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# The vaginal microbiota varies by self-identified ethnicity and HIV status throughout pregnancy and relates to preterm birth risk in a United Kingdom observational cohort

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## Abstract

Background

Low-diversity, *Lactobacillus*-dominated vaginal microbiota are associated with immune protection in pregnancy, whereas community state type (CST) IV and transitional CST III are linked to inflammation and preterm birth (PTB). Pregnant women living with HIV (PWLWH) in the UK have higher preterm birth rates than pregnant women without HIV (PWWH). We previously identified an association between PTB and CSTs III and IV in PWLWH from the UK HIV Preterm Birth Network study. However, participants in that cohort predominantly self-identified as having a Black ethnic background, and it remains unclear whether these associations differ according to HIV status and social factors. This larger analysis compares vaginal microbiota composition, inflammation, and PTB outcomes across self-identified Black and White PWLWH and PWWH.

Results

103 women (55 Black PWLWH, 14 Black PWWH, 15 White PWLWH, and 19 White PWWH) provided second trimester vaginal samples. Twelve women experienced PTB, 10 of whom were Black PWLWH (18.2% PTB rate). Black pregnant women had higher Chao1 alpha diversity than White PWWH (p=0.002). CSTs III and IV were more prevalent in Black women and White PWLWH, while CST I was less frequent. Among Black women, HIV was not associated with Chao1 diversity or inflammation, whereas among White women, HIV was linked to heightened Chao1 (p=0.007) and increased cervicovaginal cytokines. In Black PWLWH who experienced PTB, most were assigned to CST III or IV in the second trimester. Nearly half of Black PWLWH (49%) underwent a CST transition during pregnancy and were more likely to transition compared to other subgroups (aOR: 2.53, 95% CI 1.02–6.52; p=0.048).

CST change trended toward increased PTB risk in Black PWLWH (aOR: 3.50, 95% CI 0.82–18.46; p=0.10), with transition between CSTs III and IV associated with the highest risk of PTB (aOR: 7.75, 95% CI 1.57–42.75; p=0.01).

Conclusions

Black PWLWH had the highest PTB rates, and CST instability during pregnancy was associated with PTB risk in this group, particularly transitions between CSTs III and IV. The high prevalence of CSTs III and IV among Black women regardless of HIV, and within White PWLWH, may reflect broader socioeconomic determinants of reproductive health.

Keywords

Vaginal microbiome, preterm birth, HIV, metataxonomics, vaginal microbiome dynamics and reproductive health

## Background

The vaginal microbiota is a dynamic ecosystem that plays a critical role in sexual and reproductive health [1]. Its composition varies over time and among individuals and can be broadly categorized into community state types (CSTs) according to bacterial taxa [1, 2, 3]. CST IV features reduced *Lactobacillus* abundance, increased *Gardnerella vaginalis* and other anaerobic taxa, and increased bacterial diversity [2, 3]. CST IV broadly aligns with the clinical condition of bacterial vaginosis (BV) and is associated with inflammation [4] and adverse sexual and reproductive health outcomes [1, 2, 5]. Notably, CST IV communities are typically less temporally stable than *Lactobacillus*-dominated CSTs [1], which may permit transient shifts into high-diversity states and increase susceptibility to infection and inflammation.

In contrast, CSTs I, II, III, and V are dominated by one of four *Lactobacillus* species (*L. crispatus* (I), *L. gasseri* (II), *L. iners* (III), and *L. jensenii* (V)) and are generally characterized by low species diversity [2, 3]. *Lactobacillus* species maintain a low vaginal pH and produce bactericidal compounds that inhibit pathogens [6, 7]. Accordingly, these CSTs have been associated with a reduced risk of BV, sexually transmitted infections (STIs) [5], and other urogenital conditions [8, 9]. The protective role of *L. iners* is disputed, as it displays limited antimicrobial activity [10] and is more prone to transition to high-diversity states [1, 2].

In the context of pregnancy, high-diversity CST IV communities have been associated with preterm birth (PTB) (delivery before 37 gestational weeks) [11] and other complications, such as miscarriage and low birth weight [12, 13], whereas low-diversity, *Lactobacillus*-dominated CSTs are widely connected to term delivery [14, 15]. Longitudinal studies further indicate that women with uncomplicated term deliveries tend to exhibit reduced bacterial diversity [16], fewer excursions into high-diversity CSTs [17], and an enrichment of *Lactobacillus*-dominated communities over the course of pregnancy [18, 19]. Conversely, microbiome instability and an increased prevalence of high-diversity states are associated with a heightened risk of ascending genitourinary infections and inflammation, which may contribute to membrane rupture [20] and the initiation of preterm labor [11, 14, 21].

The prevalence of CSTs is often reported to differ by geography and ethnicity, where ethnicity is understood as a demographic categorization that reflects environmental and cultural factors and ancestry. Studies in both nonpregnant and pregnant populations have revealed differences in the CST distribution across ethnic groups, with a higher prevalence of high-diversity microbiota observed in cohorts of Black women than in those of White women [2, 3, 16, 19, 22]. Consistent with these patterns,

the global prevalence of BV also varies by region, with estimates of approximately 23% in Europe, 29% in South Asia, and some of the highest rates reported in southern and eastern Africa, with 34% in Tanzania and 58% in South Africa [23, 24].

However, differences in the vaginal microbiota have been attenuated after accounting for smoking, douching**,** and sexual behaviors, suggesting that ethnicity may act as a proxy for underlying social, behavioral, or structural factors [25, 26, 27]. These contextual factors are particularly relevant in settings with a high burden of human immunodeficiency virus (HIV).

Women living with HIV also have a high prevalence of CST IV and nonoptimal CST III [28, 29, 30]. It is known that BV increases the risk of HIV transmission through the recruitment of HIV target cells to the genital mucosal interface [31, 32, 33, 34]. Indeed, BV prevalence is often reported to be high in regions with high HIV prevalence [31]; however, HIV itself may play a role in the incidence and persistence of high bacterial diversity [29, 35, 36].

Women living with HIV also have high rates of PTB, which are up to 4-fold higher than those of women without HIV and the general population [37, 38, 39, 40]. The etiology of PTB is not fully understood and is likely multifactorial, including a high co-occurrence of risk factors, such as ethnicity and structural racism, alongside immune dysfunction [41, 42]. It has been hypothesized that combination antiretroviral medication (ART) may increase the risk of PTB; however, this has not been borne out with newer, less toxic, integrase inhibitor-based regimens [43, 44]. Others have examined the role of adverse placental pathology [45].

We and others have shown that pregnant women living with HIV (PWLWH) have a high prevalence of nonoptimal CSTs IV and III and that PTB was observed exclusively in these CSTs [4, 46]. A limitation of our earlier work was that 81% of participants in

the UK HIV Preterm Birth Network (UHPBN) cohort self-identified as Black, compared with 23% of PWWH in the term delivery comparator group. Differences in CSTs observed between the groups may therefore reflect differences in ethnic composition rather than HIV status itself.

Here, we present updated analyses of UHPBN with further cases of PWLWH and self-reported, ethnically matched controls contemporaneously recruited to the same study. Using long-read 16S rRNA gene sequencing, we further delineate the impact of both ethnicity and HIV status on the vaginal microbiota throughout pregnancy. Self-reported ethnicity is not conceptualized here as a biological determinant, but rather used as a contextual social variable that may reflect differences in structural conditions, healthcare access, migration history and other lived experiences that impact the vaginal microbiota that were not captured in this observational study [27, 42].

Stratification by HIV status and self-identified ethnicity is clinically relevant in the United Kingdom, where women of Black ethnicity experience higher rates of PTB than White women [47, 48], indicating a significant gap in maternal care. PWLWH in the UK also have higher rates of PTB than PWWH [49, 50]. Analyses by ethnicity and HIV status may reveal patterns of microbiome variation during pregnancy that correspond to PTB risk and are intended to generate hypotheses for future prospective studies that incorporate detailed social, environmental, and structural data.

# Methods

## Study Design

This is a secondary analysis of the UHPBN study, which continued to recruit participants until August 2023 and included 135 women. Eighty-seven PWLWH were recruited from 8–14 weeks gestation in HIV specialist clinics of ten London hospitals, and 48 PWWH were recruited from a general antenatal clinic at a single center. Eligible

149 women were 18 years of age or older and had known HIV status with a singleton
150 pregnancy conceived via natural conception (confirmed on ultrasound). The women
151 included in these secondary analyses had available vaginal samples and self-
152 identified as Black/Mixed-Black heritage or White, with ethnicity classified according
153 to the United Kingdom census definitions: White (European descent) and Black
154 (African and Caribbean descent).

155 **Sampling and data collection**

156 The participants donated a high-level vaginal swab +/- a paired sample of
157 cervicovaginal fluid (CVF) collected with a SOFTDISC® (The Flex Company, USA) for
158 microbiota and immune assays at three time points across the second and third
159 trimesters (timepoint 1 range: mean 20 weeks; timepoint 2: mean 23 weeks; and
160 timepoint 3: mean 29 weeks, as previously described) [4].

161 All the samples were snap frozen and stored at −80°C within 4 hours of collection until
162 further processing. Demographic, medical, and obstetric data were recorded for all
163 women. The baseline CD4+ T-cell count and plasma HIV RNA concentration at
164 antenatal screening (12-14 weeks) and delivery and ART regimen were also
165 documented for PWLWH.

166 **DNA extraction and full-length 16S rRNA gene sequencing**

167 DNA was extracted from either high vaginal swabs or CVF via enzymatic digestion
168 along with beating of the cell membranes prior to the use of the QIAmp DNA Mini Kit
169 (Qiagen, UK) as previously described [19]. Genomic DNA was diluted to 2 ng/μL prior
170 to sequencing. The full-length (V1-V9, 1.5 kb) 16S rRNA gene was sequenced via
171 PacBio dual-barcoded 16S rRNA gene primers (27F/1492R) using KAPA HiFi HotStart
172 (Roche Diagnostics, USA) chemistry, as per the PacBio 16S rRNA gene amplification

protocol [51]. Libraries were created with the SMRTbell kit and sequenced on the PacBio Sequel IIe platform (PacBio, USA).

**Sequence analysis and CST assignment**

Demultiplexed reads were primer trimmed and processed with the DADA2 package in R to infer amplicon sequence variants (ASVs). The decontam package [52] was used to identify and remove contaminant ASVs using DNA extraction and sequencing negative controls. Taxonomy was assigned from phylum to genus using the SILVA reference database, and [addSpecies] implemented species-level annotations along with the SILVA species FASTA. *Lachnospiraceae BVAB1* (recently renamed Candidatus *Lachnocurva vaginae*) was identified by comparing representative ASV sequences against the STIRRUPS-curated vaginal database [53]. *BVAB1* (Ca*. Lachnocurva vaginae*) was identified based on the best STIRRUPS match, along with meeting prespecified identity confidence criteria. Samples with fewer than 200 reads, singleton species, and those found in less than 5% of the samples were removed from analysis. Relative abundance, Chao1 alpha diversity, and Bray-Curtis beta diversity were determined using the *phyloseq* package [54] in R (version 4.5.0). Chao1 richness was used as the primary alpha diversity measure as the focus of the analysis was on differences in observed taxa richness across groups.

