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Inflammation and microbiota fingerprint: Delphi's oracle for NAFLD-related HCC?

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Dysfunctions of the gut bacterial flora have an important impact on liver diseases. Quantitative or qualitative ruptures of intestinal homeostasis, collectively termed dysbiosis, constitute an increasingly recognized associated factor for chronic liver diseases, including non-alcoholic fatty liver disease (NAFLD). The evidence that gut maladaptation is a common feature in NAFLD settings led Ponziani et al. to investigate whether specific variations of the flora and inflammatory cues are enriched in NAFLD patients presenting with HCC (Ponziani et al. this issue). The authors included prospectively 21 patients with NAFLD-related cirrhosis and HCC, 20 patients with NAFLD-related cirrhosis without HCC and 20 healthy controls. In addition to age, cirrhotic's cohorts were also matched on severity of liver disease and portal hypertension. While observing similar dysfunctions of gut permeability within cirrhotic patients, the authors reported an increased intestinal-derived permeability in cirrhotic subjects with HCC, revealed by the increase in lipopolysaccharides and zonulin-1 plasma level compared to healthy control. The pattern of cytokines further supports an inflammatory *milieu* specific of cirrhotic patients with and without HCC. Myeloid-derived suppressor cells (mMDSC), which are bone marrow-derived cells whose diagnostic and prognostic significance has been recently reported in HCC patients, are thought to promote tumor progression primarily by suppressing the antitumor

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immunity and favoring tumor angiogenesis (1). Ponziani et al. found that pro-inflammatory mediators such as IL8, IL13, CCL3, CCL4, and CCL5 were significantly increased in the presence of HCC and positively correlated with the levels of circulating activated monocytes and monocytic MDSC (figure 1).

When Ponziani and colleagues performed the 16S RNA-based metagenomic analysis of the gut microbial composition they found cirrhotic patients to have a lower gut microbial diversity compared to controls, independent of the presence of HCC. The authors suggest a framework where cirrhosis affects the gut microbial composition. According to their results, the dysbiotic fingerprint of the whole cirrhotic cohort (with and without HCC) is characterized by alterations of specific bacterial *genera*, as defined by the relative abundance in *Enterobacteriaceae*, *Streptococcus*, *Bacteroides*, and *Ruminococcus* among others, and the reduction in *Bifidobacterium* and *Akkermansia*.

The authors proposed that the reduction of specific bacterial *genera* such as *Akkermansia* and *Bifidobacterium* sensitizes the inflammatory risks of NAFLD patients and correlate with increased CCL5 and calprotectin levels. Importantly, Ponziani et al. have shown unappreciated taxonomic differences when comparing the microbiota of cirrhotic patients with or without HCC, such as the increased abundance of *Bacteroides* and *Ruminococcaceae*, potentially spurring disease progression via pro-tumorigenic cues.

Studies point out that hepato-metabolic disease risk involves multiple strains of bacteria and since the gut flora composition can differ from person to person, it means that single bacterial species might not explain the full responsibility of the disease. A promising strategy might be to promote the growth of healthy bacterial strains rather than target the bad ones. This is supported by some early clinical evidence that specially formulated probiotics (cocktails of “good” bacteria) can bump the microbiome back into balance (2, 3). It is still early days for this research, but the premises are exciting since almost 30% of the metabolites in the blood are accountable by from intestinal bacteria.

