Multi-omics Reveals Metabolic and Microbial Markers of Serious Non-AIDS Event Risk in People with HIV

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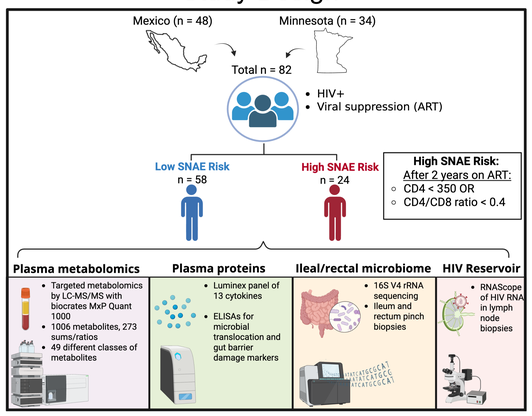
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BACKGROUND

Serious non-AIDS events (SNAEs), including non-AIDS malignancies, cardiovascular disease, and hepatic complications, remain major causes of mortality in treated HIV infection. These outcomes are driven by persistent immune activation, systemic inflammation, and metabolic dysfunction despite effective viral suppression with antiretroviral therapy (ART). To investigate mechanisms underlying SNAE pathogenesis, we performed a cross-site multi-omic analysis integrating plasma proteins, plasma metabolites, and mucosal microbiomes (ileum and rectum) in 82 ART-treated people with HIV (PWH) from the United States and Mexico.

METHODS

Study Design



Patients with a CD4⁺ T cell count <350 or a CD4/CD8 ratio < 0.4 after 2 years of ART were classified as being at high risk of SNAEs, all other patients were classified as low risk. Plasma metabolomics was conducted via LC-MS/MS using the biocrates MxP 1000 assay. Plasma cytokines and markers of microbial translocation or gut barrier damage were measured by Luminex and ELISA assays. The microbiome of ileal and rectal biopsies was characterized by 16S V4 rRNA sequencing. The number of HIV RNA-producing cells in lymph node biopsies was quantified by RNAScope using HIV RNA specific probes and quantitative image analysis.

Ileal abundance of *Akkermansia muciniphila* is reduced in PWH at high risk of SNAEs, positively correlates with the CD4/CD8 ratio, and negatively correlates with plasma IL-6 and HIV RNA-producing cells in the lymph node.

RESULTS

Table 1. Baseline characteristics by country

Characteristic (Median [Q1, Q3])	Mexico (n = 48)	US (n = 34)	p-value
CD4⁺ T cell count (cells/mm³)	478 [329, 650]	643 [441, 787]	0.006[A]
CD8⁺ T cell count (cells/mm³)	685 [445, 872]	800 [506, 1,087]	0.058[A]
CD4/CD8 ratio	0.77 [0.55, 0.99]	1.00 [0.58, 1.20]	0.5[A]
Nadir CD4⁺ T cell count (cells/mm³)	109 [31, 306]	280 [170, 434]	0.003[A]
Time living with HIV (years)	12 [7, 15]	13 [4, 22]	0.2[A]
Age (years)	53 [48, 57]	46 [34, 59]	0.037[A]
Sex at birth (n [%])			>0.9[B]
Female	2 (4.2%)	1 (2.9%)	
Male	46 (96%)	33 (97%)	
Race/ethnicity (n [%])			<0.001[B]
Black	0 (0%)	14 (41%)	
Hispanic	48 (100%)	4 (12%)	
Other	0 (0%)	4 (12%)	
White	0 (0%)	12 (35%)	

Clinical characteristics of patients enrolled in the study, stratified by country of enrollment. [A]Welch Two Sample t-test. [B]Fisher's exact test

Table 2. Baseline characteristics by serious non-AIDS event risk group

Characteristic (Median [Q1, Q3])	Low SNAE risk (n = 58)	High SNAE risk (n = 24)	p-value
CD4⁺ T cell count (cells/mm³)	639 [526, 764]	300 [251, 368]	<0.001[A]
CD8⁺ T cell count (cells/mm³)	710 [483, 933]	716 [405, 1,180]	0.4[A]
CD4/CD8 ratio	0.91 [0.68, 1.32]	0.38 [0.29, 0.81]	0.001[A]
Nadir CD4⁺ T cell count (cells/mm³)	235 [94, 401]	72 [33, 196]	0.002[A]
Time living with HIV (years)	12 [7, 15]	10 [5, 17]	0.7[A]
Time on ART (years)	12 [5, 16]	10 [5, 16]	>0.9[A]
Age (years)	52 [43, 56]	53 [46, 59]	0.1[A]
Sex at birth (n [%])			0.6[B]
Female	3 (5.2%)	0 (0%)	
Male	55 (95%)	24 (100%)	
Race/ethnicity (n [%])			0.8[B]
Black	10 (17%)	4 (17%)	
Hispanic	36 (62%)	16 (67%)	
Other	4 (6.9%)	0 (0%)	
White	8 (14%)	4 (17%)	
Country (n [%])			0.5[B]
Mexico	32 (55%)	16 (67%)	
US	26 (45%)	8 (33%)	

Clinical characteristics of patients enrolled in the study, stratified by SNAE risk group. [A]Welch Two Sample t-test. [B]Fisher's exact test

Geography is a major effect on the plasma metabolome. PWH from the US have much higher lipid concentrations than from Mexico

