# *Neither black nor white – do altered intestinal microbiota reflect chronic liver disease severity?*

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**REPLY TO** ***Wei Y, et al.: Gut 2019;0:1–9. doi:10.1136/gutjnl-2018-317836***

In their outstanding study, Wei et al. (1) compared the intestinal microbiota (IM) of well-defined autoimmune hepatitis (AIH) patients to healthy controls. They reported significantly reduced IM alpha diversity and disparate IM compositional heterogeneity (beta-diversity) with enrichment of Streptococcus, Veillonella, Klebsiella, and Lactobacillus in AIH. The excellent achievement of this study is to have characterized the IM of untreated patients with AIH. However, differentiation between IM changes caused by AIH in particular and by chronic liver disease (CLD) in general was not possible, because this study lacked a control group with CLD other than AIH.

In a pilot study, we analyzed the IM of patients with CLD of mixed etiology in comparison to healthy controls to clarify alterations across different stages of CLD (**Table 1** + **Supplementary data**). In contrast to the study by Wei et al., patients with recent intake of antibiotics were excluded. Additionally, only patients on proton pump inhibitors (PPI), which are frequently taken by CLD patients, were included to avoid a bias due to mixed PPI use, because PPI can alter the IM, in particular the abundance of Veillonellaceae and Streptococcaceae (2). Based on investigations of normalized relative reads (nRR) per IM taxon as well as IM alpha and beta diversity analyses, we detected Veillonella and Streptococcus, among other IM members, with increasing abundance (**Figure 1 A+B**), while IM alpha diversity displayed a stepwise decrease between healthy controls, non-cirrhotic and cirrhotic CLD patients (**Figure 1C**). IM beta diversity analysis indicated disparate IM compositional heterogeneity (**Figure 1D**). Moreover, IM alpha diversity correlated inversely with bacterial translocation (BT), as measured by soluble CD14 (**Figure 1E**), which, as expected, showed increased levels in CLD (**Figure 1F**).

Thus, part of IM alterations identified by Wei et al. – e. g. concerning Veillonella and Streptococcus – may not be specific for AIH, which is discussed by the authors themselves with respect to other autoimmune liver diseases. Furthermore, PPI use, which was – as stated – not controlled for by Wei et al., may lead to similar IM changes (3). However, some of these changes are additionally enhanced by the severity of cirrhosis (4), highlighting the complex interplay between widespread PPI use and cirrhosis severity on the IM.

Wei et al. identified IM changes related to the grading of AIH activity, but not to stage of fibrosis. Our findings indicate that at least among PPI users, changes in Veillonella and Streptococcus are aggravated with progressing CLD severity. The interplay between stage of CLD and IM is supported by studies in chronic HCV infection concerning Veillonella, Streptococcus and Lactobacillus (5) and Streptococcus, Lactobacillus and Klebsiella (6). Stage-dependent decreases of IM alpha diversity were also reported from non-alcoholic fatty liver disease (7). Possibly, a particular strength of the study by Wei

et al – including a homogenous set of patients with newly diagnosed AIH – may have hampered the sub-analysis regarding fibrosis stage, because the majority (51/66; 77.3%) of patients had intermediate fibrosis, so that the detection of alterations in mild or advanced fibrosis might have been missed.

In conclusion, our data and previous reports indicate that some IM alterations identified by Wei et al. may not be AIH-specific but could also be associated with PPI use and may increase with CLD severity. As a tight interplay between the IM and CLD via BT exists (8), gut-liver axis modulation is a promising approach for CLD management (9,10). Therefore, studies such as by Wei et al. are highly welcome to unravel IM alterations in well-characterized cohorts of patients with a specific CLD. However, further studies are needed to dissect IM alterations regarding etiology and stage of CLD.

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**FIGURE / TABLE LEGENDS**

**Figure 1:**

This figure shows results of chronic liver disease (CLD)-stage related normalized relative reads (n-RR) per intestinal microbiota (IM) taxon, IM alpha and beta diversity as well as bacterial translocation (BT)-associated analyses.

CLD stage-dependent distribution of n-RR per IM taxon are shown for non-cirrhotic and cirrhotic CLD as well as healthy controls (**A**+**B**) as: i) cumulative columns (**A, left panel**) and ii) heat map (**A, right panel**: different grades of blue and red indicate relative decreases and increases, respectively). Analysis of corresponding n-RR levels regarding stages of CLD are shown (**B**; *Tukey Multiple Test* corrected *One-way ANOVA* analysis: CLD (cirrhosis) vs. healthy and CLD (non-cirrhotic) – $p < 0.001$, respectively; CLD (non-cirrhotic) vs. healthy – $p < 0.01$). Linear regression analysis is indicated by a dashed line; statistical results are displayed underneath the plot.

Analysis of Chao-1 index levels (marker of IM alpha diversity) is displayed (**C**; healthy vs. CLD (cirrhosis) – $p < 0.001$). Linear regression analysis is indicated by a dashed line; statistical results are shown underneath the plot.

Principal component analysis (PCoA) regarding IM beta diversity analysis of stages of CLD and healthy controls is shown (**D**): statistical results (permutational analysis of variance, PERMANOVA) is indicated at the lower right quadrant of the panel; color-coded data points indicate relating CLD degrees: blue – healthy, green – CLD (non-cirrhotic), and red – CLD (cirrhosis).

Association analysis of IM alpha diversity (Chao-1) and bacterial translocation (marker: soluble CD14, sCD14) with the respective linear regression line is shown (**E**); statistical results are shown underneath the plot (**E**); data points indicate the relating status: blue – healthy, green – CLD (non-cirrhotic), and red – CLD (cirrhosis)). Levels of sCD14 were plotted with respect to stages of CLD (**F**: healthy vs. CLD (non-cirrhotic) and CLD (cirrhosis) – $p < 0.05$, respectively).

Levels of statistical significance are indicated as following: * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

**Table 1:**

This table shows patients´ characteristics of the study cohort.

Data of healthy controls and of CLD stage-related sub-groups – CLD (non-cirrhotic) and CLD (cirrhosis) – is shown. Displayed numbers indicate mean levels with range (where applicable) and standard deviation (SD), respectively.

[1] = *Tukey´s Multiple test*-corrected *One-Way-ANOVA* comparing stages of CLD (exclusive healthy controls); indicated are significantly different levels (*****).

[a] = not detected / available

Abbreviations for *Table 1:*

AIH: autoimmune hepatitis, ALD: alcoholic liver disease, ALT: Alanine transaminase, AST: Aspartate transaminase, BMI: body mass index, CLD: chronic liver disease, CRP: C-reactive protein, HBV: hepatitis B virus, HCV: hepatitis C virus, INR: internal normalized ratio, MELD: model of end stage liver disease, NAFLD: non-alcoholic fatty liver disease, NASH: non-alcoholic steatohepatitis, PBC: primary biliary cholangitis; PPI: proton pump inhibitor; PSC: primary sclerosing cholangitis.