The samples were classified into vaginal CSTs using the VALENCIA algorithm [3] in Python (version 3.13). ASVs were formatted to the expected taxon keys within VALENCIA at either the species or genus level if species were not available. CSTs were collapsed into the following groups: CST I (*L. crispatus* dominated, including I-A and I-B); CST II (*L. gasseri* dominated); CST III (*L. iners* dominated, including III-A and III-B); CST IV (diverse, anaerobe dominated, including IV-A, IV-B, and IV-C0-4); and CST V (*L. jensensii* dominated).

**Cytokine quantification**

A subset of women provided second trimester CVF samples. CVF was diluted in extraction buffer with a protease inhibitor cocktail as previously described [55]. Ten cytokines (IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, and TNF-α) were measured via multiplex chemiluminescent assays (V-plex Human Pro-inflammatory cytokine panel, Meso Scale Discovery®), and the analyses were restricted to 76 samples within the range of the standard curve.

**Statistical Analyses**

All the analyses were performed in R, and the figures were generated using ggplot2 [56] and EnhancedVolcano [57]. Unless otherwise specified, p-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate method (FDR), and FDR-adjusted $p < 0.05$ was considered statistically significant. In analyses with many simultaneous comparisons or limited subgroup sample sizes, both nominal and FDR-adjusted p-values are presented for transparency. Participants were stratified by HIV status and self-reported ethnicity into four groups: Black PWLWH, Black PWWH, White PWLWH, and White PWWH. Maternal age and body mass index (BMI) were included as covariates in the regression models unless otherwise stated.

**Participant characteristics**

Maternal, immunologic (for PWLWH) and obstetric characteristics were compared between subgroups using Kruskal-Wallis test for continuous variables and Fisher's test for categorical variables. Dunn's post hoc pairwise tests were performed if significance was reached.

**Vaginal microbiota**

Alpha diversity was compared across subgroups with a Kruskal–Wallis test, followed by Wilcoxon rank-sum tests between subgroups. Beta diversity was assessed with

223 global and pairwise permutational multivariate analysis of variance (PERMANOVA)
224 tests via the pairwiseAdonis package [58]. Differences in CST prevalence at the
225 second-trimester visit were evaluated using pairwise Fisher's exact tests. Logistic
226 regression was used to estimate the odds of assignment to grouped optimal
227 *Lactobacillus*-dominated CSTs (I+II+V). Estimated marginal means (EMMs) were
228 derived using the emmeans package [59], and pairwise contrasts between groups
229 were evaluated with Tukey-adjusted p values. Differentially abundant species were
230 compared pairwise between the HIV and ethnicity subgroups via the ANCOM package
231 [60]. Among PWLWH, generalized logistic models were used to examine the
232 associations between CD4 count (per 100-cell increase), detectable HIV (> 50 copies),
233 and ART duration and type with assignment to optimal grouped *Lactobacillus* CSTs,
234 testing for interactions with ethnicity.

235 **Genital Inflammation**

236 Paired microbiota and cytokine data from cervicovaginal fluid were available for 40/55
237 Black PWLWH, 11/15 White PWLWH, 6/13 Black PWWH and 6/18 White PWWH.
238 Cytokine concentrations were $\log_{10}(x+1)$ transformed to reduce skewness and handle
239 zeros. The sample size varied by cytokine, as measurements outside of the standard
240 curve were excluded. Cytokines were compared between groups with Wilcoxon
241 rank-sum tests. For each cytokine, linear models including subgroup, maternal age,
242 and BMI were fitted, and EMMs were calculated to assess adjusted pairwise group
243 differences. Principal component analysis (PCA) was conducted via the pcaMethods
244 package to summarize inflammation within each sample, and the nipals method was
245 used to handle missing data [61].

246 Spearman correlations were calculated between principal component 1 (PC1) and
247 alpha diversity, age, BMI, CD4 count, and HIV viral load. To assess inflammatory

differences within bacterial states, samples were stratified by CST, and PC1 scores were compared across HIV and ethnicity subgroups via Wilcoxon rank-sum tests, Pearson's correlation and Fisher's tests were used to explore potential associations between demographic and bacterial factors and PTB. Generalized logistic models were used to assess the individual impact of each cytokine on the odds of assignment to CST I+II+V. Cytokine effects were evaluated both overall and with subgroup interaction terms in separate models. A similar model was also used to assess the impact of PC1 on CST assignment.

**Longitudinal Analysis**

A second sample was available for 95 women (Black PWLWH, n = 49, White PWLWH, n = 14, Black PWWH, n = 14, White PWWH, n = 18). CST transitions were collapsed into optimal (I+II+V) and potentially suboptimal (III+IV) states. To increase statistical power, White PWLWH, Black PWWH, and White PWWH were combined and compared with Black PWLWH to assess the odds of CST change. Additional models examined i) the odds of CST change between individual subgroups, ii) the independent effects of ethnicity and HIV status on CST change, and iii) the likelihood of assignment to CST I+II+V at either timepoint.

Further analyses were performed to evaluate whether the initial CST influenced subsequent CST changes across subgroups to assess differences in microbiota stability over time. Associations between overall CST changes, specific transitions (I+II+V ↔ III+IV, III ↔ IV), and PTB were examined in models without covariates.

# Results

## Study participant characteristics

Of the 87 PWLWH and 48 PWWH in the UHPBN study, second trimester vaginal samples were available for analysis at 20–21 weeks gestation from 70 PWLWH and 33 PWWH who identified as Black or White. This included 55 Black PWLWH, 14 Black PWWH**,** 15 White PWLWH, and 19 White PWWH **(Table 1)**. The remaining women either identified as Indian, Arab, Asian, Latino, or Chinese or did not have an available sample at this timepoint. All PWWH were recruited from a general antenatal clinic at the same center, whereas PWLWH were recruited from eight different HIV clinics. Recruitment locations were not significantly different between the PWLWH ethnic subgroups (p = 0.37) (**Supplementary Figure 1**).

Black PWLWH were older than Black PWWH (p = 0.02) and White PWLWH (p = 0.02) (**Table 1**). White PWWH had a significantly lower BMI than Black PWLWH (p = 0.002). Gravidity, alcohol use, and obstetric complications did not differ between groups, although limited tobacco use was reported in all groups except White PWWH. The median gestational age at delivery was similar across groups (39–40 weeks).

There were 12 PTBs, 10 of which occurred in Black PWLWH (PTB rate of 18%). There was one stillbirth in the Black PWLWH group. There were no PTBs in the White PWLWH and one each in PWWH ethnic subgroups.

Among PWLWH, CD4 cell counts at the first antenatal visit were comparable between Black and White women (541 vs. 676 cells/$mm^3$, p = 0.52), and detectable plasma HIV viral loads were observed in 10/55 Black and 1/15 White women (p = 0.43) (**Table 2**). One of fifteen (6.7%) White PWLWH started ART during pregnancy, whereas 15/55 (27.3%) Black PWLWH initiated ART during pregnancy.

## Table 1: Baseline characteristics by ethnicity and HIV status

Percentages by group | P values from Kruskal–Wallis/Fisher's tests | Missing data only in the Black PWLWH group

| Summary by Group | Black PWLWH (n=55) | Black PWWH (n=14) | White PWLWH (n=15) | White PWWH (n=19) | P Value | Missing data, n |
|---|---|---|---|---|---|---|
| Median maternal age (years) (IQR)[1] | 36.00 (31.00–38.00) | 30.00 (23.25–33.75) | 31.00 (29.00–35.50) | 33.00 (29.00–34.00) | 0.003 | 0 |
| Median baseline body mass index (weight/height^2) (IQR)[2] | 27.91 (23.41–30.30) | 27.05 (22.86–29.68.53) | 24.22 (21.56–34.20) | 22.23 (20.95–23.98) | 0.004 | 3 |
| Median gestational age at visit (weeks) (IQR) | 21.00 (20.00–22.00) | 21.00 (20.00–22.75) | 20.00 (19.50–22.00) | 20.00 (20.00–21.00) | 0.51 | 0 |
| Median gestational age at delivery (weeks) (IQR) | 39.05 (37.82–40.02) | 39.10 (38.25–40.25) | 39.10 (38.50–40.05) | 40.00 (39.20–41.00) | 0.05 | 3 |
| Current alcohol use, n (%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | 0 |
| Current tobacco use, n (%) | 1 (1.8%) | 4 (28.6%) | 1 (6.7%) | 1 (5.3%) | | 0 |
| First pregnancy, n (%) | 15 (27.3%) | 4 (28.6%) | 8 (53.3%) | 8 (42.1%) | 0.23 | 0 |
| ≥1 prior pregnancy, n (%) | 40 (72.7%) | 10 (71.4%) | 7 (46.7%) | 11 (57.9%) | 0.23 | 0 |
| Preterm birth (< 37 weeks gestational age at delivery), n (%) | 10 (18.2%) | 1 (7.1%) | 0 (0%) | 1 (5.3%) | | 3 |
| Experienced obstetric complication, n (%) | 29 (52.7%) | 8 (57.1%) | 8 (53.3%) | 5 (26.3%) | 0.15 | 5 |

[1] Age was significantly different between Black PWLWH and PWWH (p = 0.02) and White PWWH (p = 0.02)

[2] From height/weight recorded at initial visit (14-15 weeks); White PWWH and Black PWLWH (p = 0.002)

## Table 2: Baseline characteristics of women living with HIV by ethnicity

Percentages by group | P values from Kruskal–Wallis/Fisher's tests | Missing data only in the Black PWLWH group

| Clinical Measurements | Black (n=55) | White (n=15) | P Value | Missing data, n |
|---|---|---|---|---|
| Median CD4 cell count (cells/mm$^3$) (IQR) | 541.00 (471.50–763.00) | 676.00 (518.00–769.50) | 0.52 | 0 |
| Detectable HIV (> 50 copies/mL), n (%) | 10 (20.0%) | 1 (6.7%) | 0.43 | 5 |
| Started ART preconception, n (%) | 40 (72.7%) | 14 (93.3%) | 0.16 | 0 |
| Started ART postconception, n (%) | 15 (27.3%) | 1 (6.7%) | 0.16 | 0 |
| Median weeks since ART initiation (if not preconception) (IQR) | 3.00 (2.11–3.96) | 11.00 | | 0 |
| **ART Class** | | | | 0 |
| Integrase inhibitor, n (%) | 14 (25.4%) | 7 (46.67%) | 0.12 | NA |
| Nonnucleoside reverse transcriptase inhibitor, n (%) | 19 (34.6%) | 6 (40.00%) | 0.76 | NA |
| Protease inhibitor, n (%) | 19 (34.6%) | 2 (13.33%) | 0.20 | NA |
| Nucleoside reverse transcriptase inhibitor, n (%) | 3 (5.5%) | 0 (0.00%) | | NA |
| **ART Backbone** | | | | 1 |
| Emtricitabine/Tenofovir, n (%) | 33 (60.0%) | 11 (73.3%) | 0.39 | NA |
| Lamivudine/Abacavir, n (%) | 15 (27.3%) | 2 (13.3%) | 0.33 | NA |
| Emtricitabine/Tenofovir alafenamide, n (%) | 2 (3.6%) | 2 (13.3%) | 0.20 | NA |
| Lamivudine/Abacavir/Zidovudine, n (%) | 2 (3.6%) | 0 (0%) | | NA |
| Tenofovir/Lamivudine, n (%) | 1 (1.8%) | 0 (0%) | | NA |
| Abacavir/Lamivudine, n (%) | 1 (1.8%) | 0 (0%) | | NA |

**White PWWH have a less diverse vaginal microbiota when compared to every other subgroup, but no significant difference was detected between Black PWLWH and PWWH**

Chao1 alpha diversity (**Figure 1**) was significantly different between subgroups (p = 0.001). White PWWH had lower alpha diversity when compared to every other group (Black PWLWH: p = 0.002, Black PWWH: p = 0.002, White PWLWH: p = 0.007). There was no statistical difference in Chao1 by HIV status within women of Black ethnicity. Beta diversity (**Figure 2**) also differed between participant subgroups (p = 0.03) and was significantly different between Black PWLWH and White PWWH (p = 0.002).

