Poster #262

Time Between HIV Acquisition and ART Initiation Impacts Gastrointestinal Microbiome in Early HIV Infection

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RATIONALE

- Starting antiretroviral therapy (ART) early in infection confers benefits on HIV-related morbidity and clinical outcomes including:
  - Smaller latent HIV reservoir
  - Preserved immunity and less inflammation
  - Fewer non-AIDS defining events
- Perturbation of the gastrointestinal (GI) microbiome (microbial dysbiosis) occurs early in untreated HIV infection.
- Treatment during chronic HIV infection does not fully reverse the HIV-associated microbial dysbiosis.
- Impairment of mucosal immunity and altered microbiota are thought to contribute to inflammation in HIV-infected persons.
- Since HIV diagnosis and ART during acute or early infection is challenging, it is important to investigate long-term individual and public health benefits of such strategies

OBJECTIVES

- Determine how the timing of ART initiation impacts the gastrointestinal microbiota during early infection.
- Determine differences in gastrointestinal bacterial composition based on both recency of infection and treatment initiation.

METHODS

- HIV-infected MSM and transgender women (TW) were diagnosed while sero-negative (acute HIV [AI]) or sero-positive and ≤ 3 mos. from last negative RNA test (recent HIV [RI]). Participants were randomized within strata to receive standard of care ART immediately (I) or after 24 weeks deferral (D) as part of the parent SABES study in Lima, Peru (N=215).
- We sequenced the V4 hypervariable region of the 16S rRNA gene from cryopreserved stool from Weeks 0 through 24 from a subset of 30 SABES participants.

Table 1. **Patient Demographic and Clinical Information**

	AI (N=8)	RI (N=12)	AD (N=5)	RD (N=5)
Age, Med (IQR)	27.5 (26, 31)	23 (21, 31)	23 (21, 31)	20 (19, 21)
ART (TDF/FTC + EFV or EVG/cobi)	EFV= 7 EVG/c=1	EFV=10 EVG/C=2	NA	NA
Estimated Days from Infection to Enrollment	18.5 (17, 21)	43 (36, 56)	21 (19, 23)	36 (33, 44)

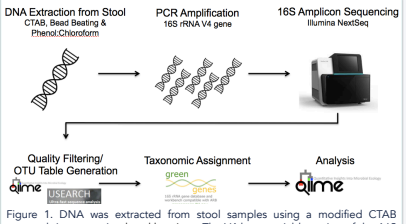


Figure 1. DNA was extracted from stool samples using a modified CTAB approach incorporating bead beating. The V4 hypervariable region of the 16S rRNA gene was amplified and sequenced on the Illumina NextSeq 500. Paired sequencing reads were quality filtered, merged, clustered at 97% sequencing similarity into operational taxonomic units (OTUs) and had chimeric and non-bacterial reads removed. High quality reads were assigned taxonomy using the Greengenes database.

TIMING OF ART MAY INFLUENCE GASTROINTESTINAL BACTERIAL COMMUNITY COMPOSITION

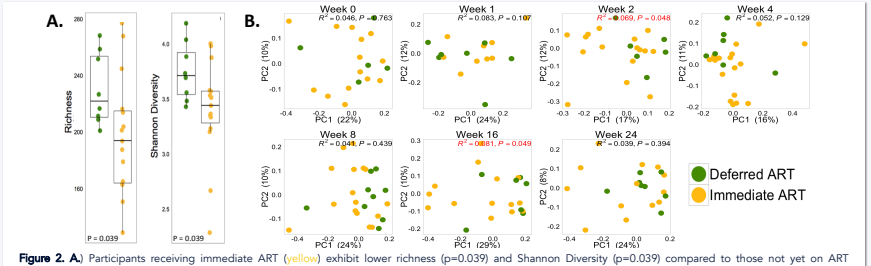


Figure 2. A.) Participants receiving immediate ART (yellow) exhibit lower richness (p=0.039) and Shannon Diversity (p=0.039) compared to those not yet on ART (Deferred, green) at 2 weeks. No differences in alpha diversity by treatment arm were observed at other time points. **B.)** Principal coordinates analysis (PCoA) **of** bacterial community composition using an unweighted UniFrac dissimilarity matrix showed significant differences between immediate (yellow) and deferred ART participants (green) at 2 (R^2=0.07; p=0.048) and 16 (R^2=0.08; p=0.049) weeks, with trends toward differences in community composition at other time points. Longitudinal analysis using PCoA tested with linear mixed effect models (LME) did not show significant differences between samples based on timing of ART initiation.

