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Effects of HIV Viremia on the Gastrointestinal Microbiome of Young Men who have Sex with Men

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Abstract

Objective: We employed a high-dimensional covariate adjustment method in microbiome analysis to better control for behavioral and clinical confounders, and in doing so examine the effects of HIV on the rectal microbiome.

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Supplemental Content

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Design: Three hundred eighty-three men who have sex with men were grouped into four HIV viremia categories: HIV negative (n = 200), HIV+ undetectable (HIV RNA <20 copies/mL; n = 66), HIV+ suppressed (RNA 20–200 copies/mL; n = 72) and HIV+ viremic (RNA >200 copies/mL; n = 45).

Methods: We performed 16S rRNA gene sequencing on rectal swab samples and used inverse probability of treatment-weighted marginal structural models to examine differences in microbial composition by HIV viremia category.

Results: HIV viremia explained a significant amount of variability in microbial composition in both unadjusted and covariate-adjusted analyses (R^2 = .011, p = .02). Alterations in bacterial taxa were more apparent with increasing viremia. Relative to the HIV negative group, HIV+ undetectable participants showed depletions in *Brachyspira, Campylobacter,* and *Parasutterella* while suppressed participants demonstrated depletions in *Barnesiella*, *Brachyspira* and *Helicobacter*. The microbial signature of viremic men was most distinct, showing enrichment in inflammatory genera *Peptoniphilus*, *Porphyromonas*, and *Prevotella* and depletion of *Bacteroides*, *Brachyspira,* and *Faecalibacterium*, among others.

Conclusions: Our study shows that, after accounting for the influence of multiple confounding factors, HIV is associated with dysbiosis in the gastrointestinal microbiome in a dose-dependent manner. This analytic approach may allow for better identification of true microbial associations by limiting the effects of confounding, and thus improve comparability across future studies.

Keywords

HIV; Microbiome; Dysbiosis; Men who have sex with men; IPTW; Propensity scores; Causal inference

Introduction

The trillions of bacteria, viruses, and fungi inhabiting the human gastrointestinal (GI) tract have a profound impact on our health and the development of disease. Disruption in the homeostasis of the these microbes, a state of “dysbiosis,” has been associated with a broad range of illnesses, including localized GI conditions, neurocognitive disorders, cancer, autoimmune disorders, and cardiovascular disease [1]. There is tremendous variability in the diversity and composition of the microbiome, even between healthy individuals [2], and the effects of different exposures, behaviors, and personal characteristics on the composition and function of the microbiome are incompletely understood.

Chronic inflammation is a hallmark of HIV infection and continues despite suppressive antiretroviral therapy (ART). The GI tract is a primary site for HIV replication resulting in significant loss of CD4+ T-cells vital to a healthy mucosal immune system. Depletion of regulatory immune cells and pathways leads to decreased epithelial barrier function allowing translocation of microbes and microbial products which contributes to the chronic inflammatory response [3, 4]. HIV replication may also result in a state of dysbiosis [5–7], which has been correlated with increases in markers of disease progression, microbial translocation, and immune activation [5, 7–9]. It has been hypothesized that HIV-associated

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immune dysfunction induces this dysbiosis, and dysbiosis causes further dysfunction [5], thereby driving persistent systemic inflammation in HIV-infected individuals [10].

Many studies have found that overall microbial diversity is reduced in HIV-infected individuals [11–13], and HIV has been associated with a shift from commensals such as *Bacteroides* to pro-inflammatory taxa such as *Prevotella* [11, 14, 15]. However, results have been inconsistent, with some studies showing the opposite or no effects of HIV on microbial diversity [16–18] and little effect on composition [19, 20]. Certainly, differences in sample collection, sequencing, post-processing, and analytic methodology may be responsible for much of the heterogeneity in results [21]. While some research has focused on the role of sampling variability and type-I error on irreproducibility of findings [22, 23], comparatively little attention has been paid to sources of systematic error such as incomparable study cohorts or confounders such as sexual behavior, substance use, diet, race/ethnicity, and age. Such confounders are highly prevalent in observational human studies of the microbiome and may have a larger effect than HIV itself [19]. Due to these limitations, the specific effects of HIV on the microbiome warrant further investigation.

Increased relative abundance of pro-inflammatory bacteria has been correlated with increased viremia, whereas the opposite has been found for potentially beneficial bacteria [9, 13, 14]. Given these findings and the effects of viral replication on mucosal immunity, it stands to reason that the level of viremia may be a significant determinant of HIV-associated dysbiosis. However, the effect of viremia has not been thoroughly explored. Numerous studies comparing cohorts of HIV-infected individuals that are either on ART or ART-naïve have shown that ART does not result in full “reconstitution” of the microbiome, even if the virus is suppressed [13, 24, 25]. Additional studies focused on elite controllers showed that the microbial composition among individuals with controlled viremia is more similar to HIV-uninfected than viremic individuals [26, 27]. However, there are likely to be important biological differences between elite controllers and other HIV-infected individuals that may limit the generalizability of these findings. In order to accurately characterize the effects of HIV on the microbiome, a more detailed examination of the effects of viremia is needed.

To this end, we compared intestinal microbial composition between HIV-uninfected, HIV-infected with undetectable viremia (HIV RNA <20 copies/ml), HIV-infected with suppressed viremia (HIV RNA ≥20–200 copies/ml), and HIV-infected viremic individuals (HIV RNA >200 copies/ml). We utilized data from a cohort comprised entirely of men who have sex with men (MSM) and employed inverse probability of treatment weighting (IPTW) to control for a robust set of clinical and behavioral confounders. We hypothesized that alterations to the microbiome would be present in all HIV-infected subgroups as compared to HIV-uninfected controls, and the severity of dysbiosis would increase with increasing viremia.