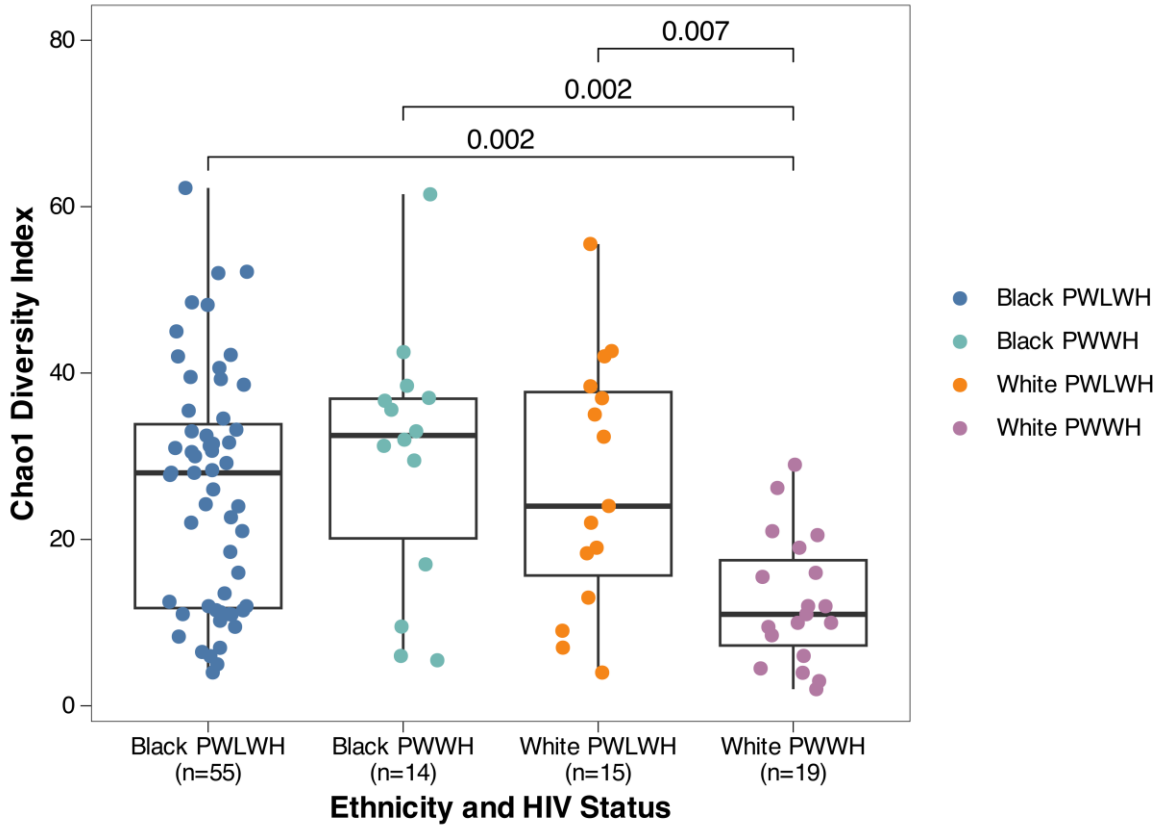



**Figure 1 - Chao1 alpha diversity across ethnicity and HIV status groups in pregnant women.** Boxplots show Chao1 alpha diversity estimates by subgroup. Points represent individual participants Only significant comparisons are shown and pairwise comparisons were adjusted via FDR correction. White PWWH had significantly lower Chao1 diversity compared to all other subgroups ($p \leq 0.002$). No significant differences were observed between the other subgroups

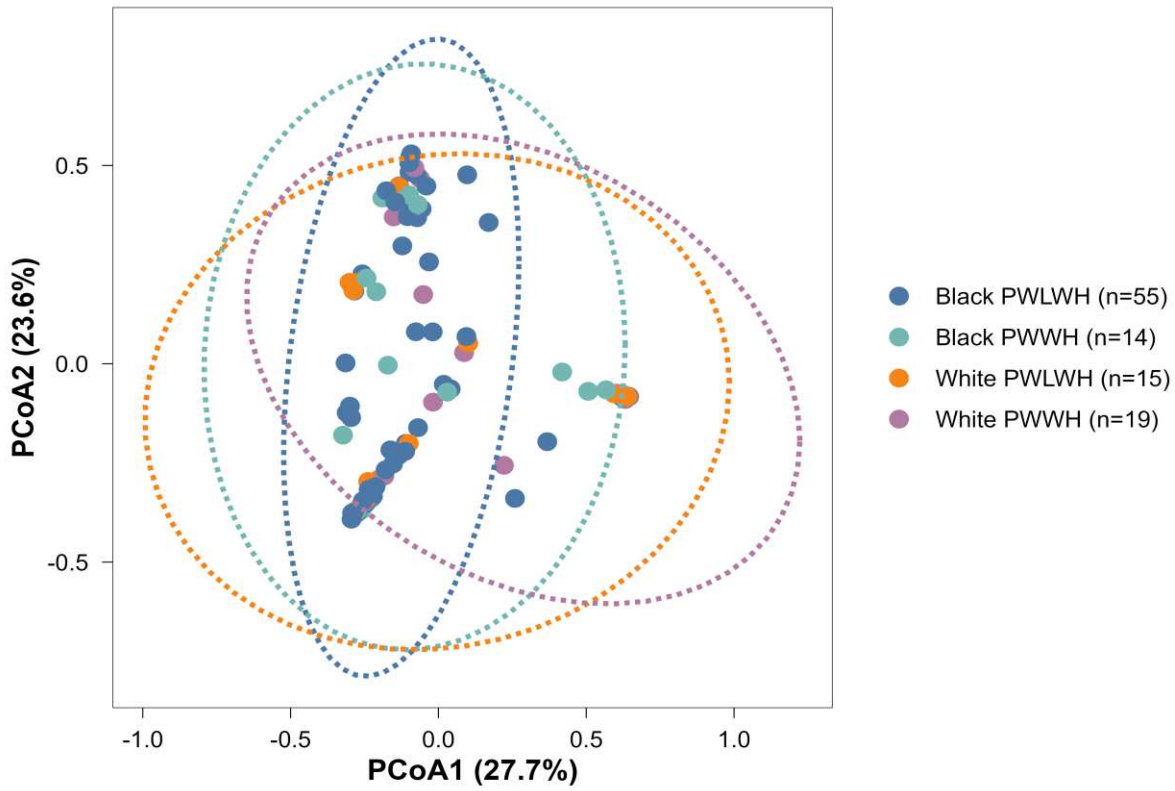



**Figure 2 - Beta diversity by ethnicity and HIV status during pregnancy.** Principal coordinate analysis of the beta diversity of the vaginal microbiota among pregnant women. Dotted ellipses represent 95% confidence ellipses around the group centroids. Overall group differences were assessed using PERMANOVA (p = 0.03), with pairwise comparisons adjusted using FDR correction. Significant differences were observed between Black PWLWH and White PWWH (p = 0.002).

## A high prevalence of *L. iners*-dominant CST III and mixed anaerobic CST IV in White PWLWH, Black PWLWH and PWWH compared to White PWWH, with CST IV-A restricted to Black Pregnant Women and CST IV-C to White PWWH

CST III was the predominant CST in White PWLWH (6/15, 40.0%), Black PWLWH (20/55, 36.4%), and Black PWWH (5/14, 35.7%) compared to 3/19 (15.8%) in White PWWH (**Figure 3**). CST IV (IV-A, IV-B, and IV-C) was slightly more common in Black pregnant women, with 5/14 (35.7%) in PWWH and 19/55 (34.6%) in PWLWH compared to 4/15 (26.7%) of White PWLWH (IV-B only) and 4/19 (21.1%) of White PWWH. CST IV-A (*G. vaginalis* and *Lachnospiraceae BVAB1*, recently renamed Ca. *Lachnocurva vaginae*) was only identified in only Black pregnant women, whereas

IV-C was observed in only White PWWH (n = 1). The largest proportion of White PWWH were assigned to CST I (9/19, 47.4%), whereas 4/15 (26.7%) of White PWLWH, 3/14 (21.4%) of Black PWWH, and 6/55 (10.9%) of Black PWLWH were assigned to CST I.

After adjusting for age and BMI, White PWWH had significantly greater odds of being in CST I+II+V than Black PWLWH (adjusted odds ratio (aOR): 6.20, 95% CI: 1.28–30.11, p = 0.02). No significant differences in the odds of CST I+II+V assignment were observed between any of the other subgroups.

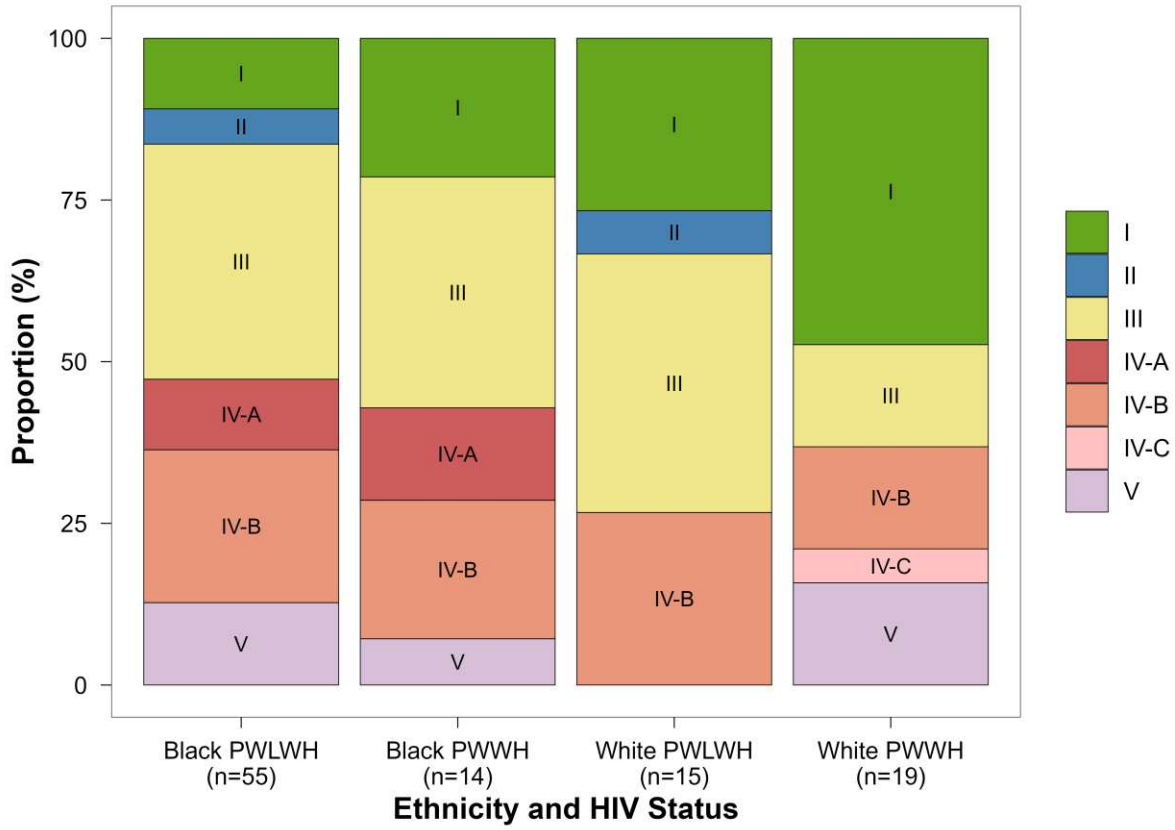



**Figure 3 - Proportion of CSTs by ethnicity and HIV status during pregnancy.** Stacked bar charts display the proportion of CST assignments (I, II, III, IV-A, IV-B, IV-C, and V) across the subgroups. CST I was most prevalent among White PWWH and least prevalent among Black PWLWH. High-diversity CST IV subtypes were prominent across all groups, particularly among Black and White PWLWH.

