A Gut Microbiome Signature for HIV and Non-Alcoholic Fatty Liver Disease

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BACKGROUND

Metabolic dysfunction-associated steatotic liver disease (MASLD), has emerged as an increasingly recognized problem among people living with HIV (PLWH). The gut-liver axis is considered to be strongly implicated in the pathogenesis of MASLD. We aimed to characterize the gut microbiota composition in PLWH and MASLD and compare it with that of two control groups: PLWH without MASLD and individuals with MASLD without HIV infection.

METHODS

- Multicenter prospective cohort study. Participants with and without HIV infection who presented hypertransaminasemia maintained in at least two determinations separated by six months were included.
- Comprehensive liver disease assessments, including abdominal ultrasounds and a screening analysis for liver disease were conducted on all subjects, who were then categorized into MASLD or non-MASLD cohorts. Moreover, a biopsy was performed on nine participants with a fibrosis score of F3 or higher, confirming steatohepatitis in four of them.
- Strict exclusion criteria were applied: individuals with active viral hepatitis, significant alcohol (over 30g daily for men, 20g for women) or specific drug abuses (cocaine, heroin, or synthetic drugs) and diagnoses of another liver disease.
- At the baseline visit, a nutritional survey was conducted, and a stool sample was collected. Samples were frozen immediately and stored until processing at −80 °C.
- DNA was extracted from fecal samples following the procedure described by Lackey et al. [1]. A two-step PCR procedure employing dual barcodes was employed to amplify a segment of the V3-V4 hypervariable region within the bacterial 16S ribosomal RNA (rRNA) genes which were amplified and sequenced with Illumina Sequencing Technology and finally clustered into operational taxonomic unit using Kraken2.
- Alpha diversity was studied by Shannon and Simpson indexes. To study how different the gut microbiota composition is between the different groups, beta diversity estimation was evaluated by principal coordinate analysis (PCoA) using Bray-Curtis dissimilarity. To further analyze differences in microbiome composition we performed a linear discriminant analysis (LDA) effect size (LEfSe).

We found a microbiome signature linked to MASLD, which has a greater influence on the overall structure of the gut microbiota than HIV status alone.

RESULTS

We included 30 HIV$^+$MASLD$^+$, 30 HIV$^+$MASLD$^-$, and 20 HIV$^-$MASLD$^+$ participants. The characteristics of the study population are provided in **Table 1**. People diagnosed with MASLD had similar characteristics, regardless of their HIV status. These participants were older, had a higher BMI, and had a higher prevalence of comorbidities such as dyslipidemia and diabetes mellitus. Participants with HIV had a higher percentage of men, and all were receiving long-term suppressive antiretroviral therapy for an average of 6 years. The severity of MASLD was comparable in both groups regardless of HIV status, measured by transient elastography (FibroScan). Among participants with MASLD, PLWH had higher alcohol consumption.

