Biliary microbiota composition determines epithelial barrier function and risk of acute bacterial cholangitis

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Background and preliminary data:

Acute bacterial cholangitis is an infection of the biliary tract, often as a consequence of biliary tract obstruction. Despite improved management, severe complications such as biliary sepsis can arise, and the mortality associated with acute cholangitis remains around 5%[1]. Causal bacterial species mainly identified are *Escherichia coli*, *Klebsiella spp* and *Enterococcus spp.* People affected by Primary Sclerosing Cholangitis (PSC), a disease of unknown etiology leading to scarring and fibrosis of the intrahepatic and extrahepatic bile ducts, are at high risk of developing acute bacterial cholangitis. We have shown using 16s rRNA sequencing from bile fluid, that even in the absence of acute bacterial cholangitis, the biliary tract is colonized by a diverse bacterial ecosystem both in non-PSC controls and people suffering from PSC[2]. People with PSC display a lower microbial diversity in the bile ducts, accompanied by an expansion of pathogens[2] (Fig.1A). Notably, the opportunistic pathogen *Enterococcus faecalis* was significantly increased in stool and bile samples of people with PSC, and the presence of *Enterococcus spp* in bile associated with lower liver transplantation-free survival in PSC[3,4] (Fig.1B). We have already collected and sequenced the genome of 49 biliary *E. faecalis* isolates from people with PSC and non-PSC controls. Genomic analysis allowed us to identify harmless and virulent *E. faecalis* strains, that harbor a vast array of virulence genes and display high proinflammatory potential *in vitro* (Fig.1C).

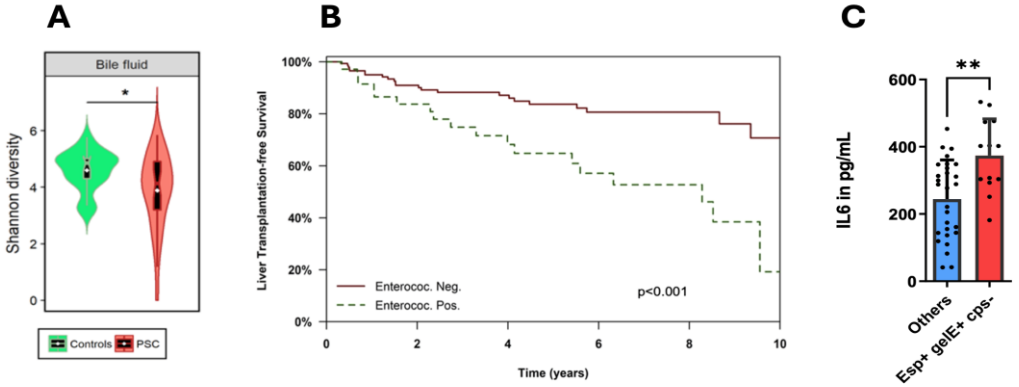

Fig1. (A) Violin plots for Shannon α-diversity index stratified by diagnosis in bile fluid. (Welch's t-test, p=0.0127); *, p<0.05. (B) Kaplan-Meier survival curve of transplant-free survival in patients with positive vs negative bile cultures. (C) Barplot showing IL-6 concentration after 24 hours of stimulation by E.faecalis lysates, stratified by the presence of virulence factors Esp, gelE and cps.

Emerging data highlight the predictive value of microbiota composition in identifying patients at risk of further infection. In liver transplant recipients, low gut microbial diversity associated with increased risk of postoperative infection, and high *Enterococcus spp* abundance had a predictive value in this setting[5]. Biliary microbes are in direct contact with cholangiocytes, the cells forming the lining of the bile ducts. Maintenance of the biliary epithelial barrier (BEB) integrity is crucial for pathogen defense and homeostasis in the liver, and we believe that microbiota composition is critical to its functionality. By understanding host-microbe dynamics at the BEB, we hope to further uncover the pathogenesis of acute bacterial cholangitis, and on a larger scale to assess the importance of the unexplored biliary microbiome in liver physiology.