**Higher CD4 count increases the odds of optimal *Lactobacillus*-dominated CST (I, II or V) within PWLWH**

Among Black and White PWLWH, higher CD4 count trended toward increasing odds of CST I+II+V assignment (aOR 1.20, 95% CI 0.97–1.52; p = 0.10). After adjusting for detectable HIV, this association reached significance (aOR 1.39, 95% CI 1.06–1.89; p = 0.03), and every 100-cell increase in the CD4 count corresponded to an approximate 39% increase in the odds of CST I+II+V assignment. Detectable HIV did not independently influence the odds of CST I+II+V assignment. Gestational age at ART initiation, time since ART initiation, ART class, or ART backbone were not associated with CST assignment overall or with self-reported ethnicity.

**Compared with White pregnant women, Black pregnant women have a greater abundance of *Lachnospiraceae BVAB1* (Ca*. Lachnocurva vaginae*)**

Species-level differences were observed by HIV status, ethnicity, and combined HIV status and ethnicity (**Figure 4**). Pregnant Black women, regardless of their HIV status, appeared to have increased abundances of *Lachnospiraceae BVAB1* (Ca*. Lachnocurva vaginae*) and *G. vaginalis*. White PWWH displayed enrichment of *L. crispatus* and depleted *L. iners*, *L. gasseri*, and *Prevotella bivia* relative to the other groups. HIV status was associated with shifts in specific taxa: Black PWLWH had higher *L. jensenii* abundance than Black PWWH, whereas the opposite was observed in White pregnant women.

In terms of differential abundance, *L. jensenii* was more abundant in Black PWLWH when compared to Black PWWH (unadjusted p = 0.01, FDR-adjusted p = 0.24) (**Supplementary Figure 2**). *Lachnospiraceae BVAB1* (Ca*. Lachnocurva vaginae*) was most abundant in Black women. Specifically, it was significantly more abundant in Black PWWH than in White PWWH (unadjusted p < 0.001, FDR-adjusted p = 0.02)

and showed higher abundance in Black PWWH than in White PWWH (unadjusted p = 0.004, FDR-adjusted p = 0.07). This species was also enriched in Black PWLWH relative to White PWLWH (unadjusted p = 0.04, FDR-adjusted p = 0.58). *Megasphaera lornae* was likewise present at higher abundance in Black PWWH than in White PWWH (unadjusted p = 0.03, FDR-adjusted p = 0.50) and White PWLWH (unadjusted p = 0.02, FDR-adjusted p = 0.33).

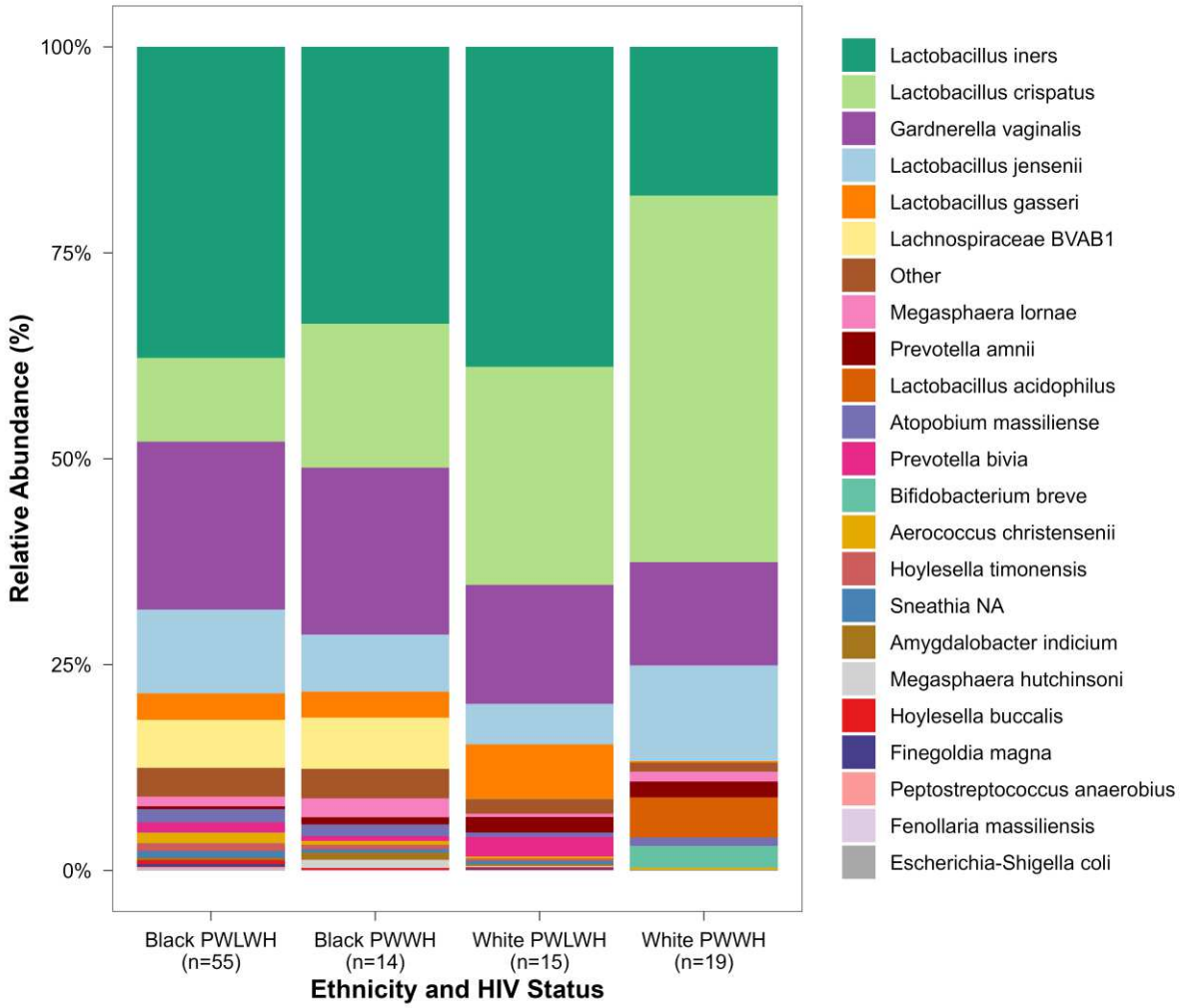



**Figure 4 – Relative abundance by ethnicity and HIV status during pregnancy.** Stacked bar charts display the proportion of species with more than 5% abundance in any given sample and are ordered by species total abundance (summed across all samples), with species arranged from most to least abundant. *L. crispatus* was notably more abundant in White PWWH, consistent with CST patterns observed in **Figure 3**.

**CVF cytokine concentrations were highest in Black women with no difference in HIV status, followed by White PWLWH, and lowest in White PWWH**

Across most measured cytokines, Black PWLWH and PWWH presented the highest concentrations, followed by White PWLWH and then White PWWH (**Figure 5**). Compared with White PWWH, Black PWWH had higher IL-10 ($p = 0.01$), IL-12p70 ($p = 0.002$), IL-13 ($p = 0.02$), IL-2 ($p = 0.005$), IL-4 ($p = 0.002$), IL-8 ($p = 0.02$), and TNF-α ($p = 0.006$).

White PWLWH had elevated IFN-γ ($p = 0.001$), IL-10 ($p = 0.005$), IL-12p70 ($p < 0.001$), IL-13 ($p < 0.001$), IL-2 ($p < 0.001$), IL-4 ($p < 0.001$), IL-6 ($p = 0.047$), IL-8 ($p = 0.007$), and TNF-α ($p = 0.006$) levels than White PWWH.

Black PWLWH had significantly higher IFN-γ ($p = 0.001$), IL-10 ($p < 0.001$), IL-12p70 ($p < 0.001$), IL-13 ($p < 0.001$), IL-2 ($p < 0.001$), IL-4 ($p < 0.001$), IL-8 ($p < 0.001$), and TNF-α ($p < 0.001$) compared to White PWWH. There were no significant differences in cytokine levels between Black PWLWH and White PWLWH, Black PWLWH and Black PWWH, or Black PWWH and White PWLWH (**Supplementary Table 1**).

After adjusting for maternal age and BMI, only IL-10, IL-12p70, IL-13, IL-2, IL-4, and TNF-α remained significantly higher in Black PWWH than in White PWWH (all $p < 0.04$). All cytokines previously identified as significant (IFN-γ, IL-10, IL-12p70, IL-13, IL-2, IL-4, IL-8, and TNF-α) remained significantly higher in Black PWLWH than in White PWWH (all $p < 0.02$).

Only IL-10, IL-12p70, IL-13, IL-2, IL-4, IL-8, and TNF-α remained significantly higher in White PWLWH than in White PWWH (all $p < 0.005$) (all p values are reported in **Supplementary Table 2**).