Table 1. Population baseline characteristics by group

	MASLD+ HIV- (n=20)	MASLD - HIV+ (n=30)	MASLD + HIV+ (n=30)	p-value
Age, median (IQR)	**56 (51, 68)**	**53 (45,56)**	**54 (43, 59)**	**0.043**
Male gender, n (%)	**11 (55)**	**26 (87)**	**27 (90)**	**0.011***
Ethnicity, n (%)				
Caucasian	14 (70)	24 (80)	18 (60)	
Latin American	3 (15)	4 (13)	10 (33)	0.218
Sub-Saharan African	0 (0)	1 (3)	0 (0)	
Other / unknown	3 (15)	1 (3)	2 (7)	
Body mass index (kg/m2), median (IQR)	**31.2 (28.8, 33.8)**	**24.0 (22.7, 27.1)**	**27.5 (25.1, 28.7)**	**0.001***
Diabetes mellitus, n (%)	**10 (50)**	**2 (7)**	**3 (10)**	**<0.001***
Dyslipidemia, n (%)	**17 (85)**	**7 (23)**	**25 (83)**	**<0.001****
Antibiotic use in past 6 months, n (%)	2 (10)	7 (23)	3 (10)	0.154
Diet (servings per week), median (IQR)				
Legumes	2 (1,2)	2 (0, 3)	2 (1, 3)	0.255
Cereals	7 (7, 7)	21 (8, 26)	7 (7, 7)	0.001**
Vegetables	5 (2, 7)	11 (5, 20)	5 (4, 7)	0.011**
White fish	1 (0, 2)	1 (1, 3)	1 (1, 2)	0.377
Blue fish	1 (1, 2)	1 (1, 2)	1 (1, 2)	0.629
Red meat (beef)	1 (1, 3)	2 (1, 3)	2 (1, 3)	0.669
Pork	2 (1, 3)	2 (1, 4)	1 (1, 2)	0.157
Poultry	3 (2, 3)	2 (1, 3)	3 (2, 5)	0.219
Dairy products	14 (11, 14)	13 (9, 19)	13 (7, 14)	0.338
Fats (oil, butter)	7 (4, 7)	17 (7, 29)	7 (6, 7)	0.001**
Alcohol	**0 (0, 1)**	**0 (0, 2)**	**3 (1, 7)**	**0.017**
Soft drinks	1 (0, 3)	1 (0, 2)	2 (0, 3)	0.689
MASLD severity				
CAP, median (IQR)	**320 (298, 359)**	**170 (134, 210)**	**275 (234, 288)**	**0.004**
kPa, median (IQR)	**6.8 (5.6, 9.5)**	**4.4 (3.4, 6.1)**	**5.3 (4.2, 6.2)**	**0.002**
Years since HIV diagnosis, median (IQR)	-	19 (9, 31)	20 (8, 26)	0.460
Years on ART, median (IQR)	-	**10.1 (7.5, 23.3)**	**5.9 (5.1, 7.0)**	**<0.001**
Nadir CD4 T-cell count, median (IQR)	-	**180 (100, 291)**	**362 (180, 524)**	**0.003**
ART regimen, n (%)				
NNRTI		**9 (30)**	**2 (7)**	
PI		**3 (10)**	**1 (3)**	**0.043**
INSTI		**15 (50)**	**25 (83)**	
NRTI backbone, n (%)				
TDF/FTC		13 (43)	6 (20)	
TAF/FTC		4 (13)	11 (37)	0.111
ABC/3TC		9 (30)	8 (27)	
Other		4 (13)	6 (17)	
HCV positive, n (%)	0 (0)	**5 (17)**	**6 (20)**	**<0.001***

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ART, antiretroviral therapy; CAP, controlled attenuation parameter; GGT, gamma-glutamyl transferase; IQR, interquartile range; INSTI, integrase strand transfer inhibitor; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein, MASLD, metabolic dysfunction-associated steatotic liver disease; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

All participants with HCV antibodies had undetectable HCV-RNA (cured hepatitis C).

*No statistically significant differences between the two groups of participants with HIV

**No statistically significant differences between the two groups of participants with MASLD

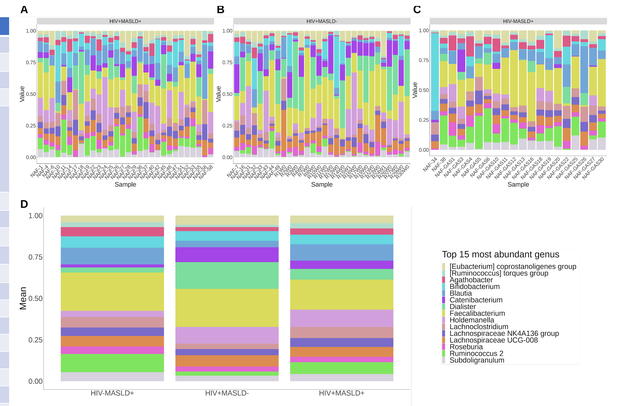


Figure 1. Taxonomic composition of the top 15 most abundant genus. Different study groups HIV+MASLD+, HIV+MASLD- and HIV-MASLD+ are represented in panels **A**, **B** and **C** respectively, where each bar denote the microbiota composition for each study subject. **D** represents the group's mean.