Methods

Study Population

Specimens for this study were obtained from an ongoing prospective cohort (The mSTUDY, NIDA U01 DA036267). The mSTUDY was approved by a UCLA Institutional Review

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Board (IRB) and all subjects provided written informed consent at study entry. Participants are recruited from community clinics in Los Angeles and complete biannual assessments including a comprehensive physical examination and medical history, urine toxicology panel, clinical laboratory tests including plasma HIV RNA, specimen collection, and detailed behavioral questionnaire. Data presented in this manuscript were collected from baseline study visits completed between August 2014 and July 2017. Additional details on sample selection and HIV RNA quantification are provided in the supplemental content.

Specimen collection and DNA preparation

The majority (76%) of rectal swabs (FLOQSwabs, Copan Diagnostics, Murrieta, CA) were collected via anoscopy under direct mucosal visualization and without preparatory enema at approximately 8 cm from the anal verge. Due to an mSTUDY protocol change, others (24%) were participant self-collected at approximately 4–5 cm from the anal verge. Collection method was taken into account in the analysis (see Tables 1 and S1). Swabs were immediately frozen neat at −80°C until processing in bulk. For DNA processing the samples were transferred to Lysing Matrix E tubes (MP Biomedicals, Burlingame, CA) containing RLT lysis buffer (Qiagen, Hilden, Germany) and bead-beated on a TissueLyser (Qiagen). DNA was then extracted using the AllPrep DNA/RNA/Protein kit (Qiagen) per manufacturer's protocol.

16S rRNA gene sequencing and data processing

Microbiome profiling was performed by sequencing of the V4 region of the 16S rRNA gene as previously described [28, 29]. Briefly, the V4 region was amplified in triplicate reactions using Golay-barcoded primers 515F/806R. Negative controls from the DNA extraction and PCR steps, as well as independent aliquots of a bacterial mock community [30] were processed alongside the samples to identify contaminant sequences and ensure data reproducibility. PCR products were then pooled and sequenced on the Illumina MiSeq platform using 2×150bp v2 chemistry. The sequences were demultiplexed with Golay error correction using QIIME v1.9.1 [31], and Divisive Amplicon Denoising Algorithm (DADA2) version 1.8 was used for error correction, exact sequence inference, read merging, and chimera removal [32]. Following contaminant removal (see supplemental content), the amplicon sequence variant (ASV) table comprised 19,955,039 total merged read pairs (mean per sample = 52,375; range 10,906 to 124,889). Taxonomic assignment was performed using RDP trainset 16 [33]. Rarefaction was performed at a depth of 10,906 reads for alpha diversity analyses. For all other analyses, estimates of relative library sizes ("size factors") were obtained by calculating geometric means of pairwise read count ratios [34].

Behavioral and clinical covariates

Demographic and behavioral covariates included in the analyses were age, race/ethnicity, employment status, country of origin, a dichotomous variable for homelessness in past month, number of receptive anal intercourse (RAI) acts in past month, a dichotomous variable for RAI within the past seven days, frequency of methamphetamine, marijuana, and cocaine use in the past 6 months, tobacco smoking, and binge drinking. All demographic and behavioral data were self-reported by participants using a computer-aided self-interview (CASI); measures are described in the supplemental content. Dichotomous variables for

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obesity (defined as BMI > 30 or waist circumference > 40 inches), and antibiotic use in the past month were also included in the analyses; these data were collected by clinical staff.

Statistical analyses

The primary analyses were unadjusted and inverse probability of treatment-weighted comparisons of microbiome diversity and composition between HIV-, HIV+ undetectable, HIV+ suppressed, and HIV+ viremic participants. The R package ‘phyloseq’ was used to calculate alpha diversity statistics, distance matrices, and for ordination. Differences in alpha diversity between groups were examined with Kruskal-Wallis tests followed by comparisons of median values using quantile regression (R package ‘quantreg’). Permutational Multivariate ANOVA (PERMANOVA) was used to test for overall differences in microbial composition between HIV groups (R package ‘vegan’). Zero-inflated negative binomial (ZINB) models were fit in order to test for differential abundance in bacterial genera between groups with multinomial least absolute shrinkage and selection operator (LASSO) models employed as a confirmatory analysis (R packages ‘pscl’ and ‘glmnet’). ZINB and LASSO model selection and analytic procedures are described in the supplemental content.

IPTW [35] is a method of confounder control where the study sample is re-weighted in order to create a “pseudo-population” in which treatment/exposure, here referring to the four HIV viremia groups, is independent of confounding variables (see supplemental content). We used IPTW to control for all variables described in the Behavioral and Clinical Covariates section. Weights were estimated using generalized boosted models (R package ‘twang’), and balance between groups was assessed by computing standardized mean differences for each covariate in the weighted sample (R package ‘tableone’). Table 1 and Table S1 provide information on covariate balance before and after weighting. Robust variance estimates for inference tests in weighted ZINB analyses were obtained via the sandwich estimator (R package ‘sandwich’). Additional detail about the IPTW estimation and modeling procedures is provided in the supplemental content. Missing covariate data were imputed using the Chained Equations method [36] (R package ‘mice’); the proportion of missing data for each covariate is shown in Table S1. In order to account for multiple testing, alpha diversity and ZINB p values were corrected with the Benjamini-Hochberg false discovery rate (FDR) method [37]; FDR adjusted p values are labelled as q values. We utilized a threshold of two-sided p or $q < 0.1$ for significance testing; accordingly, we also display false coverage rate (FCR)-adjusted 90% confidence intervals [38] where relevant. All statistical analyses were completed using R v.3.4.3

Results

Sample characteristics

N = 383 participants were included in this study; 200 were HIV-, 66 were HIV+ undetectable (HIV RNA <20 copies/ml), 72 were HIV+ suppressed (HIV RNA ≥20–200 copies/ml), and 45 were HIV+ viremic (HIV RNA >200 copies/ml). All participants were MSM with an average age 31 (standard deviation = 7). Most were Hispanic (49%) or non-Hispanic Black (39%). Table 1 provides further detail on participant characteristics. Generally, HIV-infected participants, especially those with higher levels of viremia, were

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more likely to be older, unemployed, recently homeless, and to report methamphetamine use and frequent binge drinking than their HIV-uninfected peers. Among the HIV-infected participants, the mean number of years since diagnosis was 7.5 (sd = 5.7), mean $\log_{10}$ viral load was 2.0 (sd = 1.2), and mean CD4 cell count was 625 cells/mm^3 (sd = 287). No participants were ART-naïve and ninety percent of participants reported current ART.