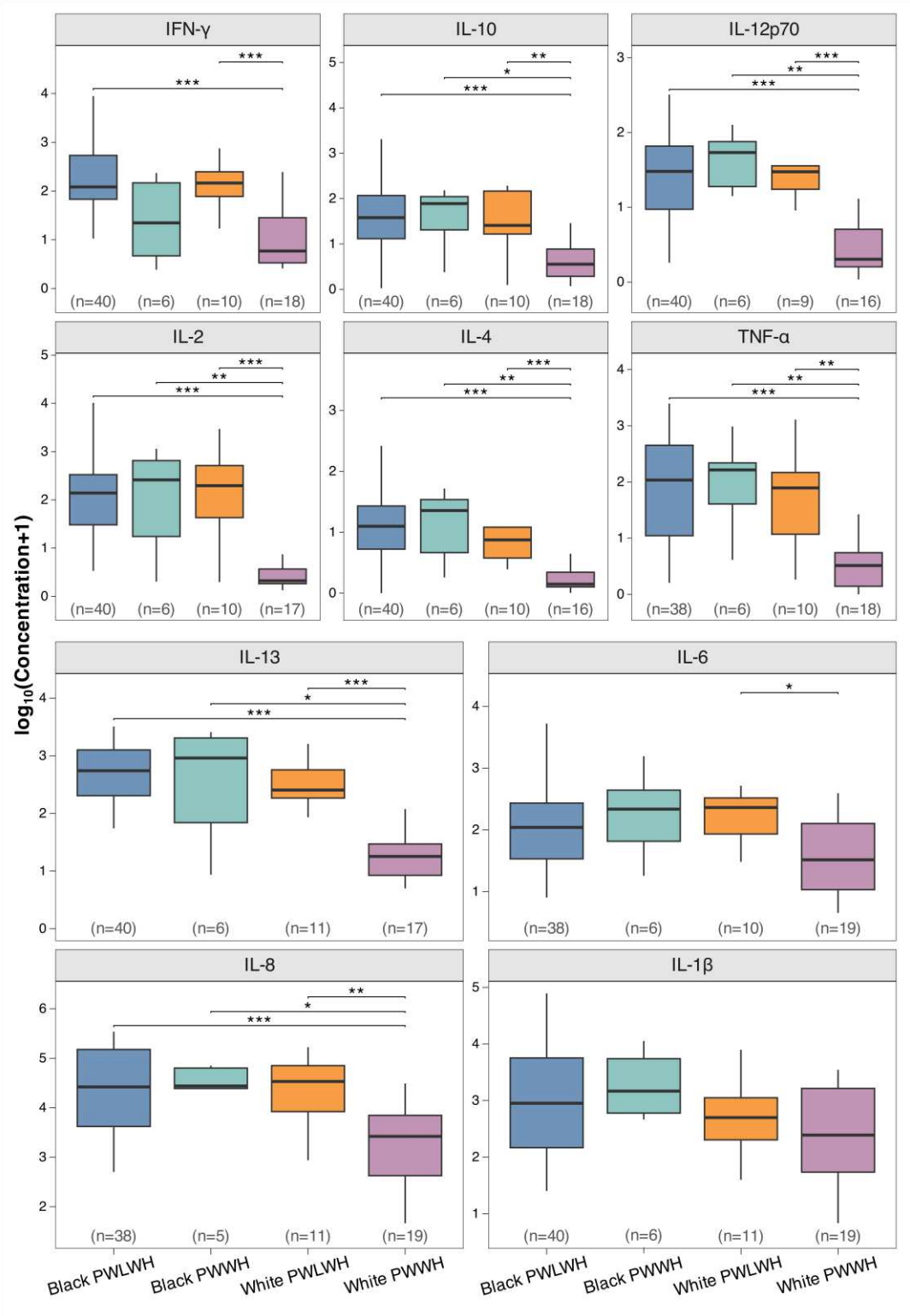



**Figure 5 - Cytokine concentrations by ethnicity and HIV status during pregnancy.** Boxplots show $\log_{10}$(concentration+1)-transformed cytokine concentrations. Pairwise comparisons were performed using Wilcoxon rank-sum tests and only FDR-significant comparisons are shown (all comparisons are provided in **Supplementary Table 1**). Asterisks indicate: $*$ $p < 0.05$, $**$ $p < 0.01$, and $***$ $p < 0.001$. Sample sizes for each cytokine are shown below the boxplots.

**HIV status in pregnant White women was associated with higher CVF inflammatory scores across optimal *Lactobacillus*-dominated CSTs (I+II+V) and *Lactobacillus* depleted CST IV**

PCA was used to explore the contribution of cytokine coexpression to CVF inflammation. PC1 explained 71.4% of the variance, and all cytokines contributed similarly to the loadings (0.26–0.34); thus, this measurement was utilized as a summative index of CVF inflammation.

Black PWLWH whose vaginal microbiota clustered into CST I+II+V had significantly more CVF inflammation (PC1) than White PWWH ($p < 0.001$, **Figure 6**), but Black PWWH did not (**Supplementary Figure 3**).

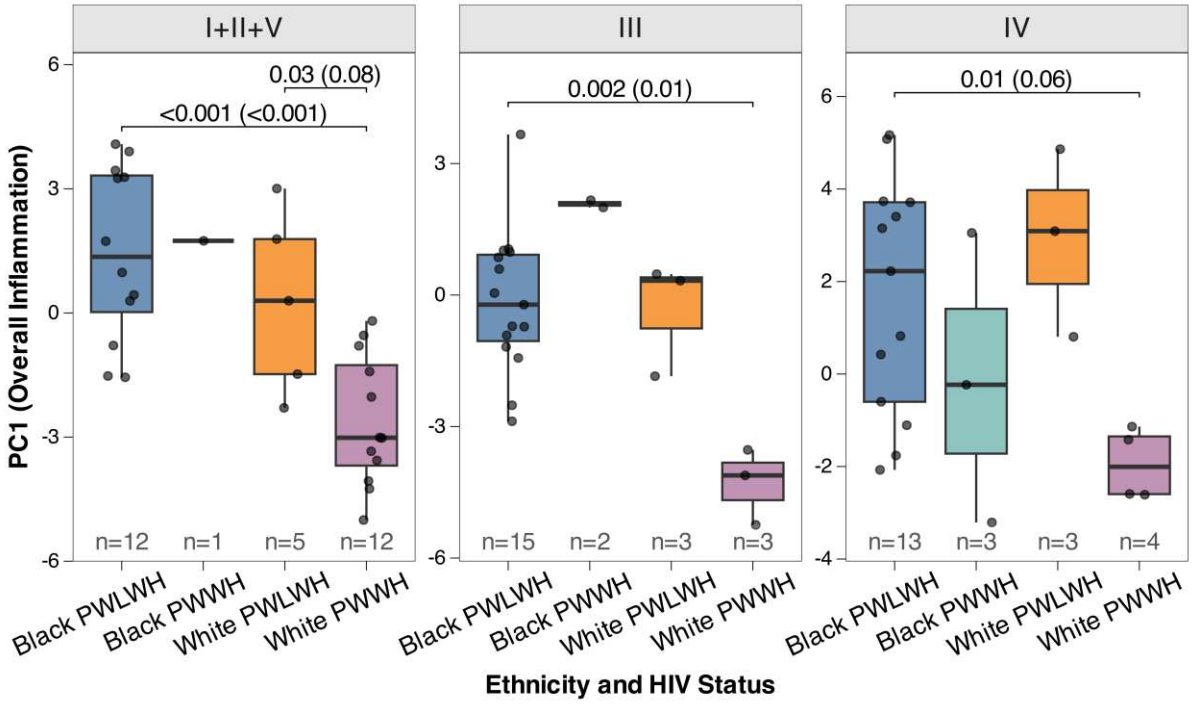



**Figure 6 - PC1 inflammation scores across ethnicity and HIV status groups stratified by CST grouping.** Boxplots show PC1 inflammation scores among pregnant women within the CST I+II+V, CST III, and CST IV groups. Pairwise comparisons were performed using Wilcoxon rank-sum tests. Only significant comparisons are shown, with unadjusted p-values displayed above each comparison and FDR-adjusted p-values in parentheses. Full p values are included in **Supplementary Figure 3**. Sample sizes varied across CST-defined subgroups and are indicated below each boxplot.

Within CST I+II+V, pairwise comparisons of cytokines revealed that the levels of IL-10 ($p < 0.001$), IL-12p70 ($p = 0.001$), IL-13 ($p < 0.001$), IL-2 ($p < 0.001$), IL-4 ($p < 0.001$), and TNF-α ($p = 0.01$) were significantly higher in Black PWLWH than in White PWWH (**Supplementary Table 3**). White PWLWH also trended toward higher PC1 values than White PWWH ($p = 0.03$, FDR-adjusted $p = 0.08$) (**Figure 6**), and specifically, higher IL-4 ($p = 0.04$) levels were observed ($p = 0.06$).

Within CST III, Black PWLWH exhibited greater CVF inflammation than White PWWH ($p = 0.01$) (**Figure 6**), with higher IL-13 ($p = 0.01$), IL-2 ($p = 0.03$), and IL-8 ($p = 0.03$) levels (**Supplementary Table 4**).

Within CST IV, PC1 appeared elevated in PWLWH regardless of ethnicity, but Black PWLWH trended toward greater inflammation than White PWWH ($p = 0.06$) (**Figure 6**). Regarding individual cytokines, Black PWLWH had higher IL-2 ($p = 0.005$), IL-13 ($p = 0.005$), and TNF-α ($p = 0.03$) levels than White PWWH (**Supplementary Table 5**).

White PWWH presented the lowest cytokine concentrations across CST-stratified and overall analyses. A significant moderate relationship between PC1 and Chao1 diversity ($\rho = 0.41$, $p < 0.001$; **Supplementary Figure 4**) was observed overall. Within the CST groups, this relationship was strongest in CST IV ($\rho = 0.47$, $p = 0.02$), trending in CST III ($\rho = 0.39$, $p = 0.06$), and not significant in CST I+II+V ($\rho = 0.21$, $p = 0.26$) (**Supplementary Figure 5**).

No significant relationships were detected between PC1 and maternal age ($\rho = 0.12$, $p = 0.31$), BMI ($\rho = 0.06$, $p = 0.61$), CD4 count ($\rho = -0.13$, $p = 0.35$), or HIV viral load ($\rho = 0.08$, $p = 0.59$) (**Supplementary Figures 6-7**).

**Black PWLWH had lower composite CVF inflammatory scores in *L. iners*-dominated CST III than other CSTs**

Among Black PWLWH, CVF inflammation tended to be greater in CSTs I+II+V and CST IV than in CST III. CSTs I+II+V and IV were not significantly different from each other (p = 0.89) (**Figure 7**). There were no significant differences in CVF inflammation by CST in Black PWWH (**Supplementary Figure 8**). Among White PWLWH, there was a nonsignificant trend (unadjusted p = 0.10, FDR-adjusted p = 0.21) toward greater CVF inflammation in CST IV than in CSTs I+II+V and III (**Supplementary Figure 8**). White PWWH observed a similar trend to Black PWLWH, with higher CVF inflammation in CST I+II+V (unadjusted p = 0.10, FDR-adjusted p = 0.15) and CST IV (unadjusted p = 0.06, FDR-adjusted p = 0.15) compared to CST III.

