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Two-year cART does not restore peripheral blood and intestinal HIV-related dysbiosis

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INTRODUCTION

Microbial dysbiosis features HIV+ individuals, both naïve and cART-treated, and is linked to anatomical/structural changes in the gastrointestinal tract, leading to microbial translocation and immune activation. Given that data on microbiota modifications during long-term therapy are lacking, we investigated gut/blood microbiota during the first 2 years of suppressive cART

PATIENTS AND METHODS

41 cART-naïve HIV-infected subjects introducing treatment were enrolled (see tab1). Peripheral blood and faecal samples were collected at baseline (T0) and following 12 (T12) and 24 months (T24) of virologically-suppressive therapy. The following parameters were studied:

- ✓ T-cell activation and maturation (flow cytometry): CD38, CD45RO, CD45RA
- ✓ Microbial traslocation: plasma sCD14, Endocab, LPS (ELISA) and 16S bacterial rDNA
- ✓ Intestinal damage: plasma IFAB-P and E-cadherin (ELISA) on plasma
- ✓ Intestinal inflammation: faecal calprotectin (ELISA-PhiCal)
- ✓ Intestinal permeability through lactulose/mannitol urinary excretion ratio (LAC/MAN test; at T0 and T12)
- ✓ Bacterial population contained in faecal and plasma samples were determined using next generation high throughput sequencing of variable regions V3-V4 of 16S rDNA bacterial gene. The detection of the sequencing fragments was performed using MiSeq Illumina® technology and analyzed with Frogs v.1.3.0 and PhiloSeq V.1.14.0. Data were presented as: (i) relative abundance at each taxonomic level (phylum, class, order, family, genus, and species); (ii) α diversity that is the mean of species diversity per sample in each group/class and (iii) β diversity that is the degree of community differentiation between groups. For these experiments 15 HIV negative subjects were recruited as controls.

STATISTICAL ANALYSES

- ✓ Data are shown as median (Interquartile Range)
- ✓ Comparisons among HIV+ patients were assessed by Friedman test and Wilcoxon test; comparisons among HIV+ patients and HIV- individuals were assessed by Kruskall-Wallis test, Mann Whitney and Fisher's exact test as appropriate
- ✓ α diversity: observed, Chao1, Shannon, Simpson and inv.Simpson (ANOVA)
- ✓ β diversity was assessed by:
  - ✓Principal Coordinate Analysis (PCoA): Jaccard, Unifrac, Weighted Unifrac and Bray-Curtis
  - ✓LEfSE analysis: differences among HIV+ patients and HIV- subjects were identified by using default values (alpha value of 0.5 for both the factorial Kruskal-Wallis test among classes and the pairwise Wilcoxon test between subclasses, threshold of 2.0 for the logarithmic LDA score for discriminative features) and the strategy for multi-class analysis set to 'all-against-all'.

Table 1. Patients' Characteristics at baseline

	HIV + (N=41)	HIV negative (N=15)	p Value
Age, years (IQR) *	42 (31.5-50.5)	29 (24-33)	0.0017
Sex, no females (%) °	4 (9,75)	0	0.56
Sexual behaviours MSM, n (%) °	28 (68,2)	5 (33.3)	0.03
BMI, n (IQR) *	22,66 (22,02-25,18)	22,71 (20,98-24,91)	0.86
Vegetarian/vegan diet, n subjects (%) °	3 (7,3)	0	0.55
Ongoing antibiotic prophylaxis, n (%) °	8 (19,5)	0	0.09
Risk factors for HIV infection Heterosexual, n (%) MSM, n (%) IV drug use, n (%)	12 (29,2) 28 (68,2) 1 (2,4)	ND	ND
Hepatitis coinfection, n (%) °	2 (4,8)	0	1
Duration of HIV infection, months (IQR) *	14 (2,5-30-5)	ND	ND
AIDS diagnosis, n (%)	6 (14,6)	ND	ND
Nadir CD4, n/mmc (IQR) ** T12 CD4 n, mmc (IQR) T24 CD4 n, mmc (IQR)	342 (233,5-431,5) 539 (442,5-686) 618 (464,5-787,5)	ND	<0.0001(nadir vs T12/T24)
CD4/CD8 T-cell ratio	21 (24,5-44-5)	ND	ND
Zenith HIV RNA load, cp/ml (IQR) log10 cp/mL (IQR)	63734 (22751-205583) 5 (4-5)	ND	ND
First cART regimen, no patients (%) NNRTI-based PI-based INI-based Other	25 (60.9) 9 (21.9) 5 (12.1) 2 (4.8)	ND	ND

*Data are median (IQR). IQR: Interquartile range, Statistical analysis: Mann Whitney Test. ° Data are n (%), Statistical analysis: Fisher's Exact. ** absolute counts n (IQR), statistical analysis Friedman test among HIV+ groups. ND, not determined; BMI Body Mass Index; MSM: man sex man; cART: combined antiretroviral therapy; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor, INI: integrase inhibitor,

Figure 1. Viral load and T-cell homeostasis in the course of cART

A significant decrease of viral load (1A) and increase in CD4/CD8 ratio (1B), CD4+ CD127+ (1C) and CD4+ CD45RA+ (1D) as well as a reduction in activated CD8+ CD38+ (1E) and CD8+ CD38+ CD45R0+% (1F) was found following therapy.