Effects of HIV viremia on overall microbial composition

The relative composition of each individual's microbiome is displayed in Figures 1A and S1, and average composition within each HIV viremia category in Figure 1B. *Prevotella* is the most highly represented bacterial genus among most participants, with increasing relative amounts of *Bacteroides, Bifidobacteria,* and *Fusobacteria* in those towards the right side of the axis (Figure 1A). Higher levels of *Alloprevotella* and *Porphyromonas* are apparent in the HIV+ viremic group, and lower levels of *Bacteroides* are apparent in all HIV + groups relative to HIV- controls (Figure 1B).

To quantitatively examine the influence of HIV viremia on differences in microbial composition between-subjects we used PERMANOVA with Bray-Curtis distance. HIV viremia explained a significant amount of variability in microbial composition in both unadjusted (R^2 = .014, p = .001) and covariate-adjusted analyses (R^2 = .011, p = .017) (Table S2). Figure 2A displays ordination of the samples by principal coordinates analysis (Bray-Curtis distance), where HIV- and HIV+ viremic groups are distinct while HIV+ undetectable and HIV+ suppressed are more similar.

Comparisons of alpha diversity suggest a tendency for HIV+ individuals to have higher diversity in metrics that do not account for evenness (observed count and Chao1 statistic) (Figure 2B). Kruskal-Wallis analyses revealed significant differences in observed and Chao1 richness by HIV group (Table S3). Quantile regression was further used to investigate these differences and revealed higher median observed and Chao1 values for HIV+ suppressed versus HIV- individuals (q = .022 in IPTW-adjusted analyses). No other significant differences were found in any group. Shannon and Simpson indices did not vary greatly between groups.

Differences in specific bacterial taxa associated with HIV viremia

Zero-inflated binomial (ZINB) models were utilized to identify bacterial genera that were differentially abundant among the HIV viremia groups. HIV+ undetectable showed significant enrichment in *Finegoldia* and *Streptococcus* and depletion in *Bacteroides, Brachyspira, Campylobacter, Helicobacter, Parasutterella,* and *Turicibacter* when compared to HIV-uninfected. After IPTW adjustment for behavioral and clinical confounders, depletions in *Campylobacter, Parasutterella* and *Brachyspira* remained significant (Figure 3A; Table S4). HIV+ suppressed participants (HIV RNA ≤200 copies/ml) had increased *Pseudoflavonifractor* and decreased *Bacteroides, Barnsiella, Brachyspira, Campylobacter, Escherichia/Shigella, Flaonifractor, Helicobacter, Oxalobacter, Parabacteroides, Turicibacter,* and *Victivallis* relative to HIV-negative subjects. Following IPTW adjustment, depletions in *Barnesiella, Helicobacter,* and *Brachyspira* remained significant (Figure 3B; Table S4).

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HIV+ viremic men (HIV RNA >200 copies/ml) had the most distinct microbial signature relative to HIV-negative, showing significant enrichment of *Corynebacterium, Dietzia, Finegolda, Murdochiella, Negativicoccus, Peptoniphilus, Porphyromonas,* and *Prevotella* as well as depletion of *Arcanobacterium, Brachyspira, Bacteroides, Campylobacter, Faecalibacterium, Helicobacter* and *Succinivibrio*. With IPTW adjustment, enrichment of *Murdochiella, Peptoniphilus, Porphyromonas,* and *Prevotella* and depletion of *Arcanobacterium, Bacteroides*, *Brachyspira, Faecalibacterium,* and *Helicobacter* were significant (Figure 3C; Table S4). For some bacteria, including *Faecalibacterium, Peptoniphilus, Porphyromonas*, *Prevotella*, and *Streptococcus,* effect size (i.e., degree of enrichment or depletion) increased with increasing viremia (Figure S2).

An IPTW-adjusted multinomial least absolute shrinkage and selection operator (LASSO) model was also used as an additional method of feature selection to compare with ZINB findings. Among HIV+ participants with undetectable or suppressed viremia, no genera were significant in both the adjusted ZINB and LASSO models. However, among the HIV+ viremic group, differences in *Bacteroides, Peptoniphilus, Porphyromonas, and Prevotella* were consistent across analytic strategies (Figure 4).

It was also of interest to determine whether HIV+ participants with low levels of viremia (HIV RNA < 200 copies/mL) had distinct microbial signatures from those who were HIV+ but undetectable (HIV RNA <20 copies/mL). One genus, *Sneathea,* was significantly different between these groups in adjusted ZINB analyses ($q < .1$). The LASSO model identified depletions in *Gemmiger* in HIV+ suppressed as compared to undetectable participants (Figure S3).

Discussion

In this study examining the effects of HIV on the rectal microbiome in a cohort of 383 young, mostly minority MSM, we found important differences in microbial composition between HIV-uninfected and HIV-infected men which varied depending on level of viremia. HIV viremia category accounted for about 1% of the variability in microbiome composition, an effect size that is consistent with previous studies [19]. As hypothesized, microbiome perturbations were most evident among HIV+ viremic men, and least evident in HIV+ men with undetectable viremia. Importantly, we utilized IPTW to account for multiple confounding factors in our analyses, which decreased the likelihood that the results we report are attributable to clinical or behavioral covariates affecting the microbiome such as sexual behavior, substance use, or obesity.