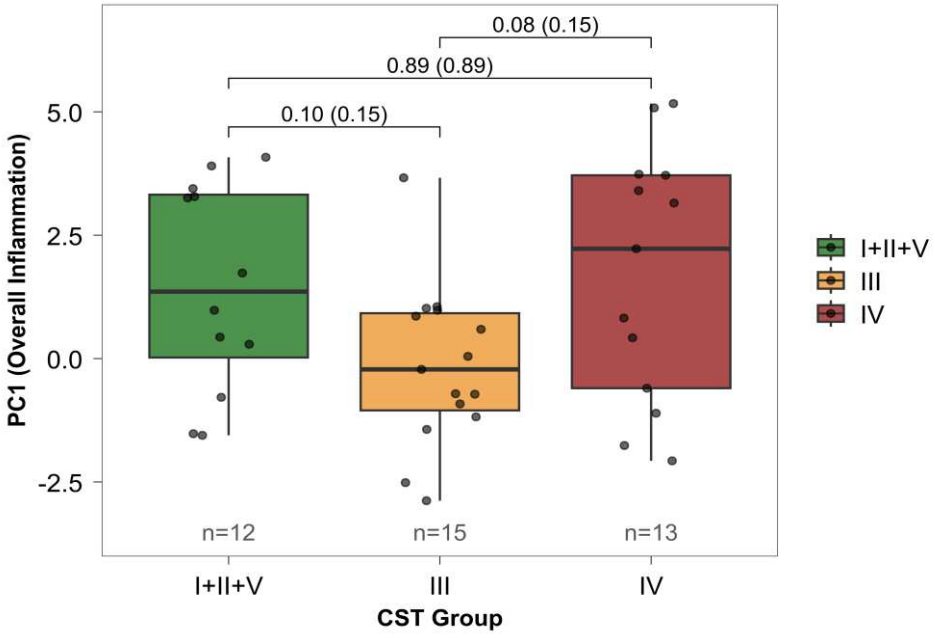



**Figure 7 - PC1 scores by CST group in Black PWLWH.** Boxplots showing PC1 inflammation scores among Black PWLWH within CST I+II+V, CST III, and CST IV groups. Pairwise comparisons were performed via Wilcoxon rank-sum tests and unadjusted p values with FDR-adjusted values in parentheses are presented above each comparison. The sample size varied by CST within subgroups and is noted below each box. CST I+II+V and IV both trended toward having increased PC1 when compared to CST III but were not significantly different from each other.

**Nearly half of Black PWLWH experienced a CST change during pregnancy and were more likely to transition CSTs than every other subgroup combined**

Ninety-five women included in the cross-sectional analysis provided an additional sample at an average gestational age of 28.01 weeks or 7.18 weeks after their mid-second trimester visit (**Supplementary Figure 9**). Weeks between visits were not significantly different between subgroups (**Supplementary Table 6**). Overall, 36/95 women (37.9%) changed CST between timepoints. CST proportions were largely consistent across timepoints, with a maximum loss or gain of two women (**Figure 8**).

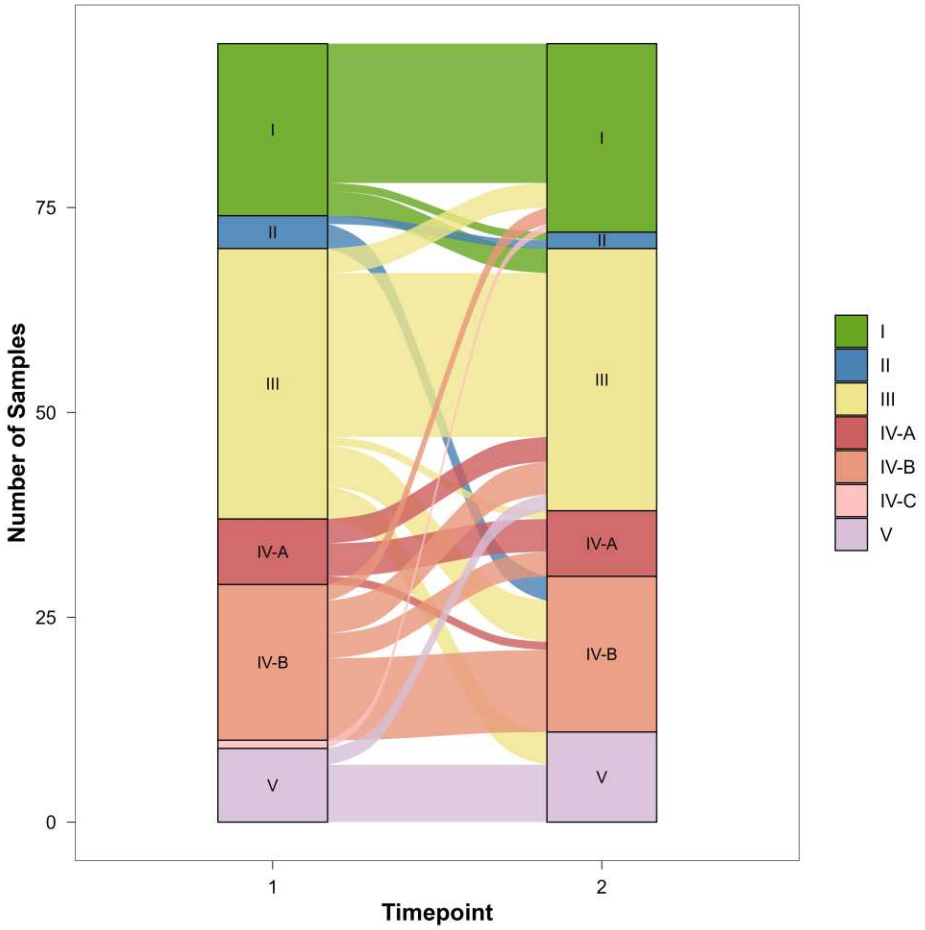



**Figure 8 - CST change from the middle-second trimester to the early-third trimester.** Two stacked bar plots show the count and proportion of each CST by timepoint (1 = 20.84-week average, 2 = 28.01-week average) in the overall group of pregnant women who provided multiple samples. Alluvial lines trace each woman's CST from the timepoint, enabling visualization of CST changes and stability**.** 36/95 women (37.9%) changed CST, but CST proportions were largely consistent across timepoints.

When stratified by subgroup (**Figure 9**), 24/49 (49.0%) of Black PWLWH experienced a CST change, with most transitions (15/24, 62.5%) occurring between CST III and CST IV (9/24, 37.5%) or into CST III or IV from CST I+II+V (6/24, 25.0%). Two of 14 (14.3%) Black PWWH exhibited a CST change. Five of the 14 (35.7%) White PWLWH changed CSTs. Among White PWWH, 5/18 (27.8%) moved CSTs, and 3/5 (60.0%) of these changes were from CSTs III+IV to CSTs I+II+V.

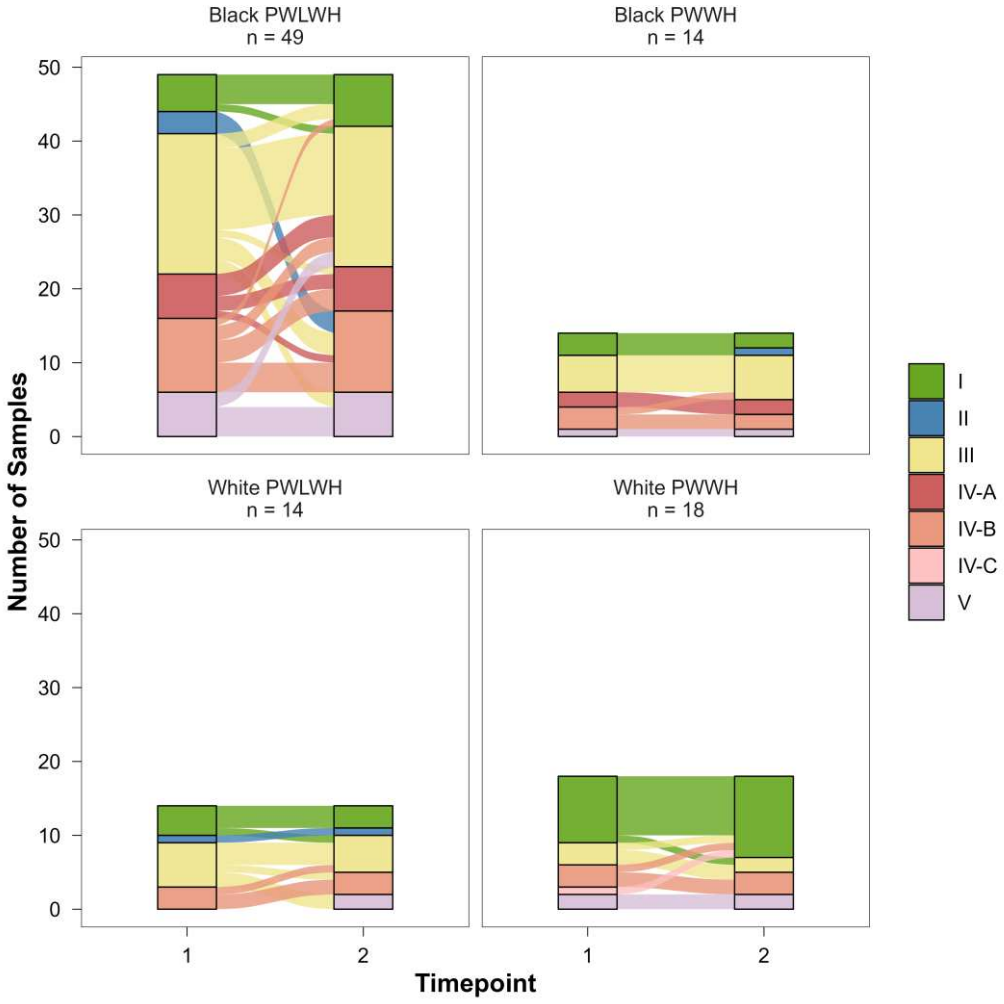



**Figure 9 - CST change throughout pregnancy by ethnicity and HIV status.** Two stacked bar plots in each subgroup show the count and proportion of each CST of pregnant women who provided multiple samples at 20.84 weeks (average) and 28.01 (average) weeks. Alluvial lines trace each woman's CST from the timepoint, enabling visualization of CST change and stability**.** 24/49 (49.0%) Black PWLWH experienced a CST change. Two of 14 (14.3%) Black PWWH exhibited a CST change. Five of the 14 (35.7%) White PWLWH changed CSTs, and 5/18 (27.8%) of White PWWH changed CSTs.

When Black PWWH, White PWLWH, and White PWWH were combined to create a comparable sample size, Black PWLWH had significantly greater odds of CST change than the combined group (aOR 2.53, 95% CI 1.02–6.52; p = 0.048). There were no significant pairwise comparisons when the subgroups were evaluated individually. In a model that considered ethnicity and HIV status individually, ethnicity was not associated with CST change. However, PWLWH trended toward higher odds of CST change compared to PWWH (aOR 2.86, 95% CI 1.01–8.93; p = 0.06). Descriptive patterns of CST stability by subgroup (**Table 3**) were consistent with models that included CST at timepoint 1 and combined HIV status and ethnicity, as there was no evidence of their interacting effect on CST stability. When considering CSTs at either timepoint, White PWWH had greater odds of having CST I+II+V at either timepoint than Black PWLWH (aOR: 9.85, 95% CI: 1.63–59.41; p = 0.01) and Black PWWH (aOR: 10.88, 95% CI: 1.21–97.67; p = 0.03).