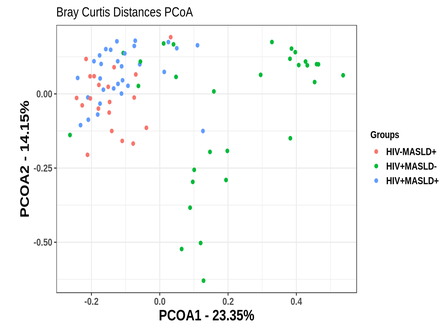


Figure 2. Abundance of the most significant genus according to LEfSe. LefSe analysis examined differences in the microbiome across three groups and selected those with a Linear Discriminant Analysis score >4. Each study group is represented by a color, where a box plot shows the distribution of the most significant genus according to LEfSe.
Abbreviations: LEfSe, Linear discriminant analysis effect size; MASLD, non-alcoholic fatty liver disease.

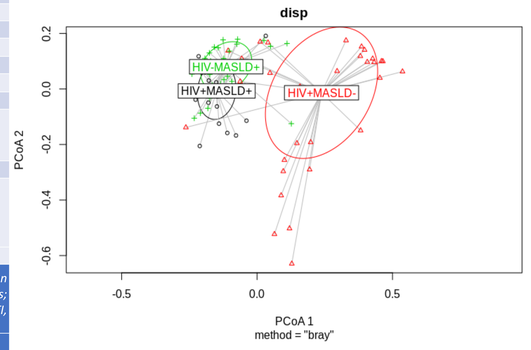


Figure 3. Beta-dispersion plot. Multivariate homogeneity of groups dispersions using Bray-Curtis dissimilarity measures. *Tukey multiple comparisons test:* HIV$^-$MASLD$^+$ vs. HIV$^+$MASLD$^+$, q-value = 0.002; HIV$^-$MASLD$^+$ vs. HIV$^+$MASLD$^-$, q-value = 0.930; HIV$^+$MASLD$^+$ vs. HIV$^+$MASLD$^-$, q-value < 0.001.

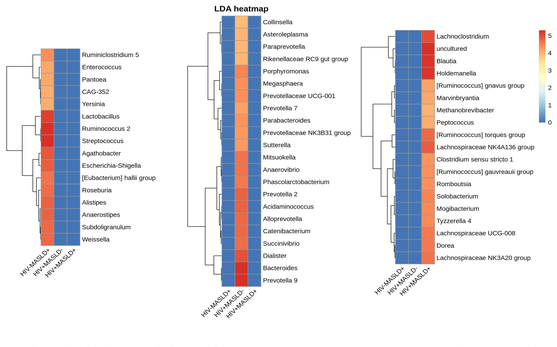


Figure 4. LDA heatmap. Estimated LDA value for those genus (heatmap rows) most likely to explain differences between the three study groups (heatmap columns), being those genus in red the most significant for each group.
Abbreviations: LDA, Linear discriminant analysis; MASLD, non-alcoholic fatty liver disease

CONCLUSIONS

We found a microbiome signature linked to MASLD, which has a greater influence on the overall structure of the gut microbiota than HIV status alone. We suggest that part of the alterations in the microbiota described as associated with HIV could be confused by the presence of MASLD, which is more prevalent in people with HIV. Future works should address this issue to advance knowledge in this field.

1. Lackey KA, Williams JE, Meehan CL, et al. What's normal? Microbiomes in human milk and infant feces are related to each other but vary geographically: The inspire study. Front Nutr **2019**; 6:45.