High diversity is generally associated with a healthy rectal microbiome [2], and reduced richness and diversity has been reported in studies comparing HIV-infected and uninfected persons [11, 12, 25, 39–41]. Still other studies report no differences in diversity associated with HIV-infection [16, 17], while others have suggested that differences in diversity may be related to sampling location [14], or HIV-treatment status [24, 40]. We found few significant differences in diversity metrics in our study, and findings did not follow a clear dose-response pattern with level of viremia. As we were able to adjust for multiple confounders in our analyses, we can be reasonably confident that previously reported determinants of

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diversity such as sexual behavior [19] and substance use [42] had limited influence on our findings. Our results suggest that once these confounding factors are taken into account, bacterial diversity and richness may not be substantially impacted by HIV infection itself.

One of the more consistent findings across studies of HIV and the microbiome has been enrichment in *Prevotella* and depletion in *Bacteroides* among both untreated and treated HIV-infected individuals [9, 11, 14]. *Prevotella* species are considered pro-inflammatory [43, 44], while *Bacteroides* species have been shown to induce regulatory T-cell differentiation and IL-10 production [45, 46]. Previous work has suggested that observed alterations to the *Prevotella/Bacteroides* ratio may have been due to sexual behavior rather than HIV [19]; however, others have shown decreased *Bacteroides* among HIV-infected MSM who were matched with MSM controls [9]. Our study examined exclusively MSM and controlled for recency and frequency of receptive anal intercourse in our analyses, therefore, our study provides additional evidence that HIV may directly alter the *Prevotella/Bacteroides* ratio independent of sexual behavior. Although we found decreased *Bacteroides* in all HIV-infected individuals, the effect was similar between undetectable and suppressed participants and only statistically significant after adjustment for confounding in the viremic group. In addition, we found increasing relative amounts of *Prevotella* with increasing levels of viremia, which were only significant in the viremic group. Our findings are consistent with previous research showing that *Prevotella* may normalize with ART [13] whereas depletions in *Bacteroides* persist even with therapy [24].

Of the 78 genera tested, ZINB and LASSO models identified *Porphyromonas* as the genus with the largest difference between HIV+ viremic and HIV- individuals. *Porphyromonas* is a well-known modifier of inflammatory cytokines [47]. In fact, *Porphyromonas gingivalis* has been identified as a potential cause of systemic inflammation and metabolic disorders associated with periodontal disease [48] and implicated in inflammatory processes leading to the development of atherosclerosis [49]. Furthermore, administration of *P. gingivalis* to mice was shown to induce GI dysbiosis and contribute to intestinal permeability [50]. The association between increasing levels of HIV viremia and *Porphyromonas* may therefore represent an important mechanism behind HIV-associated chronic inflammation deserving of further study.

Of particular interest in our study is the examination of low level viremia individuals who are not undetectable (HIV RNA ≥20–200 copies/ml). It is notable that this group, while distinct from HIV-uninfected, was very similar to the undetectable viremia (HIV RNA <20 copies/ml) group in ordination analysis. Only a single genus, *Sneatha*, was statistically significantly different between these two groups when directly compared. Clinically, persistent low level viremia may increase risk of subsequent virologic failure [51], but a recent large study showed no difference in progression to AIDS or incidence of non-AIDS events in persons with low level viremia compared to undetectable [52]. Our analysis suggests that microbial composition is similar between those with low level and undetectable viremia, but remains distinguishable from HIV-uninfected individuals. While those with low level viremia can still have microbial translocation and inflammatory biomarkers [53], the overall decreased dysbiosis in low level viremia may correspond to reduced chronic inflammation which lessens clinical progression.

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Our results should be interpreted with consideration of the following limitations. First, we did not have diet information on our cohort. We included race/ethnicity, country of origin, employment status, obesity, and homelessness (all of which may impact dietary intake) in our covariate adjustment set to mitigate this limitation to the best of our ability. Second, the IPTW procedure will only achieve perfect balance between exposure groups in nonparametric settings with large sample sizes relative to the number of relevant confounders, and there is potential for residual confounding even by variables we controlled for in our study. However, we note that many of the most significant confounders (e.g. age, antibiotic use, sexual behavior, alcohol use, obesity) were well-balanced after weighting. The ability of 16S sequencing to provide species-level resolution is limited, thus we conducted analyses at the genus level. We acknowledge that differentially abundant genera do not necessarily indicate differences in functionally important species. Finally, we did not have time since ART initiation for our cohort, and thus cannot determine if participants were viremic because they recently started treatment or because treatment was failing.

Our study also has numerous strengths. Primarily, we utilized data from a large cohort of regionally, socioeconomically, and behaviorally similar individuals, increasing the internal validity of our findings. We employed a novel technique, IPTW, to incorporate a large amount of clinical and behavioral data into our analyses. With IPTW, analyses are “marginal structural models” instead of conditional on covariates, as in multiple regression. Modeling microbiome data marginally offers several advantages including the ability to control for many confounding factors without inducing overfitting bias [54] or losing efficiency due to overstratification [55]. Addressing sources of systematic error using IPTW may improve reproducibility in future studies of HIV and the microbiome. We also stratified our HIV-infected participants by level of viremia, allowing us to examine differences between HIV-uninfected and HIV+ undetectable, suppressed, and viremic individuals. This stratification leads to better understanding of the relationship between active viral replication and dysbiosis, namely, that dysbiosis increases with increasing viremia. Finally, we were able to replicate our major findings using two distinct analytic strategies. Genera identified as differentially abundant in both analyses may be more likely to be true discoveries.

This study contributes to a growing body of literature describing the effects of HIV on microbial dysbiosis. We show that, even when taking into account the influence of multiple confounding factors, HIV is associated with intestinal dysbiosis in a dose-dependent manner. Although great strides have been made in the management of chronic HIV infection, the life expectancy among HIV-infected individuals remains reduced relative to their HIV-negative peers [56]. This reduction has largely been attributed to increased rates of inflammation-related comorbidities observed among people living with HIV [57], and the microbiome likely plays a key role in modulating interactions between HIV and the immune system. Therefore, understanding the ways in which HIV and the microbiome interact may be a crucial step towards developing intervention strategies to reduce the burden of HIV-associated morbidity and mortality.