Hypothesis:

We hypothesize that biliary dysbiosis and expansion of virulent *Enterococcus sp* in the bile leads to biliary barrier dysfunction and is thus a risk factor for acute bacterial cholangitis in PSC and post liver transplantation.

Aims and Work Programme:

1. To investigate the relationship between the biliary microbiome and occurrence of acute bacterial cholangitis in a well characterized cohort of people affected by PSC and post liver transplantation.
2. To investigate the potential of biliary commensals and pathogens to modulate the biliary epithelial barrier function.

In Aim #1, we will rely on 16s rRNA sequencing analyses as well as our large and ongoing collection and whole genome sequencing data of bile samples and biliary isolates. We will:

- Perform 16s rRNA sequencing of consecutive bile sample collected from our PSC and post-transplant cohort and analyze the stability of microbial communities over time and course of disease.
- Assess whether microbial patterns are predictive for the occurrence of acute bacterial cholangitis.
- Assess whether *E. faecalis* strain diversity in the bile i.e presence of harmless and virulent strains is associated with higher risk of acute bacterial cholangitis.
- Investigate how antimicrobial therapies affect biliary microbial composition, and thus potentially the risk of bacterial cholangitis

In Aim #2, we will investigate the crosstalk of biliary commensals and pathogens with cholangiocytes, *ex vivo* using PSC biosamples and *in vitro* using biliary organoid co-culture systems. We plan to:

- Perform 16s rRNA sequencing of bile samples and mRNA sequencing of paired biliary brushes collected during endoscopic procedures, to perform correlation analyses of biliary microbiota and cholangiocyte transcriptomic profiles.
- Challenge biliary organoids with live bacteria and assess the potential of these bacteria to induce changes in cholangiocyte barrier function, as assessed by transepithelial electrical resistance measurements and production of antimicrobial peptides in the culture supernatant assessed by ELISA.
- Perform translocation assays to assess the capacity of biliary bacterial strains to translocate across the biliary barrier.

Project-related publications:

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(2) Liwinski, T.; Zenouzi, R.; John, C.; Ehlken, H.; Rühlemann, M. C.; Bang, C.; Groth, S.; Lieb, W.; Kantowski, M.; Andersen, N.; Schachschal, G.; Karlsen, T. H.; Hov, J. R.; Rösch, T.; Lohse, A. W.; Heeren, J.; Franke, A.; Schramm, C. Alterations of the Bile Microbiome in Primary Sclerosing Cholangitis. *Gut* **2020**, *69* (4), 665–672.

(3) Zigmond, E.; Zecher, B. F.; Bartels, A.-L.; Ziv-Baran, T.; Rösch, T.; Schachschal, G.; Lohse, A. W.; Ehlken, H.; Schramm, C. Bile Duct Colonization With Enterococcus Sp. Associates With Disease Progression in Primary Sclerosing Cholangitis. *Clin Gastroenterol Hepatol* **2023**, *21* (5), 1223-1232.e3.

(4) Awoniyi M, Wang J, Ngo B, Meadows V, Tam J, Viswanathan A, Lai Y, Montgomery S, Farmer M, Kummen M, Thingholm L, Schramm C, Bang C, Franke A, Lu K, Zhou H, Bajaj JS, Hylemon PB, Ting J, Popov YV, Hov JR, Francis HL, Sartor RB. Protective and aggressive bacterial subsets and metabolites modify hepatobiliary inflammation and fibrosis in a murine model of PSC. *Gut* **2023** Apr;72(4):671-685. doi: 10.1136/gutjnl-2021-326500.

(5) Lehmann, C. J.; Dylla, N. P.; Odenwald, M.; Nayak, R.; Khalid, M.; Boissiere, J.; Cantoral, J.; Adler, E.; Stutz, M. R.; Cruz, M. D.; Moran, A.; Lin, H.; Ramaswamy, R.; Sundararajan, A.; Sidebottom, A. M.; Little, J.; Pamer, E. G.; Aronsohn, A.; Fung, J.; Baker, T. B.; Kacha, A. Fecal Metabolite Profiling Identifies Liver Transplant Recipients at Risk for Postoperative Infection. *Cell Host & Microbe* **2024**, *32* (1), 117-130.e4.