**Table 3: CST Stability by Ethnicity and HIV Status**

Counts (%) of stable and changed CSTs by CST at Timepoint 1

| | I | | II | | III | | IV | | V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Stable | Changed | Stable | Changed | Stable | Changed | Stable | Changed | Stable | Changed |
| Black PWLWH | 4 (80.0%) | 1 (20.0%) | 0 (0%) | 3 (100%) | 11 (57.9%) | 8 (42.1%) | 10 (62.5%) | 6 (37.5%) | 4 (66.7%) | 2 (33.3%) |
| Black PWWH | 2 (66.7%) | 1 (33.3%) | 0 (0%) | 0 (0%) | 5 (100%) | 0 (0%) | 4 (80%) | 1 (20%) | 1 (100%) | 0 (0%) |
| White PWLWH | 3 (75.0%) | 1 (25.0%) | 1 (100%) | 0 (0%) | 3 (50.0%) | 3 (50.0%) | 2 (66.7%) | 1 (33.3%) | 0 (0%) | 0 (0%) |
| White PWWH | 8 (88.9%) | 1 (11.1%) | 0 (0%) | 0 (0%) | 1 (33.3%) | 2 (66.7%) | 2 (50%) | 2 (50%) | 2 (100%) | 0 (0%) |

**The majority of Black PWLWH who experienced PTB had a vaginal microbiota assigned to CST III or IV at their initial second trimester visit, with differential abundance analyses revealing unique discriminatory anaerobic species, including *Hoylesella buccalis* and *Hallella colorans***

Black PWLWH accounted for most PTBs in this cohort (10 of 12, 83.3%). Among Black PWLWH who provided a sample at the initial timepoint, 10/55 (18.2%) delivered preterm. Subsequent analyses compared the microbiota of women who gave birth preterm and at term within this group only. Seven of the ten PTBs in Black PWLWH were spontaneous. Three were iatrogenic: one due to preeclampsia at 34 weeks, one due to emergency cesarean section for reduced fetal movements at 36 weeks, and one due to emergency cesarean section at 34 weeks for fetal distress.

Among the PTBs that occurred in Black PWLWH, four were to women whose vaginal microbiota was assigned to CST III, five were in CST IV, and one was in CST V at the initial timepoint during the middle second trimester (mean 20.96 weeks, **Supplementary Figure 9**). Gestational age at delivery showed modest but significant negative correlations with gravidity ($\rho = -0.30$, $p = 0.03$) and BMI ($\rho = -0.29$, $p = 0.04$). However, PTB was not associated with gravidity (first pregnancy vs second or more), BMI (overweight vs not). All other associations with PTB or gestational age at delivery were not significant.

Differential abundance (**Figure 10**) of Black PWLWH who gave birth preterm versus at term revealed that *H. buccalis* (unadjusted $p < 0.001$, FDR-adjusted $p = 0.002$), *H. colorans* (unadjusted $p < 0.001$, FDR-adjusted $p = 0.01$), *Gemelliphila asaccharolytica* (unadjusted $p = 0.03$, FDR-adjusted $p = 0.16$), and *Peptoniphilus lacrimalis* (unadjusted $p = 0.05$, FDR-adjusted $p = 0.19$) were more abundant in PTBs. *Aerococcus* species (unadjusted $p = 0.004$, FDR-adjusted $p = 0.04$), *P. disiens*

(unadjusted p = 0.007, FDR-adjusted p = 0.05), and *L. crispatus* (unadjusted p = 0.04, FDR-adjusted p = 0.19) were more abundant in term births.

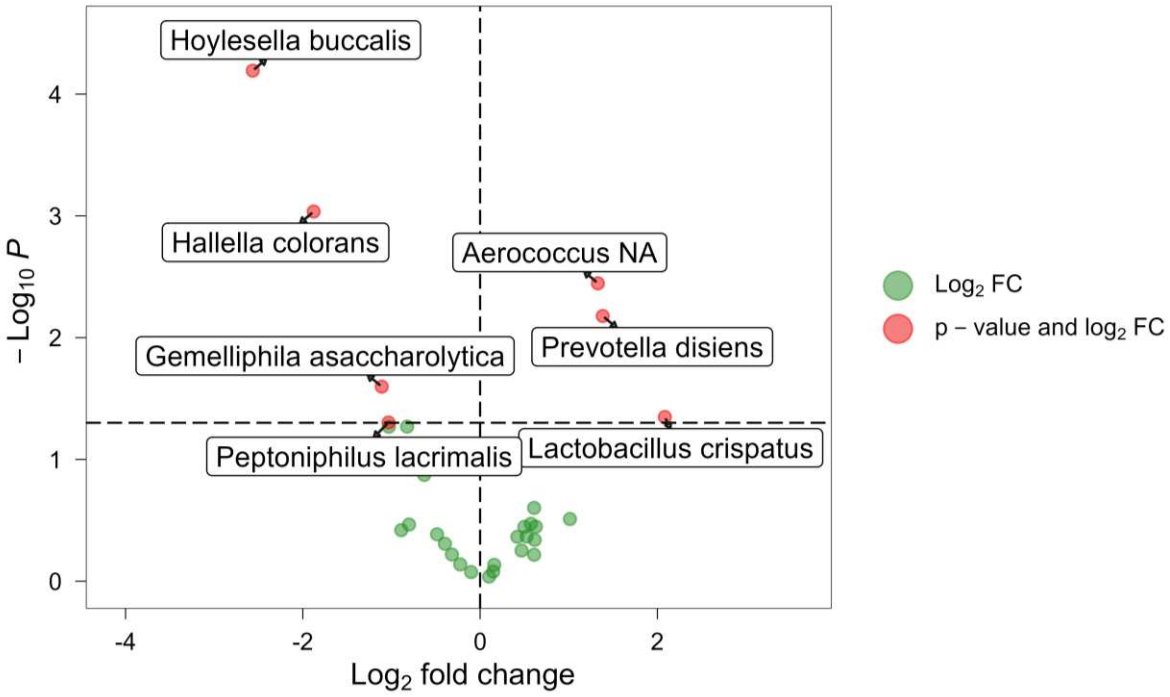



**Figure 10. Differential abundance by birth outcome in Black PWLWH.** This volcano plot displays the species that were identified to be more abundant in Black PWLWH who gave birth at term (negative log2-fold change) and preterm (positive log2-fold change) taken from the trimester 2 (20-21 weeks cross section). Each dot represents a species. The green points indicate a fold change difference that was not significant, whereas the red points that are labeled with the species name have a significantly different fold change (unadjusted p < 0.05). Anaerobes such as *Hoylesella buccalis* (unadjusted p < 0.001, FDR-adjusted p = 0.002) and *Hallella colorans* (unadjusted p < 0.001, FDR-adjusted p = 0.01) were identified as being significantly more abundant in women who gave birth preterm.

**Black PWLWH who gave birth preterm trended toward higher odds of CST change but were significantly more likely to transition between CSTs III and IV**

All the Black PWLWH who gave birth preterm provided a second sample, enabling observation of CST dynamics during pregnancy. Two transitioned from CST III to IV, three from CST IV to CST III, and one from CST III to CST V. Three women remained in CST IV throughout the timepoints, and one woman stayed in CST III.

CST change trended toward increasing odds of PTB (aOR 3.50, 95% CI 0.82-18.46; p = 0.10). There was no significant effect of transitioning from CST I+II+V to III+IV or from CST III+IV to CST I+II+V. Transitioning between CST III+IV was associated with an increased risk of PTB (aOR 7.75, 95% CI 1.57-42.75; p = 0.01), but the sequence of CST III and IV did not affect the risk.

## Discussion

This new analysis of the UHPBN study leverages a uniquely structured cohort enriched for PWLWH and matched by self-reported ethnicity, enabling investigation of the interplay between the vaginal microbiota, inflammation, and PTB in a larger, well-characterized cohort. This study design also permits stratified analyses by both HIV status and ethnicity simultaneously**,** rather than examining these factors in isolation. Our longitudinal design, with near-complete repeat sampling across pregnancy, further enables detailed characterization of microbiota dynamics over time across these intersecting groups. Finally, the availability of comprehensive birth outcome data for most participants, combined with high-resolution long-read 16S rRNA gene sequencing, provides a detailed and reliable assessment of bacterial taxonomy in relation to pregnancy outcomes.

Within our unique cohort, we observed that the vaginal bacterial composition, CST distribution, and local inflammation varied by both HIV infection status and self-identified ethnicity. Bacterial (alpha and beta) diversity and inflammation were highest among Black women regardless of HIV status and decreased from White PWLWH to White PWWH, with differences by HIV status being more pronounced between White PWLWH to White PWWH. These patterns were accompanied by differences in CST composition and stability, with potential implications for PTB risk.

595 Consistent with prior research, Black pregnant women, regardless of their HIV status,
596 presented greater relative abundances of *G. vaginalis* and *Lachnospiraceae BVAB1*
597 (Ca. *Lachnocurva vaginae*) than White pregnant women [14, 16]. Accordingly, CST IV-
598 A, characterized by dominance of *G. vaginalis* and *Lachnospiraceae BVAB1*
599 (Ca*. Lachnocurva vaginae*), was identified exclusively among these subgroups of our
600 cohort.
601 Compared with White PWWH, Black PWWH had significantly greater alpha diversity
602 and a greater proportion of CSTs III and IV, which aligns with the findings of previous
603 studies that compared vaginal microbiota composition by ethnicity [3, 14, 19].
604 Among Black women, Chao1 alpha diversity was slightly higher in PWLWH than in
605 PWWH, but both groups had similar proportions of CSTs III and IV. This result differs
606 from those of studies in Malawi [62], Zambia [46], and Zimbabwe [36], where PWLWH
607 showed higher alpha diversity and greater abundance of anaerobe-rich CSTs than
608 PWWH. Our previous multiethnicity UK-based study also reported greater alpha
609 diversity and prevalence of CST III and IV in PWLWH compared to a reference control
610 group of PWWH using short read 16S rRNA gene sequencing [4]. Our new findings
611 are similar to those of a study of nonpregnant Black and White American women that
612 revealed only modest differences in the microbiota by HIV status in Black women [63].
613 The authors reported that BV prevalence was greater among African American women
614 in their cohort and that while BV and HIV status were correlated in all ethnic groups,
615 this association was weakest in the African American subcohort [63]. In our cohort,
616 Black PWLWH and PWWH had nearly identical proportions of CST IV (34.5% vs
617 34.7%), which broadly corresponds to BV incidence (estimated to be 23% in Europe)
618 [23, 24]. Taken together, these findings lead us to conclude that other unmeasured

619 factors may have played a greater role than HIV status alone in shaping the microbiota
620 of Black pregnant women in our UK study.
621 To our knowledge, this is one of the first studies to characterize the vaginal microbiota
622 in White pregnant women stratified by HIV status. The elevated alpha diversity and
623 increased prevalence of CSTs III and IV in White PWLWH compared with White
624 PWWH are consistent with our prior report comparing PWLWH vs PWWH, which was
625 not stratified by ethnicity [4]. Replication of these findings in a subgroup representing
626 the majority ethnicity of the UK population, in whom there may be fewer confounding
627 risk factors for PTB, provides additional evidence supporting a role for HIV infection in
628 driving vaginal dysbiosis
629 Higher CD4 counts in PWLWH were associated with greater odds of assignment to
630 *Lactobacillus*-dominated CSTs I+II+V during the second trimester, irrespective of
631 ethnicity, which provides further weight to the hypothesis that immune restoration has
632 a protective effect on maintaining low diversity. This finding concurs with previous
633 findings that lower CD4 counts are associated with BV [35, 64]. Although previous
634 research has identified a link between HIV viral load, increased bacterial diversity, and
635 BV, we did not observe a relationship between viral load and CST assignment [63, 65].
636 However, in our cohort, most women (77%) began ART preconception, and only 16%
637 had detectable viral loads at screening, which may have limited our ability to detect
638 associations.
639 Analysis of individual cytokine concentrations revealed patterns consistent with alpha
640 diversity trends: Black pregnant women presented the greatest degree of inflammation
641 irrespective of HIV status, followed by White PWLWH and then White PWWH. Prior
642 studies have identified associations between bacterial diversity, CST IV, and
643 inflammation in PWLWH [4, 36]. The comparable cytokine concentrations between