Supplementary Material

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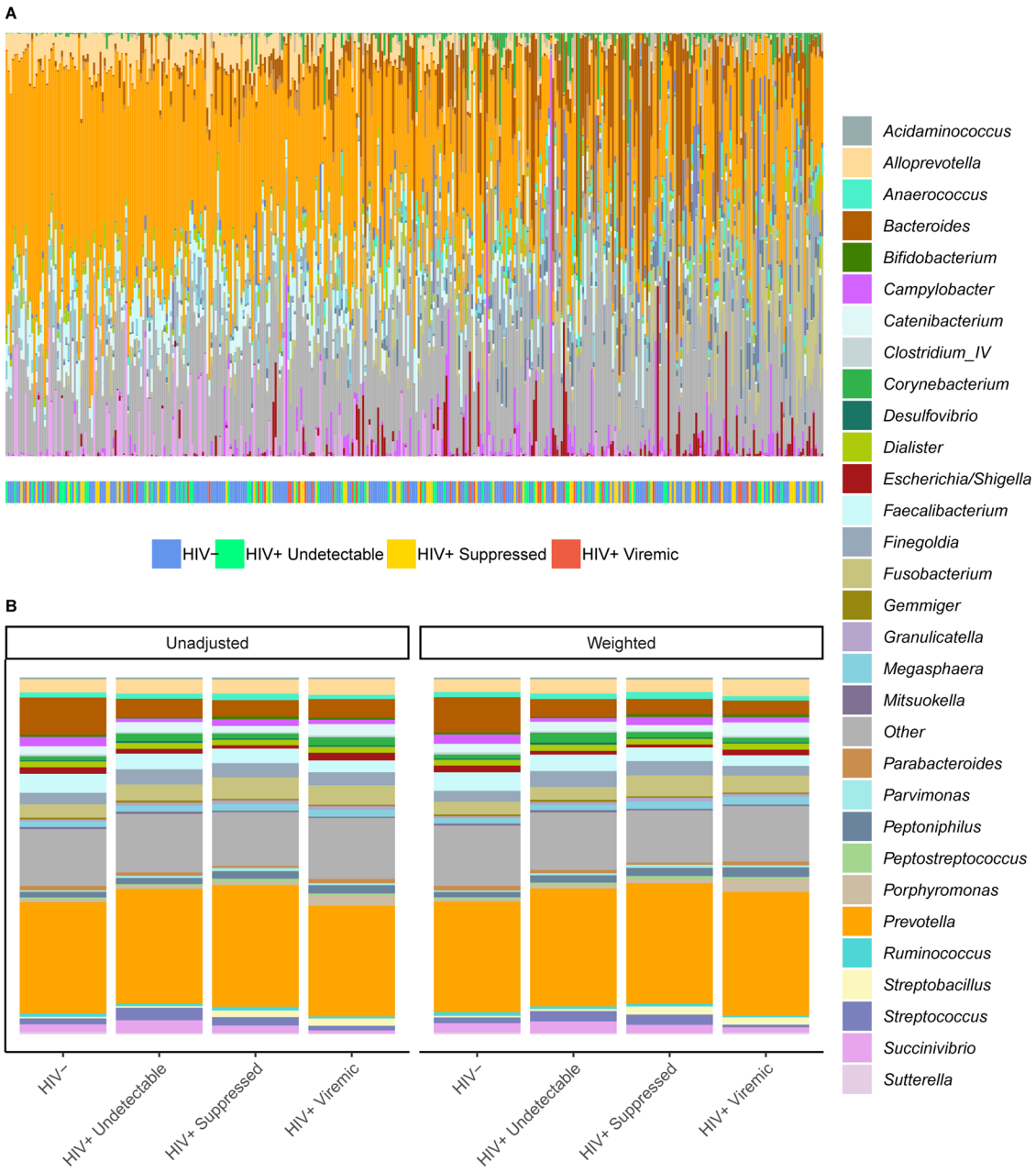

Figure 1.

Rectal microbial composition of study participants, N = 383. (A) Columns represent the relative composition of each subject's microbiome at the genus level. HIV status of the subjects is indicated by a colored line below their microbial composition. Subjects are ordered by the first principal coordinate of a Bray-Curtis pairwise distance matrix. Genera representing less than 1% of the composition on average across samples were combined into "Other." (B) Average microbial composition within each HIV viremia category. Unadjusted and inverse probability of treatment weighted compositions are shown. Bacterial genera representing less than 1% of the overall relative composition or present in less than 20% of the samples were grouped into "Other."