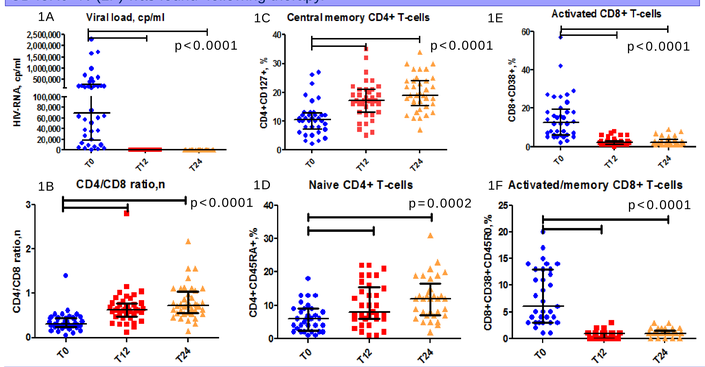


Figure 2. Microbial Traslocation, gut barrier permeability/damage and inflammation parameters in the course of cART

No modifications of plasma sCD14 (2A), 16S-rDNA (2E) and LPS (not shown) were measured, albeit an increase of EndoCab (2C). A rise in IFAB-P (2B) and no variations of E-cadherin (not shown), LAC/MAN ratio (2D) and calprotectin (2F) were also observed. Of note however, pathologic LAC/MAN ratios and calprotectin (green dotted line shows reference values) decreased on cART.

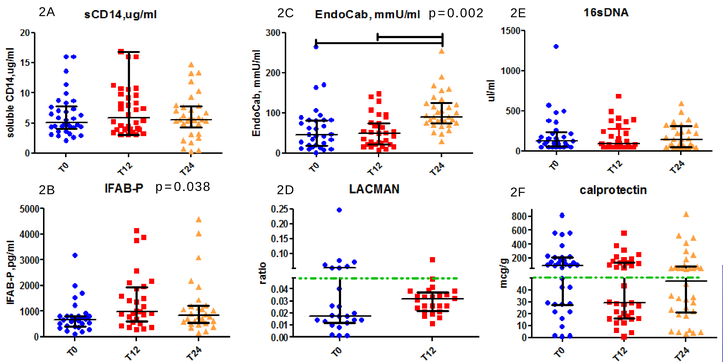


Figure 3. Relative abundance barplots taxa class from faecal samples

Visual comparison of the relative abundance barplots indicates higher levels of fecal Negativicutes (class) at T12 and T24 compared with T0 and higher levels of fecal Gammabroteobacteria (class) in HIV groups compared to HIV-. No clear trends are apparent from visual comparison of plasma sample profiles (not shown).

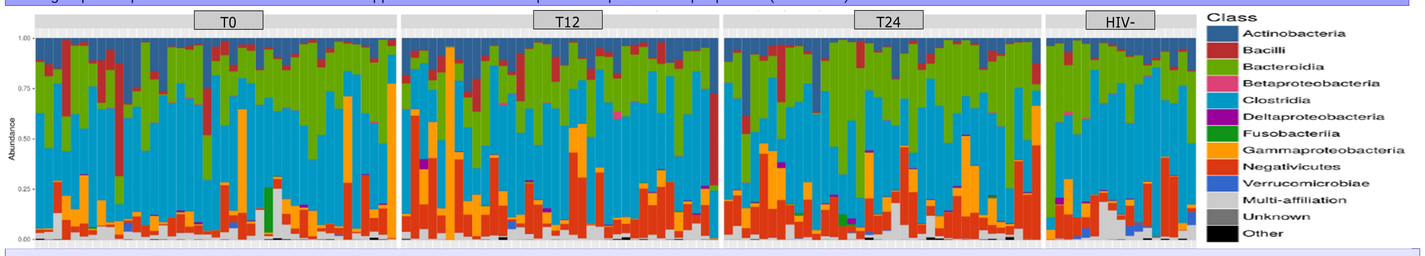


Figure 4. Alfa-diversity in blood and faecal samples

Blood alpha diversity analysis shows a significant increase of diversity in HIV- compared to HIV+ groups according to Shannon and Simpson (4A). In contrast, faecal alpha diversity analysis shows a significant decrease of diversity in HIV- compared to HIV+ groups according to Observed and Chao1 (4B). No major differences were detected in HIV+ according to treatment status and length of cART.

