Blood Microbiota Correlates with Inflammation and ART-mediated Immune Restoration

Sergio Serrano-Villar[1], Mariano Matarranz[2], Jose I. Bernardino[3], Alejandro Vallejo[1], Carolina Gutiérrez[1], Otilia Bisbal[2], María Lagarde[2], Nadia Madrid[1], Rafael Rubio[2], Cristina Gómez-Ayerbe[1], María Jesús Vivancos[1], Alfonso Cabello[4], Matilde Sánchez-Conde[1], Vicente Estrada[5], Santiago Moreno[1]

1 Hospital Universitario Ramón y Cajal, Madrid, Spain. 2 Hospital Universitario Doce de Octubre, Madrid, Spain. 3 Hospital Universitario La Paz, Madrid, Spain. 4 Fundación Jiménez Diaz, Madrid, Spain. 5 Hospital Universitario Clínico San Carlos, Madrid, Spain.

*Corresponding author
Ctra. de Colmenar Km 9,100, 28034 Madrid
Email: sergio.serrano@salud.madrid.org

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ABSTRACT #242

INTRODUCTION

•HIV affects mucosa-associated lymphoid tissues and leads to bacterial translocation from the gut, which sustains chronic inflammation.
• The compositional profile of the translocated bacteria remains undefined.
•We aimed to characterize the compositional profile of the blood microbiota of HIV-infected late presenters at diagnosis and after 48 weeks of ART.

METHODS

- Case-control study. We included 20 healthy controls and 40 HIV-infected individuals with <350 CD4+ T-cells/uL or AIDS at diagnosis who were followed during the first 48 weeks of antiretroviral therapy (ART).
- **16S rDNA targeted metagenomic sequencing of V3-V4 regions** using MiSeq Illumina and the FROGS metagenomic workflow.
  - After demultiplexing of the bar-coded Illumina paired reads, single read sequences are cleaned and paired for each sample independently into longer fragments. Operational taxonomic units (OTUs) are produced with via single-linkage clustering and taxonomic assignment is performed in order to determine community profiles.
  - The following specific filters have been applied for this analysis in order to obtain the best results:
    - The last 10 bases of reads R1 were removed (lower quality preventing good read pairing)
    - The last 40 bases of reads R2 were removed (lower quality preventing good read pairing)
    - Amplicons with a length < 350 nt or a length > 500 nt are removed.
    - OTUs with abundance lower than 0.005% of the whole dataset abundance are removed.
- **We measured plasma biomarkers of inflammation, bacterial translocation and immune activation (expression of HLADR and CD38) in circulating T cells.**

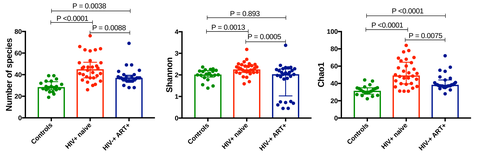
RESULTS

General characteristics of the study population

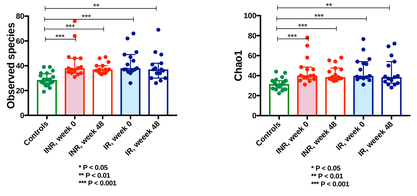
Variable	Controls	INR	IR	P value
	n=20	n=15	n=15	(INR vs. IR)
Age, mean (SD), years	39.1 (11.9)	41.9 (10.8)	37.9 (13.9)	0.194
Male, n (%)	15 (75%)	14 (93.4)	13 (86.7)	0.543
Body mass index, mean (SD), kg/m2	23.7 (4.2)	23.2 (3.3)	23.2 (3.2)	0.793
Time since HIV diagnosis, median (P25-P75), months	-	3.7 (0.8-26.3)	2.6 (1.2-6.2)	0.697
Risk factor, n (%)	-			
IDU	-	1 (6.7)	0 (0)	0.721
MSM	-	12 (80)	12 (80)	
HTX	-	1 (6.7)	1 (6.7)	
Unknown	-	1 (6.7)	2 (13.3)	
AIDS diagnosis, n (%)	-	3 (20)	1 (6.7)	0.307
HIV RNA, median (P25-P75), copies/mL	-	4.5 (4.2-5.5)	4.8 (4.4-5.2)	0.851
CD4+ T cell counts, median (P25-P75), cells/uL	-			
Week 0 (Naive)	-	234 (62-297)	262 (89-298)	0.756
Week 48 (On ART)	-	416 (214-549)	398 (344-638)	0.290
CD8+ T cell counts, median (P25-P75), cells/uL	-			
Week 0 (Naive)	-	740 (658-1037)	1055 (648-1454)	0.309
Week 48 (On ART)	-	1168 (697-1414)	741 (567-1441)	0.245
CD4/CD8 ratio, median (P25-P75), cells/uL	-			
Week 0 (Naive)	-	0.20 (0.09-0.35)	0.22 (0.08-0.32)	0.885
Week 48 (On ART)	-	0.32 (0.22-0.70)	0.65 (0.25-0.99)	0.165
HCV coinfection, n (%)	-	2(13.3)	1 (6.7)	0.543
Use of antibiotic in past 3 months, n (%)	-	3 (20)	5 (33.3)	0.409
First-line ART used, n (%)	-			
INSTI-based	-	9 (60)	8 (53.3)	0.357
PI-based	-	3 (20)	1 (6.7)	
NNRTI-based	-	3 (20)	6 (40)	