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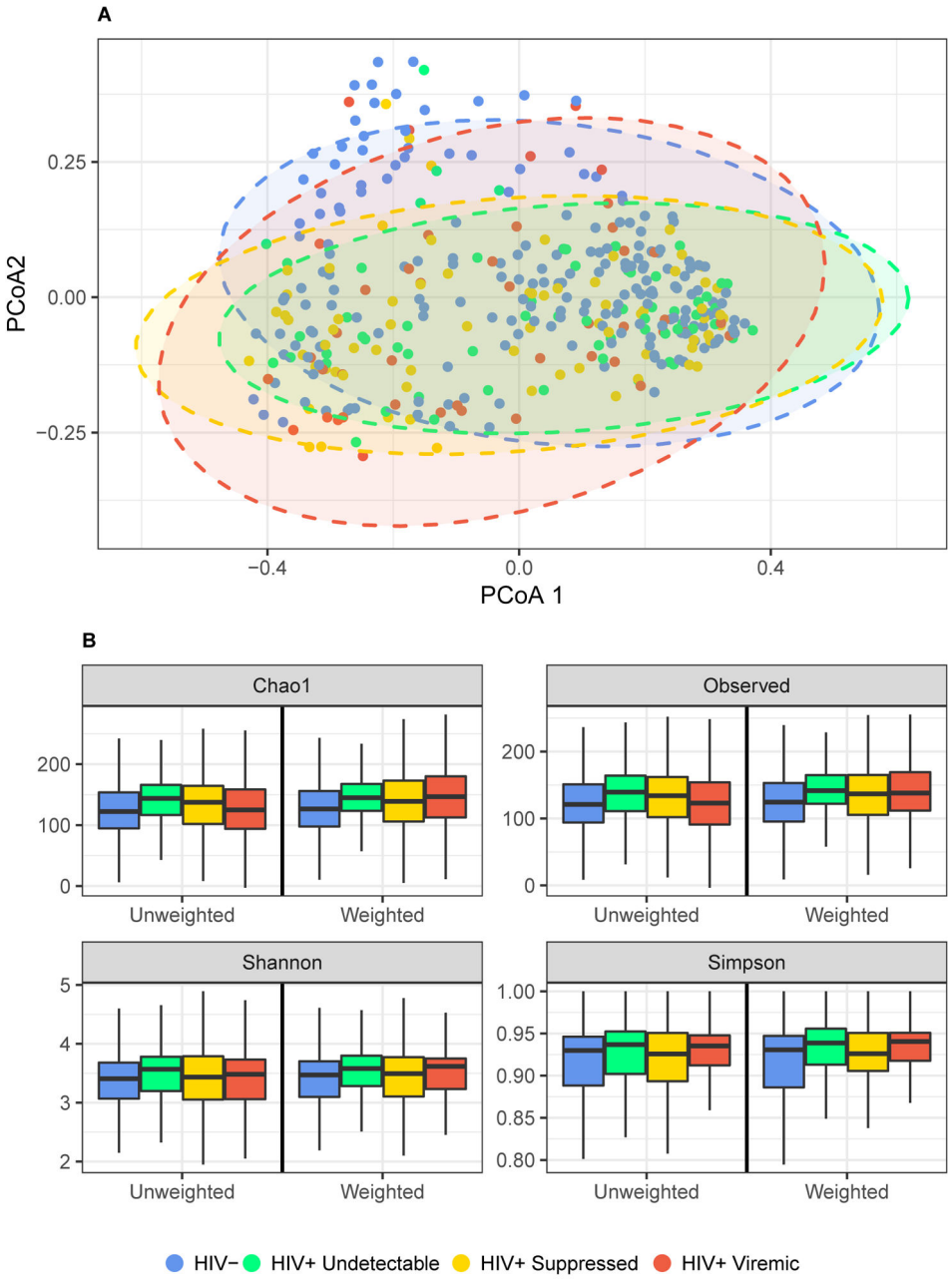

Figure 2.

Associations between HIV viremia and overall microbial composition. (A) Ordination of the Bray-Curtis distance between samples using principal coordinates analysis. PCoA = Principal coordinate axis. Ellipses are 95% confidence regions for each group assuming points follow a multivariate *t* distribution. (B) Boxplots of richness metrics. Boxes represent the lower, median, and upper quartile of the data and whiskers are 1.5*interquartile range.

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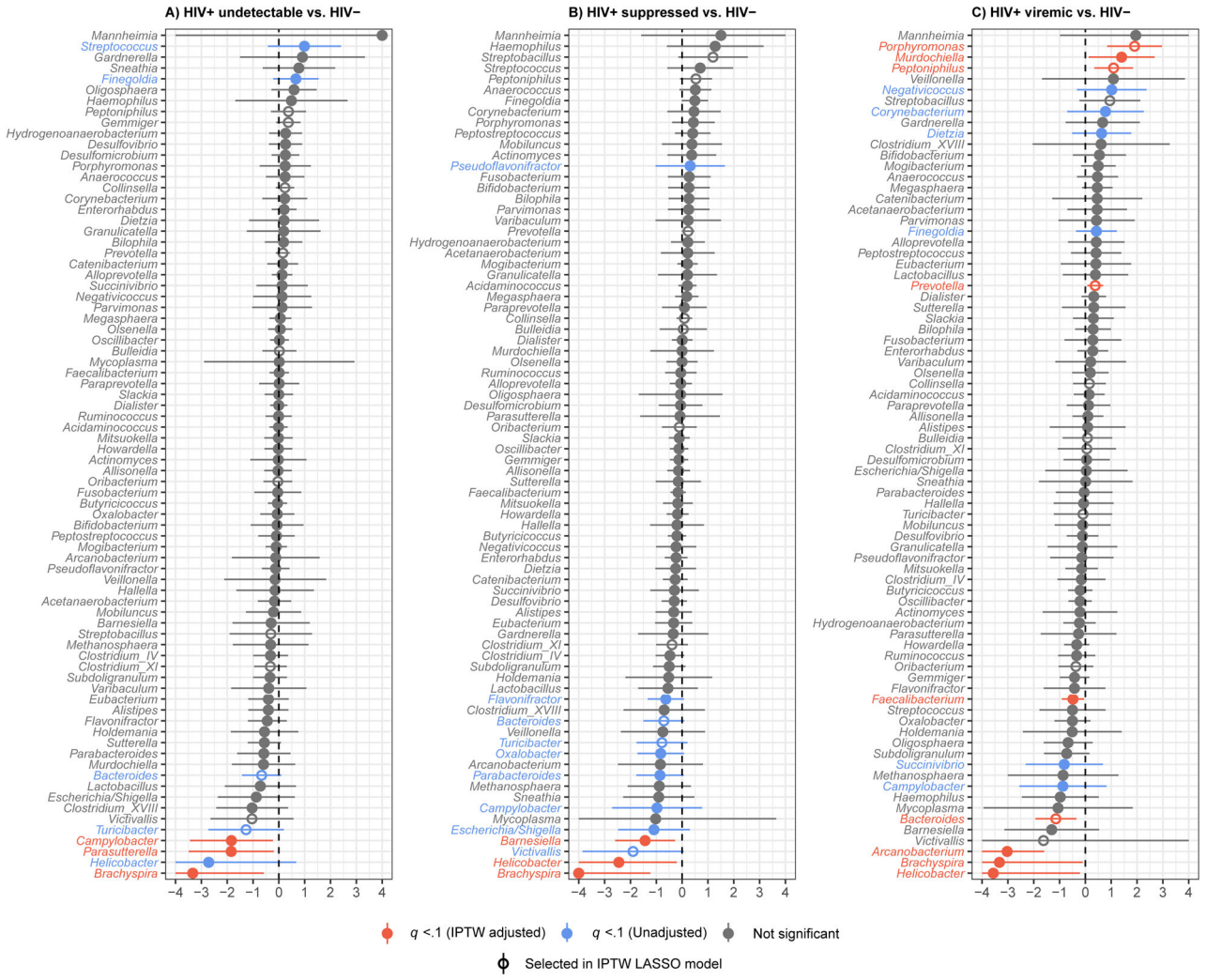