DIFFERENCES IN GASTROINTESTINAL BACTERIAL COMMUNITY COMPOSITION EXIST IN HIV-INFECTED MSM AND TW BASED ON HIV-INFECTION RECENCY

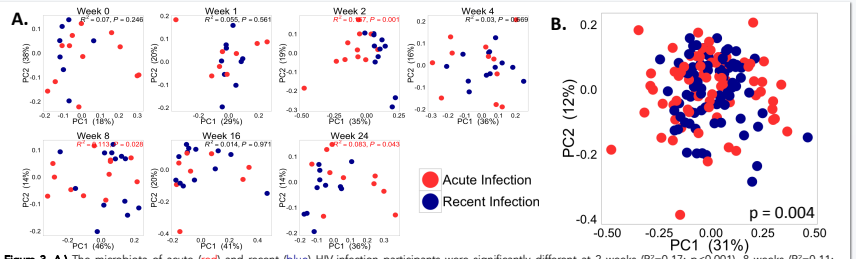


Figure 3. A.) The microbiota of acute (red) and recent (blue) HIV-infection participants were significantly different at 2 weeks (R^2=0.17; p<0.001), 8 weeks (R^2=0.11; p=0.028), and 24 weeks (R^2=0.083; p=0.043) after randomization, by PCoA using a weighted UniFrac dissimilarity matrix. **B.)** Longitudinal PCoA analysis using a weighted UniFrac dissimilarity matrix and tested using LME to account for repeated measures demonstrated significant differences in microbiota composition between Acute and Recent infection status (LME p = 0.004) throughout the sampling period.

MICROBIAL COMMUNITIES IN PERSONS WITH ACUTE AND RECENT INFECTION WERE DIFFERENT IF TREATMENT WAS DEFERRED, BUT WERE SIMILAR AMONG BOTH IMMEDIATLEY TREATED GROUPS

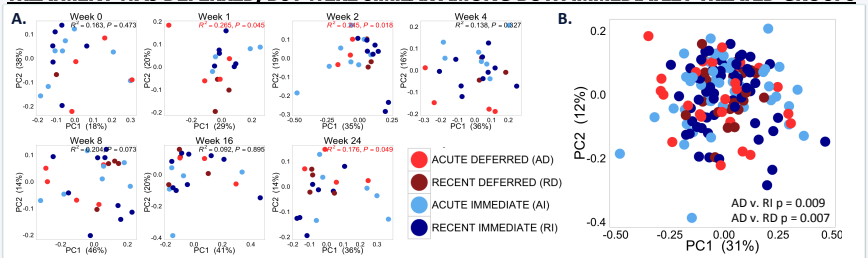


Figure 4. A.) PCoA using a weighted UniFrac dissimilarity matrix yielded significant differences in microbial community composition at 1 week (R^2=0.265; p=0.045), 2 weeks (R^2=0.245; p=0.018) and 24 weeks (R^2=0.176; p=0.049) after randomization when participants were stratified based on both infection and treatment status (AD, RD, AI, and RI). **B.)** Longitudinal PCoA of all samples tested using LME to account for repeated measures showed that there were significant differences between community profiles of AD and RI (LME, p=0.009) and AD and RD (LME, p=0.007) but no significant differences in the other comparisons.

DISCUSSION

- The gastrointestinal microbiota profiles of HIV-infected MSM or TW participating in a longitudinal study in Peru were determined to be significantly different at certain time points when stratified cross-sectionally based on both HIV-infection recency and timing of ART, but differences by group were not maintained throughout longitudinal sampling in early infection.
- Bacterial community composition differed in longitudinal analysis between participants with recently acquired or acute HIV infection.
- Stratification of participants based on both time since HIV infection and treatment arm provided the strongest separation of microbial community composition in both the cross-sectional and longitudinal analyses.
- Participants with acute infection not yet on ART (AD) had significantly different microbial community structure than either treated or untreated individuals with recent infection (RI or RD) suggesting that microbial changes may be very pronounced in acute HIV infection but stabilize post-acute infection.
- Results of this study may be limited by small sample size in some groups

FUTURE DIRECTIONS

- Comparison of microbiota profiles of study participants before and after ART initiation to age-matched MSM and TW HIV-uninfected controls to determine the impact of ART on restoration of pre-infection microbiota profiles.
- Determination of whether microbiota profiles associated with HIV infection recency or treatment initiation correlate with immunological data in this cohort.
- Analysis of follow-up stool samples after 1.5-3.5 years on ART for each group.

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