RESULTS

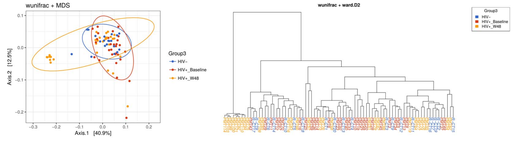
Significant Impact of HIV and ART on Alpha Diversity Indexes



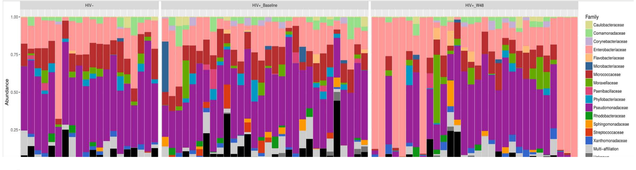
No association between immune recovery and alpha diversity



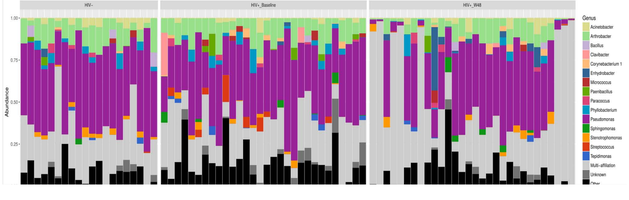
No clear separation between groups in Beta Diversity analyses



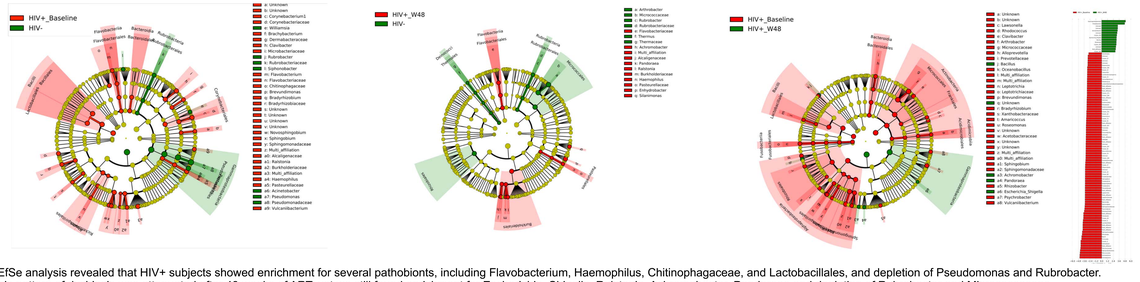
Relative proportion of taxa at the family level (top 15 families)



Relative proportion of taxa at the genus level (top 15 genus)



LEfSe cladograms of pairwise analysis from the LDS effect size data ((log(LDA Score)>2.0)



LEfSe analysis revealed that HIV+ subjects showed enrichment for several pathobionts, including Flavobacterium, Haemophilus, Chitinophagaceae, and Lactobacillales, and depletion of Pseudomonas and Rubrobacter. This pattern of dysbiosis was attenuated after 48 weeks of ART, yet we still found enrichment for Escherichia, Shigella, Ralstonia, Achromobacter, Pandoreae and depletion of Rubrobacter and Micrococcaceae.

