs placebo. Thus, FXR-FGF19 are potent regulators of circulating bile acids, stressing the importance of the gut-liver axis as a novel 'hepatostat' [65].

Another drug that can be used in PBC patients is rifampicin, a pregnane X receptor (PXR) agonist and cytochrome P450 enzyme inducer used as second-step management for pruritus in cholestatic diseases, including PBC [66]. There are no reports on the longitudinal analysis of IGM from PBC patients under rifampicin. However, rifampicin together with isoniazid and pyrazinamide (HRZ) which is an effective treatment against *Mycobacterium tuberculosis* infection, has been tested for detailed analysis of changes in the IGM. Rifampicin alone or in combination produces a long-lasting dysbiosis [67]. Thus, it should be expected that changes in IGM may occur also in PBC patients treated with rifampicin and this would potentially lead to alteration of immunological responses.

In conclusion, recognition of the intestinal microbiota composition will guide future therapeutic efforts in the clinical setting with the view of combination therapy with agents already employed against pathogenic mechanisms of the disease [68]. Bile acids have a potential role in modulating IGM and vice-versa. More recently, the bile acid receptors FXR and TGR5 have become major pharmacological targets for PBC. Nonbile acid FXR agonists (Tropifexor, Cilofexor, EDP-305) have been tested in phase two-thirds trials in PBC. However, further studies are needed to test their potential effect on the intestinal microbiome. Furthermore, the utilization of microbiota as a diagnostic biomarker in PBC remains so far not convincing enough so far. While the case of PSC again has been proposed as a peculiar condition with an association with a panel of taxa and even a single genus (*Veillonella*) [69], fecal microbiota profiles as diagnostic biomarker has not proved to be a useful tool in PBC.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. De Luca F, Shoenfeld Y. The microbiome in autoimmune diseases. Clin Exp Immunol. 2019;195:74–85.
•• **Recent useful review.**
2. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020;30:492–506.
3. Maroni L, Ninfole E, Pinto C, et al. Gut-liver axis and inflammasome activation in cholangiocyte pathophysiology. Cells. 2020;9:736.
4. Brenner DA, Paik YH, Schnabl B. Role of gut microbiota in liver disease. J Clin Gastroenterol. 2015;49:S25–7.
5. Chopyk DM, Grakoui A. Contribution of the intestinal microbiome and gut barrier to hepatic disorders. Gastroenterology. 2020;159:S0016–5085(20)34839–3. Online ahead of print.
•• **Recent useful review.**
6. Alvaro D, Carpino G, Craxi A, et al. Primary Biliary Cholangitis management: controversies, perspectives, and daily practice implications from an expert panel. Liver Int. 2020;40:2590–2601. Online ahead of print.
7. Selmi C, De Santis M, Cavaciocchi F, et al. Infectious agents and xenobiotics in the aetiology of primary biliary cirrhosis. Dis Markers. 2010;29:287–299.
8. Floreani A, Leung PSC, Gershwin ME. Environmental basis of autoimmunity. Clin Rev Allerg Immunol. 2016;50:287–300.
9. Mattner J, Savage PB, Leung P, et al. Liver autoimmunity triggered by microbial activation of natural killers T cells. Cell Host Microbe. 2008;3:304–315.
10. Bogdanos DP, Baum H, Okamoto M, et al. Primary biliary cirrhosis is characterized by IgG3 antibodies cross-reactive with the major mitochondrial autoepitope and its lactobacillus mimic. Hepatology. 2005;42:458–465.
11. Burroughs AK, Rosenstein IJ, Epstein O, et al. Bacteriuria and primary biliary cirrhosis. Gut. 1984;25:133–137.
12. Bogdanos DP, Baum H, Butler P, et al. Association between the primary biliary cirrhosis specific anti-sp100 antibodies and recurrent urinary tract infection. Dig Liver Dis. 2003;35:801–805.
13. Gershwin ME, Selmi C, Worman HJ, et al. The USA PBC Epidemiology Group. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. Hepatology. 2005;42:1194–1202.

14. Daniels JA, Torbenson M, Anders RA, et al. Immunostaining of plasma cells in primary biliary cirrhosis. Am J Clin Pathol. 2009;131:243–249.
15. Moreira RK, Revetta F, Koehler E, et al. Diagnostic utility of IgG and IgM immunohistochemistry in autoimmune liver disease. World J Gastroenterol. 2010;16:453–457.
16. Feld JJ, Meddings J, Heathcote EJ. Abnormal intestine permeability in primary biliary cirrhosis. Dig Dis Sci. 2006;51:1607–1613.
17. Floreani A, Baragiotta A, Pizzuti D, et al. Mucosal IgA defect in primary biliary cirrhosis. Am J Gastroenterol. 2002;97:508–510.
18. Sheth P, Delos Santos N, Seth A, et al. Lipopolysaccharide disrupts tight junctions in cholangiocyte monolayers by c-Src-, TLR4-, and LBP-dependent mechanism. Am J Physiol Gastrointest Liver Physiol. 2007;293:G308–G318.