This study advances our knowledge of intestine-derived inflammation and HCC. The authors define a specific set of inflammatory regulators such as CCL3, CCL4, and CCL5 associated with cirrhosis and HCC. They also highlight the clinical significance of correlating cytokine/chemokine profile with the recruitment of cellular infiltrates hastening tumor progression via immunosuppressive effects. Their findings corroborate previous studies defining the pro-tumorigenic role of these cytokines in both animal models and clinical settings (4-7). These observations follow on previous seminal studies performed with similarities and also remarkable differences both in terms of study design and operational procedures. Exploiting a quantitative-metagenomic based resource Qin and colleagues compared the gut-metagenomic signature of 98 Chinese patients with cirrhosis predominantly due to viral hepatitis and alcoholic cirrhosis with 83 healthy controls, finding an enrichment of taxonomical species of buccal origin, indicative of an invasion of the gut from the mouth in liver cirrhosis (8). Further, Bajaj and coworkers characterized the association of gut microbiome with hepatic decompensation in 219 cirrhotic patients, although 17% of patients presented with NAFLD-related conditions and only 68% were of Caucasian origin precluding comparison with the study of Ponziani et al. (9). Loomba and colleagues integrated stool metagenomic profiling with the assessment of serum analytes via metabolomics in 86 patients with biopsy-proven NAFLD to examine the diagnostic potential of microbiome-derived signature for the presence of advanced fibrosis (10). All these studies recorded changes in the gut microbiota of patients with different states known to predispose to liver diseases.

Additionally, dysbiosis seems to associate with an increase in the size of the population of bacteria that produce ethanol. So, although the name NAFLD contains the term 'non-alcoholic', the reality of the condition is more complicated and alcohol from bacteria may still play a part in its origins. An alluring and so far unexplored frame prompted by Ponziani et al. might come from the correlation of the phenotypic severity with the deregulation of ethanol-producing bacterial population.

The generalizability of the results of Ponziani et al. is uncertain since this is a single center study with a relatively small cohort size. Their cohort selection followed tight selection criteria, ethnicity and diet amongst many others, aimed to minimize the impact of these confounding factors though such stringency may also limit the holistic interpretation of microbial composition's shifts. Nevertheless, the contribution of this work elegantly corroborates the compelling evidence of a link between inflammation, changes in the gut microbiota composition

and NAFLD-related cirrhosis and HCC. The details and relative importance of the proposed mechanisms by which dysbiosis causes liver damage via inflammation are still unclear. The intestinal microbiota profiling has not yet provided a clear causative relationship between dysbiosis and HCC development. Still, the potential of microbiome-based metagenomic signatures is enormous and interventions to prevent this dreadful disease warrant further studies to pin down the importance of these associations.

This work sets the stage to a new way of monitoring and preventing liver cirrhosis, indicating that stool- and plasma-based test may represent a powerful diagnostic tool for disease detection, adjunctive or more optimistically substitutive to current invasive approaches for staging liver diseases. It is plausible that the serum/plasma based tests, as the cytokine analysis performed by the authors, may be used in conjunction with the bacterial metagenomic signature to elevate the non-invasive screening potential of detecting advanced fibrosis, cirrhosis and ultimately HCC risk.

Legend Figure 1. The presence of a hepatocellular carcinoma in patients with cirrhosis due to non-alcoholic fatty liver disease is associated with changes in the gut and in the blood compartments. In the gut, the authors report an increased intestinal permeability, increased levels of fecal calprotectin and modifications in the composition of the microbiome. In the blood, they found increased levels of pro-inflammatory mediators such as interleukin 8 and 13 and C-C motif chemokine ligand 3, 4 and 5.

References

1. Motz GT, Coukos G. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. *Nat Rev Immunol* 2011;11:702-711.
2. Wong VW, Won GL, Chim AM, Chu WC, Yeung DK, Li KC, Chan HL. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. *Ann Hepatol* 2013;12:256-262.
3. Li J, Sung CY, Lee N, Ni Y, Pihlajamaki J, Panagiotou G, El-Nezami H. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proc Natl Acad Sci U S A* 2016;113:E1306-1315.

4. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, Vrbanac AF, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature* 2017;551:340-345.
5. Crispe IN. Hepatocytes as Immunological Agents. *J Immunol* 2016;196:17-21.
6. Grakoui A, Crispe IN. Presentation of hepatocellular antigens. *Cell Mol Immunol* 2016;13:293-300.
7. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012;482:179-185.
8. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59-64.
9. Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940-947.
10. Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, et al. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2017;25:1054-1062 e1055.

Figure 1.