644 Black PWLWH and Black PWWH in our study likely reflect their similar alpha diversity
645 and CST III and IV proportions. This finding contrasts with that of a Zimbabwean cohort
646 of 42 PWLWH, matched 7:1 with PWWH, who did not observe a difference in
647 inflammation by HIV status or an association with alpha diversity despite a higher
648 prevalence of both CST IV and PTB in PWLWH, suggesting a role of other confounding
649 factors [46].
650 Exploration of vaginal mucosal cytokines in PWLWH and PWWH highlighted that IFN-
651 γ levels were higher in PWLWH compared with PWWH in both Black and White
652 participants, but this difference reached statistical significance only among White
653 women (White PWLWH vs PWWH p = 0.001; Black PWLWH vs PWWH p = 0.28,
654 **Supplementary Table 1**). This pattern likely reflects HIV-associated mucosal immune
655 activation, although the weaker signal in Black women may reflect sample size or
656 background inflammatory differences [66]. In contrast, a trend toward lower IL-1β
657 concentrations in White PWWH than in Black PWWH (p = 0.11, **Supplementary Table**
658 **1**), but no difference in HIV status was observed. In our previous analyses, IL-1β was
659 associated with vaginal microbiota diversity and *G. vaginalis* in PWLWH [4], a finding
660 that was replicated by others [36].
661 PC1-derived inflammation analyses were underpowered, particularly in PWWH;
662 however, overall inflammation patterns persisted. When analyzed by CST, Black and
663 White PWLWH exhibited similar levels of inflammation, whereas White PWWH had
664 lower levels. Alpha diversity was significantly associated with PC1 overall, but this
665 relationship persisted only in CST IV (with a trend in CST III). This finding led us to
666 conclude that inflammation in anaerobe-dominant communities could be diversity
667 driven. However, this conclusion requires confirmation using models *in vivo*.

668 The Black PWLWH in our study that were assigned to CSTs I+II+V and CST IV tended
669 to have greater inflammation than those who were in CST III. In contrast, the Zambian
670 Preterm Birth Prevention Study reported greater inflammation in the anaerobe-
671 dominant CST IV compared with *Lactobacillus*-dominant CSTs among PWLWH [36].
672 Our observations may be influenced by small sample sizes or host-driven immune
673 effects. These differences may also result from the PC1 methodology, which captured
674 shared variance across cytokines rather than individual concentrations. In spite of this,
675 the lower inflammation observed in CST III in this group is an interesting finding given
676 the ongoing debate about the protective role of *L. iners,* particularly in women for whom
677 it is a prevalent CST [67].
678 Compared with all the other subgroups combined, Black PWLWH had higher odds of
679 CST transition during pregnancy, but the pairwise comparisons were not significant,
680 likely due to limited power. Prior studies have reported higher transition rates among
681 Black pregnant women regardless of HIV status, but statistical significance was not
682 achieved, suggesting that the observed transitions may reflect the baseline CST
683 distribution [16, 19]. Indeed, our previous analysis, which was limited to PWLWH,
684 revealed that CST instability was highest in women with a microbiota dominated by
685 the coexistence of both *L. iners* and *G. vaginalis* (67% transition) than in those with
686 zero transitions in the *L. crispatus*-dominant group [4].
687 PTB was highest in Black PWLWH (18.2%), followed by Black PWWH and White
688 PWWH (one case in each group - 7.1% and 5.3%, respectively), and no cases were
689 reported in White PWLWH. Although the small subgroup sizes limits interpretation,
690 these rates among PWWH are consistent with UK population data, where a recent
691 study of over 1 million births reported slightly higher PTB rates among Black compared
692 with White women (6.0% vs 5.7%) [47]. However, after accounting for socioeconomic

693 deprivation, the authors found that ethnic differences in PTB risk were attenuated,
694 suggesting that broader structural and social inequalities may contribute to these
695 disparities. The authors further proposed that residual differences may reflect factors
696 not captured within routine datasets, including structural racism, practitioner bias,
697 discrimination-related stress, and inequities in healthcare access and support services
698 [47].
699 Within PWLWH, HIV-related clinical factors may have contributed to the different PTB
700 rates. A greater proportion of Black PW received protease inhibitor-based regimens
701 and had a detectable viral load at screening. Both factors have previously been
702 associated with PTB [38, 39, 50, 68, 69]. Unfortunately, the heterogeneous ART
703 exposure in the prospective cohort limits the power of analyses to demonstrate
704 differences according to the timing of treatment, regime type, and presence of
705 detectable viremia.
706 Among Black PWLWH, most PTBs occurred in women classified as having CST III or
707 IV at the middle–second trimester, which is consistent with our previous findings
708 across PWLWH [4]. *L. crispatus* was more abundant in term births, which aligns with
709 studies within communities of varying ethnicities and HIV statuses [4, 14, 25].
710 Anaerobic taxa, including *H. colorans*, *H. buccalis*, *Aerococcus* spp., and *P. disiens*,
711 were observed variably, underscoring the complex, multifactorial bacterial
712 contributions to birth outcomes [4, 14, 70].
713 In concordance with existing research, we observed no significant association
714 between overall CST stability versus transition and PTB risk [62, 71]. However, Black
715 PWLWH who transitioned specifically between CST III and IV had higher odds of PTB
716 compared with those with other transitions or stable CSTs. This finding supports
717 previous studies linking *L. iners*/CST III and CST IV with PTB [4, 71], as well as more

718 recent evidence showing no association between CST transition overall and PTB risk
719 [62]. However, albeit in a cohort of PWWH who initiated pre-exposure prophylaxis
720 during pregnancy, this same recent study identified increased PTB risk among women
721 who transitioned from non-CST IV -- most commonly CST III -- to CST IV during
722 pregnancy [62]. Taken together, these findings allow us to conclude that transitions
723 involving CST IV, particularly from CST III to CST IV, may be associated with increased
724 PTB risk rather than CST instability more broadly.
725 This study has several limitations. Despite the inclusion of additional matched controls
726 in the White PWLWH and Black PWWH groups, the cohort was skewed toward Black
727 PWLWH. Compared with White pregnant women, Black pregnant women had higher
728 BMIs, and Black PWLWH were significantly older than PWWH. Similarly, gravidity was
729 slightly higher among Black pregnant women. Although age and BMI were included as
730 covariates, residual confounding is possible. The observational design, small
731 subgroup sizes, and incomplete cytokine data reduced the statistical power,
732 particularly for inflammation, CST change, and PTB analyses.
733 The UK cohort, which was derived from a high-income country, potentially limits
734 generalizability to other populations. However, recruitment sites served
735 socioeconomically heterogeneous London boroughs, some of which rank among the
736 more deprived nationally according to the English Indices of Deprivation [72]. These
737 indices measure income, employment, health, crime, barriers to housing and services,
738 and the living environment by area [72]. Contemporaneous residential postcode data
739 were unavailable, so area-level deprivation could not be evaluated at the participant
740 level. Exploratory considerations of borough-level deprivation indices based on clinic
741 location revealed that clinics recruiting PWLWH were in local authorities with greater
742 average deprivation than the clinic recruiting PWWH, which may explain our limited

differences in HIV status in Black women. Deprivation scores did not differ significantly between sites recruiting Black and White PWLWH groups, which could reflect shared sociodemographic factors that influence the microbiota and why differences between these groups were moderate. However, as these estimates correspond to clinic locations rather than participant residences, they should be interpreted cautiously.

This study originally sought to investigate differences solely by HIV status and therefore did not collect sociodemographic and behavioral data (e.g., douching, exposure to xenobiotics, and education) that recent research has identified as relevant to vaginal microbiota ecology and PTB risk [25, 73, 74]. Accordingly, the variation in CST distribution, alpha diversity, inflammatory markers, and PTB observed in this cohort may be influenced by unmeasured structural and behavioral determinants rather than intrinsic biological differences between ethnic groups.

## Conclusions

To our knowledge, this is the first UK study to examine vaginal taxonomic and inflammatory dynamics during pregnancy in a cohort stratified by self-identified ethnicity and HIV status. The UHPBN study represents one of the largest pregnancy cohorts enriched for women with HIV and examines the relationships among the vaginal microbiota, HIV status, and birth outcome. Our findings highlight variations in bacterial composition, CST stability, and inflammation across ethnicities and HIV subgroups while underscoring the importance of host, behavioral, and clinical factors in shaping these patterns. Future studies integrating multidimensional social, environmental, and microbiome data will be essential to identify modifiable drivers of microbial and inflammatory dynamics that contribute to adverse pregnancy outcomes and to inform translational therapies [75, 76] that address these risks.

# Declarations

## Ethics approval and consent to participate

The study was approved by the NHS Health Research Authority National Research Ethics Service Committee (REC 13/LO/0107). All women provided oral and written informed consent.

## Consent for publication

Not applicable.

## Availability of data and materials

16S rRNA gene sequencing data are deposited in the European Nucleotide Archive (ENA) under accession number PRJEB114705 and are available at: https://www.ebi.ac.uk/ena.

The code used to perform all analyses, along with community state type (CST) assignment data and ASV tables generated after preprocessing, are available at: https://github.com/emilywatters/VMB-HIV-Ethnicity.

## Competing interests

PRB and DAM hold a patent regarding Lactobacillus crispatus composition for use in pregnant subjects. International application number: PCT/GB2022/050453. International publication number: WO 2022/175680.

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**Authors' contributions**

C-ES, PRB, GPT, and DAM conceived and designed the study. C-ES and RQ recruited participants and collected samples and metadata. C-ES, RQ, YSL, and DC carried out experimental work. AS, DC, MS and JRM processed the sequencing data, and HF provided CST assignments. EW and C-ES analyzed and interpreted the data. EW generated all figures and tables. EW and C-ES wrote the first draft of the manuscript. All authors critically revised the manuscript and approved the final submitted version.

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## Abbreviations

Adjusted odds ratio (aOR)

Amplicon sequence variant (ASV)

Antiretroviral therapy (ART)

Bacterial vaginosis (BV)

Benjamini–Hochberg false discovery rate (FDR)

Body mass index (BMI)

Cervicovaginal fluid (CVF)

Community state type (CST)

Estimated marginal means (EMMs)

Human Immunodeficiency Virus (HIV)

Permutational multivariate analysis of variance (PERMANOVA)

Pregnant women living with HIV (PWLHIV)

Pregnant women without HIV (PWWH)

Preterm birth (PTB)

Principal component 1 (PC1)

Principal component analysis (PCA)

Sexually transmitted infections (STIs)

UK HIV Preterm Birth Network study (UHPBN)

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