19. Tabibian JH, O'Hara SP, Splinter PL, et al. Cholangiocyte senescence by way of N-ras activation is characteristic of primary sclerosing cholangitis. Hepatology. 2014;59:2263–2275.
20. Maldonado R, Sa-Correira I, Valvano M. Lipopolysaccharide modification in gram-negative bacteria during chronic infection. FEMS Microbiol Rev. 2016;40:480–493.
21. Wang A-P, Migita K, Ito M, et al. Hepatic expression of toll-like receptor 4 in primary biliary cirrhosis. J Autoimmun. 2005;25:85–91.
22. Seki E, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. Hepatology. 2008;48:322–335.
23. Wahlstrom A, Sayin SI, Marschall H-U, et al. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metab. 2016;24:41–50.
24. Sayin SI, Wahlstrom A, Felin J, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab. 2013;17:225–235.
25. Pean N, Doignon I, Tordjmann T. Gut microbiota and bile acids: an old story revisited (again). Clin Res Hepatol Gastroenterol. 2014;38:129–131.
    - **Review focusing on the link between bile acids and gut microbiota.**
26. Isaacs-Ten A, Echeandia M, Moreno-Gonzalez M, et al. Intestinal microbiome-macrophage crosstalk contributes to cholestatic liver disease by promoting intestinal permeability. Hepatology. 2020. in press. DOI:10.1002/hep.31228
27. Sato K, Meng F, Fava G, et al. Functional roles of gut bacteria imbalance in cholangiopathies. Liver Res. 2019;3:40–45.
28. Sabino J, Vieira-Silva S, Machiels K, et al. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. Gut. 2016;65:1681–1689.
29. Kummen M, Holm K, Anmarkrud JA, et al. The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls. Gut. 2017;66:611–619.
30. Ruhlemann M, Liwinski T, Heinsen F-A, et al. Consistent alterations in faecal microbiomes of patients with primary sclerosing cholangitis independent of associated colitis. Aliment Pharmacol Ther. 2019;50:580–589.
31. Lv L-X, Fang D-Q, Shi D, et al. Alterations and correlations of the gut microbiome, metabolism and immunity in patients with primary biliary cirrhosis. Environ Microbiol. 2016;18:2272–2286.
    - **Important study on gut microbiome in PBC.**
32. Tang R, Wei Y, Li Y, et al. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. Gut. 2018;67:534–541.
33. Hiramatsu K, Harada K, Tsuneyama K, et al. Amplification and sequence analysis of partial bacterial 16S ribosomal RNA gene in gallbladder bile from patients with primary biliary cirrhosis. J Hepatol. 2000;33:9–18.
34. Pereira P, Aho V, Arola J, et al. Bile microbiota in primary sclerosing cholangitis: impact on disease progression and development of biliary dysplasia. Plos One. 2017;12:e0182924.
35. Kong L, Lu Y, Zhang S, et al. Role of nutrition, gene polymorphism, and gut microbiota in non-alcoholic fatty liver disease. Discov Med. 2017;24:95–106.
36. Zhao L-N, Yu T, Lan S-Y, et al. Probiotics can improve the clinical outcomes of hepatic encephalopathy: an update meta-analysis. Clin Res Hepatol Gastroenterol. 2015;39:674–682.
37. Shukla S, Shukla A, Mehboob S, et al. Meta-analysis: the effect of gut flora modulation using prebiotics, probiotics and symbiotics on minimal hepatic encephalopathy. Aliment Pharmacol Ther. 2011;33:662–671.
38. Kuiper EM, Hansen BE, de Vries RA, et al. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. Gastroenterology. 2009;136:1281–1287.
39. Zhang D-Y, Zhu L, Tseng Y-J, et al. The protective effect and mechanism of the FXR agonist obeticholic acid via targeting gut microbiota in non-alcoholic fatty liver disease. Drug Des Devel Ther. 2019;13:2249–2270.
40. Ubeda M, Lazio M, Munoz L, et al. Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. J Hepatol. 2016;64:1049–1957.
41. Parmer C, Hung A, Yan KG, et al. Changes in intestinal microbiome in rats with cirrhosis and ascites: comparison between obeticholic acid (OCA) and placebo. Hepatology. 2019;70(suppl. 1):1098A (Abs 1821).
42. Verkebe L, Farre R, Verbinnen B, et al. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. Am J Pathol. 2015;185:409–419.
43. Kang SH, Lee YB, Lee J-H, et al. Rifaximin treatment is associated with reduced risk of cirrhotic complications and prolonged overall survival in patients experiencing hepatic encephalopathy. Aliment Pharmacol Ther. 2017;46:845–855.
44. Farkkila M, Karvonen AL, Nurmi H, et al. Metronidazole and ursodeoxycholic acid for primary sclerosing cholangitis: a randomized placebo-controlled trial. Hepatology. 2004;40:1379–1386.