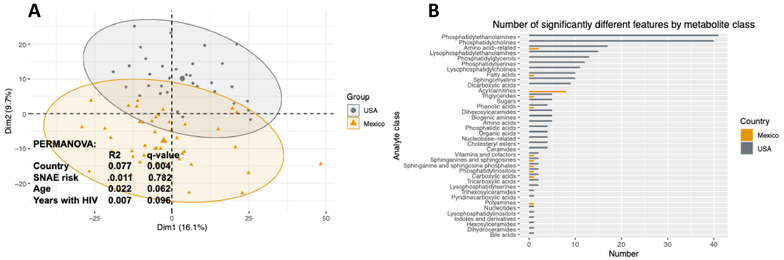


Figure 1. Principle component analysis of plasma metabolome features tested by PERMANOVA (A). List of of the number of increased differentially abundant (q < 0.25) features in each country by metabolite class, tested by multiple linear regression (B).

Increased dicarboxylic acids in PWH at high risk of SNAEs

Increased IL-6 in PWH at high risk of SNAEs

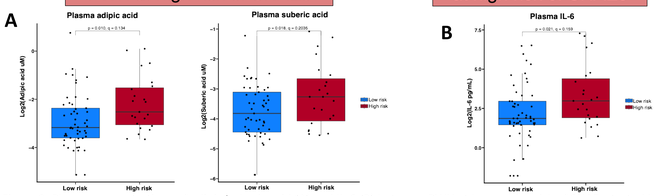


Figure 2. Plasma concentrations of adipic/suberic acid (A) and IL-6 (B) between PWH at low or high risk of SNAEs, tested by multiple linear regression adjusted by country of origin, age, and years living with HIV.

PWH at high risk of SNAEs have reduced commensal microbes in ileal and rectal biopsies

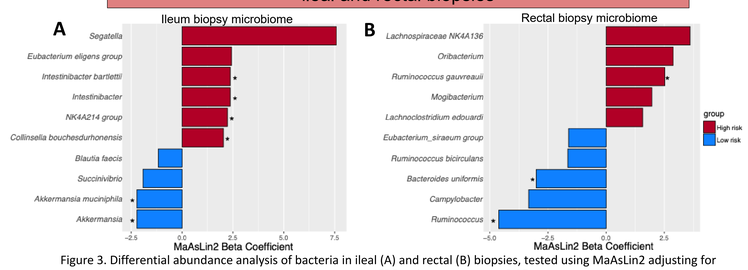


Figure 3. Differential abundance analysis of bacteria in ileal (A) and rectal (B) biopsies, tested using MaAsLin2 adjusting for country, age, and years living with HIV. Asterisks denote an FDR-adjusted p-value < 0.25.

RESULTS CONT.

Ileal *Akkermansia muciniphila* abundance linked to increased CD4/CD8 ratios and reduced IL-6 and HIV viral RNA

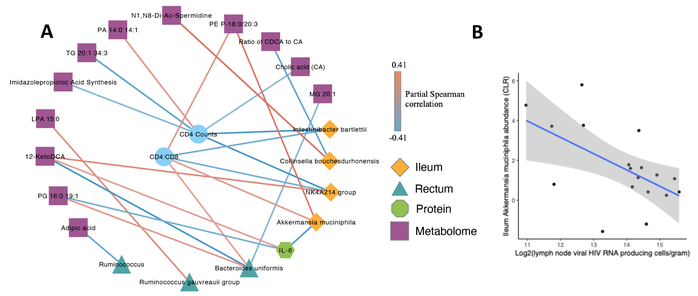


Figure 4. Partial Spearman correlations between biomarkers of each omic layer and T cell criteria, all edges represent significant correlations (FDR-adjusted p-value <0.25) (A). Spearman correlation between ileal abundance of *A. muciniphila* and HIV RNA-producing cells in lymph node biopsies (B).

CONCLUSIONS

- Geography was a major driver of multi-omic variation, particularly in the plasma metabolome, with U.S. PWH exhibiting higher levels of multiple lipid classes.
- Independent of geography, PWH at high SNAE risk showed a consistent signature of systemic inflammation (IL-6), mitochondrial dysfunction (dicarboxylic acids), and microbial dysbiosis (reduced commensals).
- Ileal *Akkermansia muciniphila* abundance was reduced in high-risk PWH and was positively associated with CD4/CD8 ratio and inversely associated with IL-6 and lymph node HIV RNA-producing cells.
- Together, these data define a shared high-risk phenotype and implicate *A. muciniphila* as a potential microbiome-targeted strategy to reduce immune activation and SNAE risk in treated HIV.

ADDITIONAL KEY INFORMATION

Acknowledgements: We are deeply grateful to the participants of this study. Funding provided by the National Institute of Health (grants AI140923, DK143536, DK091538, AI177584, and AI147912) and UMN's Department of Surgery funds to Dr. Nichole Klatt. CIENI-INER is supported by the Mexican Government (Programa Presupuestal P016; Anexo 13 del Decreto del Presupuesto de Egresos de la Federación).

PLAIN LANGUAGE SUMMARY:

People with HIV at high risk for serious comorbidities have increased inflammatory protein levels, alterations in gut microbiome composition, and metabolic perturbations. Targeted microbiome-based therapies may help to reduce inflammation and comorbidity risk.