Figure 3.

Comparisons of individual bacterial genera between HIV viremia categories. Forest plots of results of zero-inflated negative binomial models comparing genus-level bacterial counts between HIV-negative and (A) HIV+ undetectable (HIV RNA <20 copies/ml), (B) HIV+ suppressed (HIV RNA >20 and ≤200 copies/ml) and (C) HIV+ viremic (HIV RNA >200 copies/ml) participants. Inverse probability of treatment-weighted effect sizes and 90% false coverage rate-adjusted confidence intervals (truncated at −4, 4) are plotted, with statistical significance ($q < 0.1$) indicated in color. Effect sizes are log ratios of normalized genera counts.

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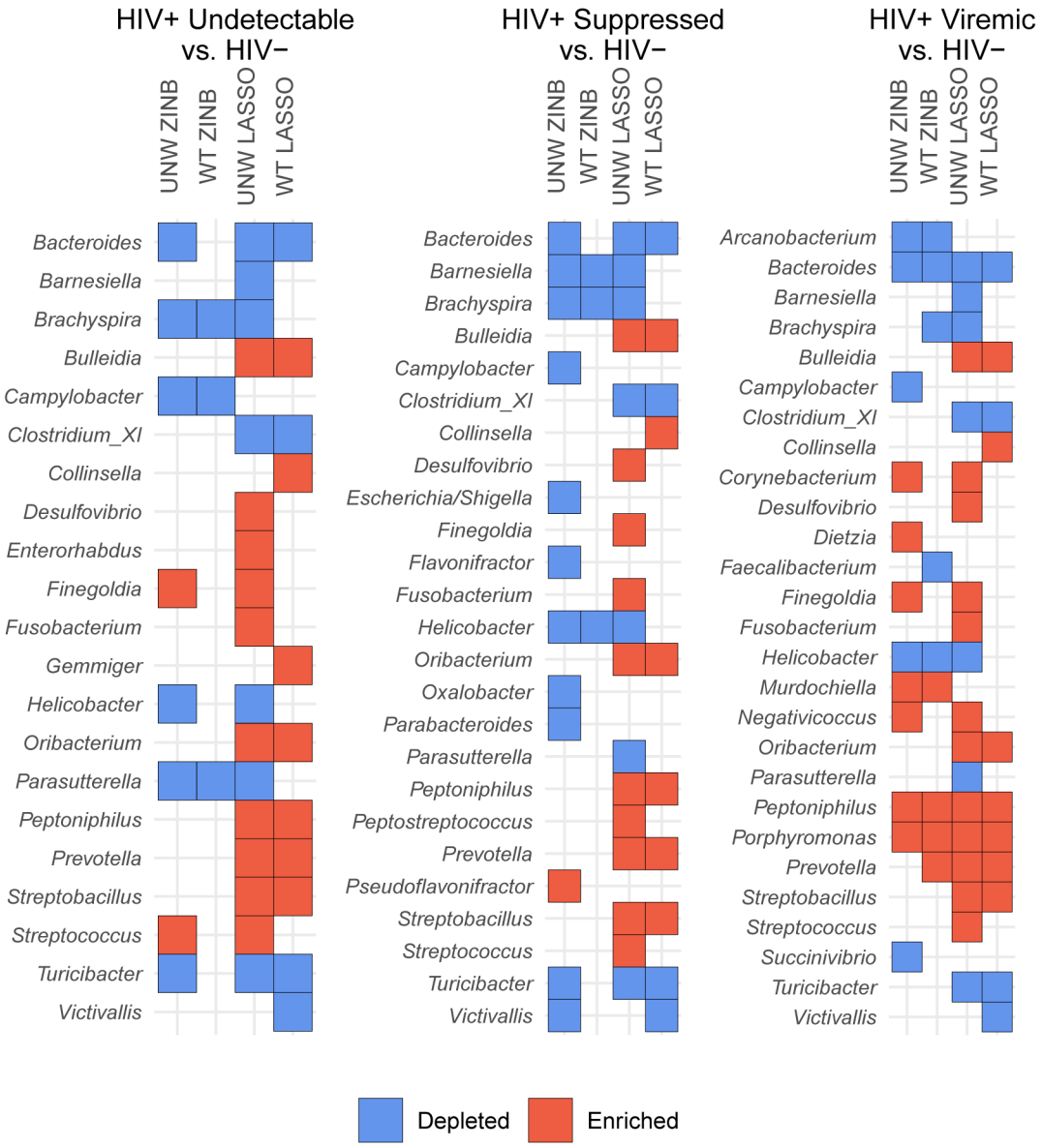

Figure 4.

Summary of zero-inflated negative binomial (ZINB) and least absolute shrinkage and selection operator (LASSO) model results. Enriched taxa are those with positive effect sizes (relative to HIV-), depleted are those with negative effect sizes. Genera with no effect in either analysis are not shown. UNW = unadjusted, WT = IPTW adjusted.

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Table 1.