45. Silveira MG, Torok NJ, Gassard AA, et al. Minocycline in the treatment of patients with primary sclerosing cholangitis: results of a pilot study. Am J Gastroenterol. 2009;104:83–88.
46. Tabibian JH, Weeding E, Jorgensen RA, et al. Randomised clinical trial: vancomycin or metronidazole in patients with primary sclerosing cholangitis – a pilot study. Aliment Pharmacol Ther. 2013;37:604–612.
47. Bajaj JS, Khoruts A. Microbiota changes and intestinal microbiota transplantation in liver disease and cirrhosis. J Hepatol. 2020;72:1003–1027.
48. Bajaj JS, Salzman N, Acharya C, et al. Microbial functional change is linked with clinical outcomes after capsular fecal transplant in cirrhosis. JCI Insight. 2019;4:e133410.
49. Allegretti JB, Kassam Z, Carrellas M, et al. Fecal microbiota transplantation in patients with primary sclerosing cholangitis: a pilot clinical trial. Am J Gastroenterol. 2019;114:1071–1079.
50. Fraher MH, O'Toole PW, Quigley EMM. Techniques used to characterize the microbiota: a guide for clinicians. Nat Rev Gastroenterol Hepatol. 2012;9:312–322.
51. Handelsman J, Rondon MR, Brady SF, et al. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol. 1998;5:R245–R249.
52. Breitbart M, Hewson I, Felts B, et al. Metagenomic analyses of an uncultured viral community from human feces. J Bacteriol. 2003;185:6220–6223.
53. Hiramatsu K, Harada K, Tsuneyama K, et al. Amplification and sequence analysis of partial bacterial 16S ribosomal gene in gallbladder bile from patients with primary biliary cirrhosis. J Hepatol. 2000;33:9–18.
54. Chen Y, Ji F, Shi D, et al. Dysbiosis of small intestinal microbiota in liver cirrhosis and its association with aetiology. Sci Rep. 2016;6:34055.
55. Nakamoto N, Sasaki N, Aoki R, et al. Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune response in primary sclerosing cholangitis. Nat Microbiol. 2019;4:492–503.
56. Liwinski T, Zenouzi R, John C. Alterations of the bile microbiome in primary sclerosing cholangitis. Gut. 2020;69:665–672.

57. Olsson R, Boberg KM, Schaffalistky De Mickadell O, et al. High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. Gastroenterology. 2005;129:1464–1472.
58. Lindor KD, Kowdley KV, Luketic VAC, et al. High dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology. 2009;50:808–814.
• **Important trial in PSC showing that high dose UDCA are harmful.**
59. Tabibian JH, Lindon KD. Ursodeoxycholic acid in primary sclerosing cholangitis: if withdrawal is bad, then administration is good (right?). Hepatology. 2014;60:785–788.
60. Arizumi T, Tazuma S, Nakazawa T, et al. The association of UDCA treatment with long-term outcome and biliary tract cancer in patients with primary sclerosing cholangitis. Hepatology. 2020;72(Suppl 1):Abs 100.
61. Kulkarni AV, Tevethia HV, Da PK, et al. Obeticholic acid in liver disease – a systematic review and meta-analysis. Hepatology. 2020;72(Suppl. 1):Abs 1685.
62. Gadaleta RM, Garcia-Irigoyen O, Cariello M, et al. Fibroblast growth factor 19 modulates intestinal microbiota and inflammation in presence of farnesoid X receptor. EBiomedicine. 2020;52:102719.
63. Russell DW. Fifty years of advanced in the bile acid synthesis and metabolism. J Lipid Res. 2009;50(Suppl):S120–S125.
64. May MJ, Wigg AJ, Leggett BA, et al. NGM282 for treatment of patients with primary biliary cholangitis: a multicenter, randomized, double-blind, placebo-controlled trial. Hepatol Commun. 2018;2:1–14.
65. Avila MA, Moschetta A. The FXR-FGF19 gut-liver axis as a novel "hepatostat". Gastroenterology. 2015;149:536–540.
66. Trivedi HD, Lizaola B, Tapper EB, et al. Management of pruritus in primary biliary cholangitis: a narrative review. Am J Med. 2017;130:744.e1–744.e7.
67. Namasivayan S, Maiga M, Yan W, et al. Longitudinal profiling reveals a persistent intestinal dysbiosis triggered by conventional anti-tuberculosis therapy. Microbioma. 2017;5:71–88.
68. Henao-Mejia J, Elinav E, Thaiss CA, et al. Role of the intestinal microbioma in liver disease. J Autoimmun. 2013;46:66–73.
• **Important review describing the relationship between the immune system, the microbiome and chronic liver disease.**
69. Ruhlemann MC, Heinsen F-A, Zenouzi R, et al. Faecal microbiota profiles as diagnostic biomarkers in primary sclerosing cholangitis. Gut. 2017;66:753–754.