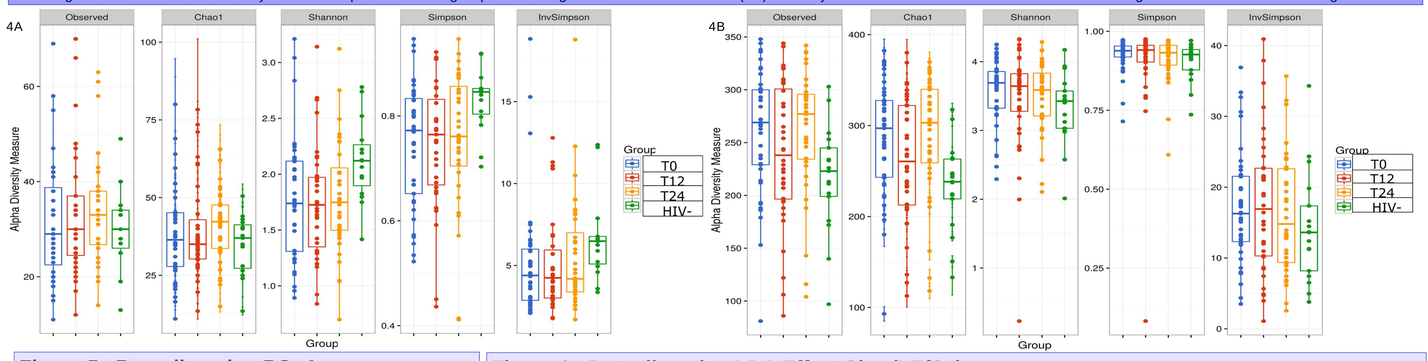


Figure 5. Beta-diversity: PCoA

Shown below are the faecal ordination plots by unweighted Unifrac (5A) and Jaccard (5B) that show a separation of HIV- subjects.
No clusters were detected in HIV+ according to treatment status and length of cART.
No clusters were observed in blood comparisons (not shown).

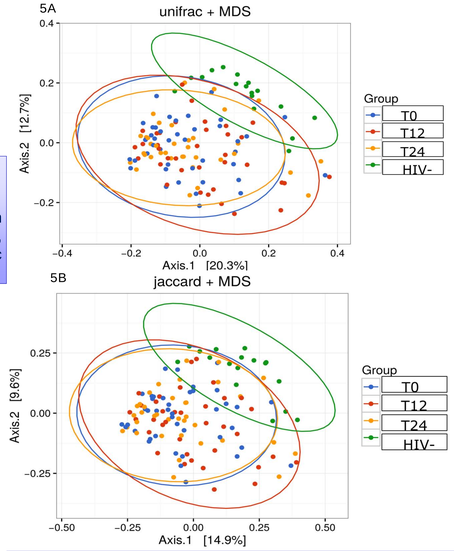
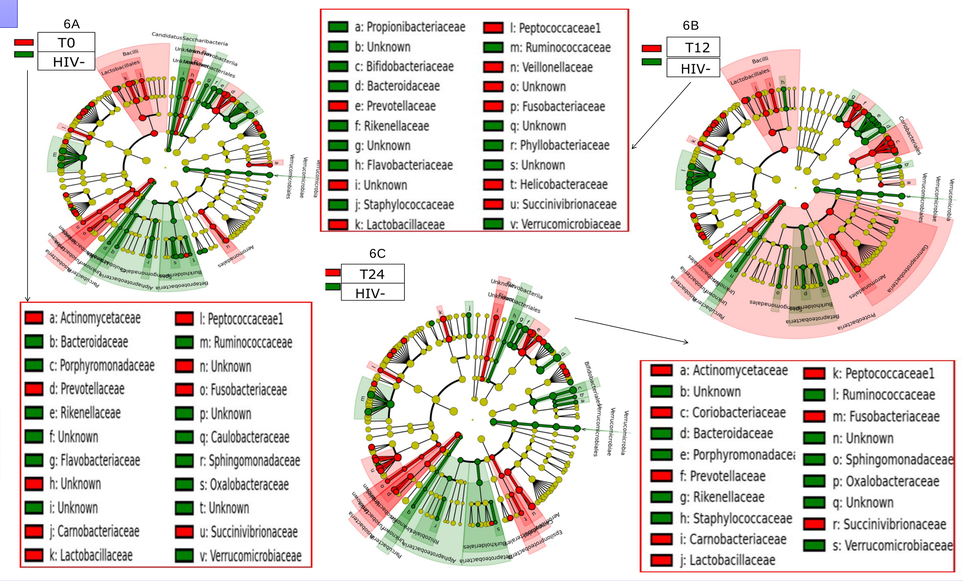


Figure 6. Beta-diversity: LDA Effect Size (LEfSe)

Figure shows the comparison between HIV - and, respectively, T0 (6A),T12 (6B) and T24 (6C). Controls present higher Bacteroidaceae, Rikenellaceae, Ruminococcaceae and Verrucomicrobiaceae families; in contrast HIV + patients have higher Prevotellaceae, Peptococcaceae, Fusobacteriodaceae and Succiniovibronaceae. Minimal shifts in LEfSE analysis were detected in blood (not shown).



CONCLUSIONS

In the present study we confirm major modifications of the microbiota within the GI tract of HIV-infected individuals which persist despite suppressive long-term cART. These findings suggest the ability of the virus to irreversibly impact the microbiological core during HIV disease and may account for enduring anatomical and functional defects in the gut.