Participant characteristics, N = 383 men who have sex with men in Los Angeles, CA

	HIV-Negative mean (sd)/ n (%)	Undetectable (HIV RNA < 20 copies/mL)	Suppressed (HIV RNA ≥20 - 200 copies/mL)	Viremic (HIV RNA > 200 copies/mL)	SMD[a] (unweighted)	SMD (weighted)
n	200	66	72	45		
Age	28.91 (6.43)	33.41 (6.55)	34.10 (6.36)	33.18 (6.86)	.41	.17
Employment					.54	.25
Student	26 (13.0)	5 (7.6)	3 (4.2)	1 (2.2)		
Unemployed	61 (30.5)	23 (34.8)	50 (69.4)	27 (60.0)		
Full/part time	113 (56.5)	38 (57.6)	19 (26.4)	17 (37.8)		
Race/Ethnicity					.48	.37
Black Non-Hispanic	82 (41.0)	17 (25.8)	25 (34.7)	26 (57.8)		
Hispanic	98 (49.0)	37 (56.1)	35 (48.6)	19 (42.2)		
Other Non-Hispanic	20 (10.0)	12 (18.2)	12 (16.7)	0		
Country of origin					.12	.08
United States	171 (85.5)	53 (80.3)	55 (76.4)	36 (80.0)		
Other	29 (14.5)	13 (19.7)	17 (23.6)	9 (20.0)		
Homeless in past 6 months	65 (32.5)	20 (30.3)	21 (29.2)	22 (48.9)	.21	.18
RAI in past 7 days	88 (44.0)	32 (48.5)	30 (41.7)	19 (42.2)	.08	.07
Number of RAI acts in past month	2.06 (4.19)	2.48 (4.68)	2.33 (5.45)	4.42 (8.03)	.19	.07
Methamphetamine use in past 6 months					.54	.20
Daily/Weekly	21 (10.5)	14 (21.2)	18 (25.0)	21 (46.7)		
Monthly/less	32 (16.0)	21 (31.8)	19 (26.4)	9 (20.0)		
Never	147 (73.5)	31 (47.0)	35 (48.6)	15 (33.3)		
Marijuana use					.30	.14
Daily/Weekly	71 (35.5)	19 (28.8)	21 (29.2)	19 (42.2)		
Monthly/less	57 (28.5)	10 (15.2)	16 (22.2)	10 (22.2)		
Never	72 (36.0)	37 (56.1)	35 (48.6)	16 (35.6)		
Cocaine use					.16	.09
At least once	53 (26.5)	11 (16.7)	21 (29.2)	13 (28.9)		
Never	147 (73.5)	55 (83.3)	51 (70.8)	32 (71.1)		
Tobacco smoking					.26	.15
>1 pack/day	10 (5.0)	3 (4.5)	5 (6.9)	2 (4.4)		
<1 pack/day	68 (4.0)	24 (36.4)	31 (43.1)	25 (55.6)		
Nonsmoker	122 (61.0)	39 (59.1)	36 (50.0)	18 (40.0)		
Binge drinking in past 6 months[b]					.40	.15
Weekly	24 (12.0)	15 (22.7)	8 (11.1)	8 (17.8)		
Monthly/less	111 (55.5)	26 (39.4)	20 (27.8)	16 (35.6)		
Never	65 (32.5)	25 (37.9)	44 (61.1)	21 (44.7)		

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	HIV-Negative mean (sd)/ n (%)	Undetectable (HIV RNA < 20 copies/mL)	Suppressed (HIV RNA ≥20 - 200 copies/mL)	Viremic (HIV RNA > 200 copies/mL)	SMD[a] (unweighted)	SMD (weighted)
Obese[c]	64 (32.0)	16 (24.2)	13 (18.1)	10 (22.2)	.17	.15
Antibiotic use	11 (5.5)	5 (7.6)	7 (9.7)	8 (17.8)	.21	.08
Sample collection method					.21	.08
Anoscopy	152 (77.0)	47 (71.2)	53 (73.6)	39 (86.7)		
Self-collected	46 (23.0)	19 (28.8)	19 (26.4)	6 (13.3)		
Years since HIV diagnosis [d]	N/A	7 (6)	7 (5)	8 (6)	N/A	N/A
HIV RNA copies/mL (median, IQR)	N/A	N/A	20 (30)	15,730 (48,680)	N/A	N/A
CD4 cells/mm^3	N/A	708.95 (279.6)	645.21 (262.9)	470.02 (280.1)	N/A	N/A
ART regimen					N/A	N/A
INSTI + NRTI	0	30 (45.5)	30 (41.7)	8 (17.8)		
NNRTI + NRTI	0	21 (31.8)	20 (27.8)	7 (15.6)		
NRTI + PI		10 (15.2)	11 (15.3)	9 (20)		
Other	0	5 (7.6)	8 (10.2)	4 (8.8)		
Missing/Not reported/NA	166 (83)	0	3 (4.2)	17 (37.8)		
PrEP user[e]	37 (19)	N/A	N/A	N/A	N/A	N/A

SMD = Standardized mean difference; RAI = Receptive anal intercourse; ART = Antiretroviral therapy; INSTI = Integrase strand transfer inhibitor; NRTI = Nucleoside reverse transcriptase inhibitor; NNRTI = Non-nucleoside reverse transcriptase inhibitor; PI = Protease inhibitor

[a] SMD is a measure of imbalance across groups; higher SMDs indicate greater imbalance. Average SMD before weighting = .29, after weighting = .15.

[b] Binge drinking defined as 6 or more drinks on one occasion.

[c] Obese defined as BMI > 30 or BMI > 25 and waist circumference > 40 inches.

[d] Years since HIV diagnosis, HIV RNA, CD4 cell count and ART regimen were not included in the inverse probability of treatment weight model (as they are generally not relevant to HIV negative participants), all other variables in the table were included.

[e] HIV negative men taking tenofovir disoproxil fumarate/emtricitabine for pre-exposure prophylaxis (PrEP).