Associations between blood microbiota signatures and the extent of immunological recovery after one year of ART

Table. Differences in T cell activation, T cell senescence, inflammatory and bacterial translocacion markers at baseline

Parameter, median (P25-P75)	INR	IR	P value
%CD4+ HLADR+CD38+ T cells	9.6 (4.7-17.3)	5.8 (2.2-13)	0.149
%CD8+ HLADR+CD38+ T cells	15.2 (10.6-24.3)	8 (5.4-19.9)	**0.033**
%CD4+ CD28- T cells	31 (19.4-44.8)	24.2 (11-29.2)	0.138
%CD8+ CD28- T cells	84.6 (80.2-85.6)	80.7 (78.6-85.2)	0.275
IL-6, pg/mL	1.4 (0.8-4)	1.7 (0.4-4)	0.967
CRP, ng/mL	70.4 (35-149)	87 (38.9-122.1)	0.756
TNF-α, pg/mL	8.5 (6.4-20.5)	11.2 (3.5-41.1)	0.662
sCD14, ng/mL	1550 (1381-1679)	1590 (1359-1693)	0.820
sCD163, ng/mL	0.9 (0.5-1.2)	0.6 (0.5-0.7)	**0.093**
IP-10, pg/mL	56.1 (26.5-155.6)	33.4 (26.4-38)	**0.037**
16S copies/ng DNA	97 (55-127)	77 (60-117)	0.756
LTA, ng/mL	1.2 (0.4-1.8)	0.7 (0.1-1.3)	**0.096**
IL-7, pg/mL	4.6 (1.2-7.6)	5.6 (1.4-12.7)	0.507
IL-10, pg/mL	4.2 (2.3-8.1)	1.8 (1.3-2.8)	**0.009**
IL-17, pg/mL	1.5 (0-3.2)	3 (0-3.5)	0.187

NMDS analysis at OTU level based on the weighted Unifrac distances

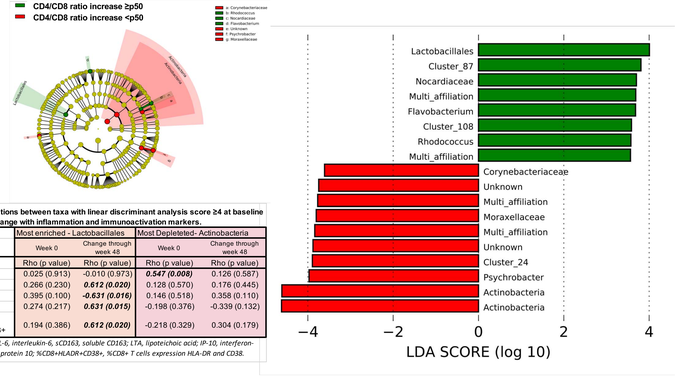


Table. Correlations between taxa with linear discriminant analysis score ≥4 at baseline and by fold change with inflammation and immunoactivation markers.

	Most enriched - Lactobacillales		Most Depleted- Actinobacteria	
	Week 0	Change through week 48	Week 0	Change through week 48
	Rho (p value)	Rho (p value)	Rho (p value)	Rho (p value)
IL-6	0.025 (0.913)	-0.010 (0.973)	***0.547 (0.008)***	0.126 (0.587)
sCD163	0.266 (0.230)	***0.612 (0.020)***	0.128 (0.570)	0.176 (0.445)
LTA	0.395 (0.100)	***-0.631 (0.016)***	0.146 (0.518)	0.358 (0.110)
IP-10	0.274 (0.217)	***0.631 (0.015)***	-0.198 (0.376)	-0.339 (0.132)
%CD8+ HLADR+CD38+	0.194 (0.386)	***0.612 (0.020)***	-0.218 (0.329)	0.304 (0.179)

Abbreviatures, IL-6, interleukin-6, sCD163; soluble CD163; LTA, lipoteichoic acid; IP-10, interferon-gamma induced protein 10; %CD8+HLADR+CD38+, %CD8+ T cells expression HLA-DR and CD38.

CONCLUSIONS

- HIV infection affects the composition of the blood microbiota by increasing the abundance of pathobionts. ART appears to attenuate these compositional abnormalities.
- Different translocated bacteria were predictive of the extent of immune recovery after 48 weeks of ART and significantly correlated with markers of inflammation, bacterial translocation and immunoactivation.
- Hence, these taxa may prove functionally relevant for HIV immunopathogenesis and deserve further investigation.

ACKNOWLEDGMENTS

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