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Unique profile of inflammation and immune activation in pregnant people with HIV in the United States

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Background: Little is known about inflammation/immune activation during pregnancy in people with HIV (PWH) and growth in their children who are HIV-exposed and uninfected (CHEU).

Methods: Using data from the Pediatric HIV/AIDS Cohort Study and an HIV-seronegative comparison group, we assessed associations of: (1) HIV status, mode of HIV acquisition [perinatally-acquired vs. non-perinatally-acquired], and type of antiretroviral therapy (ART) with inflammation/immune activation in pregnancy; (2) inflammation/immune activation in pregnancy with growth (weight-for-age, length-for-age, and weight-for-length Z-score) of CHEU at 12 months. Interleukin(IL)-6, high-sensitivity C-reactive protein(hs-CRP), soluble(s) TNF-alpha receptor 1&2(sTNFR1, sTNFR2), sCD14, and sCD163 were measured between 13-27 weeks' gestation. Linear regression models were fit to estimate differences between groups for each log-transformed biomarker, adjusted for confounders.

Results: 188 pregnant PWH (39 perinatally-acquired, 149 non-perinatally-acquired) and 76 HIV-seronegative persons were included. PWH had higher IL-6, sTNFR1, sCD14, and sCD163 and lower sTNFR2, compared to HIV-seronegative persons in adjusted models. Among PWH, sCD163 was higher in those with perinatally-acquired vs. non-perinatally-acquired and on PI-based vs. INSTI-based ART. Higher maternal concentrations of IL-6, sTNFR2, and hs-CRP were associated with poorer growth at 12 months.

Conclusions: Maternal HIV status is associated with a distinct profile of inflammation/immune activation during pregnancy, which may influence child growth.

Key Words: inflammation; immune activation; HIV; pediatrics; pregnancy; HIV-exposed uninfected

INTRODUCTION

Successful expansion of antiretroviral therapy (ART) in pregnant people with HIV (PWH) has improved maternal outcomes and reduced perinatal transmission of HIV. As a result, there is a growing population of children who are HIV-exposed and uninfected (CHEU).[1] Unfortunately, CHEU have a higher risk of poor health outcomes compared to their HIV-unexposed counterparts. Globally, studies have found that CHEU have higher rates of infectious morbidity than HIV-unexposed children, and experience poorer growth and survival outcomes,[2–7] yet underlying mechanisms are poorly understood.

The *in-utero* environment for CHEU is unique, influenced by the complex interplay between maternal HIV and ART during gestation. Only a few studies have examined the role of maternal inflammation during pregnancy and downstream effects on child health outcomes, particularly in CHEU.[8–11] A study of CHEU and HIV-unexposed children in India noted higher maternal concentrations of C-reactive protein (CRP) were associated with weight-for-age and weight-for-length Z-score through 12 months of life,[11] and a study in Tanzania found that maternal plasma

tumor necrosis factor-alpha (TNF-α) was associated with lower birthweight.[10] Knowledge about how inflammation exposure *in-utero* relates to birth and infant outcomes could identify targets for interventions to optimize health for CHEU.

PWH with perinatally-acquired HIV may experience distinct inflammatory alterations associated with chronic immune activation from lifelong HIV infection requiring ART since birth.[12] However, few studies have assessed the association of mode of HIV acquisition (perinatally-acquired or non-perinatally-acquired) or ART regimen with inflammation in pregnancy. Several studies in non-pregnant adults, however, have compared the association between specific ART regimens and markers of inflammation/immune activation in ART-naïve individuals initiating treatment.[13–15] Integrase strand transfer inhibitors (INSTIs) appear to be associated with reduced inflammation compared to non-nucleoside reverse transcriptase inhibitors (NNRTIs), including lower levels of high-sensitivity CRP (hs-CRP) and soluble CD14 (sCD14).[14,15] However, it remains unclear if INSTIs have a beneficial effect on inflammation/immune activation compared to other antiretroviral drug classes, such as protease inhibitors (PIs). In a study of non-pregnant ART-naïve participants there was no significant evidence that reduction in inflammation/immune activation was different between those randomized to INSTIs vs. PIs.[16] However, given the implications of immune activation in pregnancy for both women and children, it is important to understand associations between ART regimens taken in pregnancy and systemic inflammation/immune activation.

Using prospectively collected data from a cohort of pregnant PWH and a second cohort of pregnant HIV-seronegative persons as a comparator group, we assessed biomarkers of maternal inflammation/immune activation measured at 13-27 weeks' gestation. Inflammation biomarkers included interleukin-6 (IL-6), hs-CRP, soluble TNF-α receptor 1 (sTNFR1), and soluble TNF-α receptor 2 (sTNFR2). sTNFR1 and sTNFR2 are circulating forms of their membrane-bound counterparts, which are essential for TNF-α-signaling.[17] Biomarkers of immune activation included sCD14 and soluble CD163 (sCD163), myeloid differentiation markers of monocyte-macrophage activation.[18,19] Further among PWH, we also evaluated associations of mode of HIV acquisition and type of ART with biomarkers of inflammation/immune activation during pregnancy. Finally, we evaluated associations of maternal inflammation/immune activation with infant outcomes, including preterm birth, small-for-gestational-age at birth, birth anthropometrics, and growth at 12-months of age.

METHODS

Study populations

We analyzed data collected from PWH during pregnancy and their CHEU, from birth to the one-year visit, enrolled in the Dynamic Cohort of the Surveillance Monitoring for ART Toxicities (SMARTT) study, a prospective cohort study designed to identify adverse effects of *in-utero*

antiretroviral exposures in infants and children, conducted by the Pediatric HIV/AIDS Cohort Study (PHACS) network. Details on the PHACS SMARTT study, which has enrolled participants since 2007 at 22 sites across the United States, including Puerto Rico, have been previously described.[20,21]

In 2016, the SMARTT study began to collect blood samples in pregnancy. Pregnancies with plasma blood samples collected at 13-27 weeks' gestation between 2016-2019 were included in this sub-study. Individuals with multi-fetal gestations were excluded. For those with repeated enrolled singleton pregnancies, the first enrolled pregnancy with an available blood sample was included.

The comparison group of healthy HIV-seronegative pregnant persons was comprised of participants enrolled in a study at Mount Sinai Medical Center in NYC from 2011-2015 (K23HD070760, PI: Jao).[22] Participants were ≥16 years of age, had pregnancies which resulted in a singleton live birth, and recruited from the midwifery clinic where individuals with low-risk pregnancies receive care. Those with a blood sample collected from 13-27 weeks' gestation were included.

The Institutional Review Board (IRB) at each study site and Harvard T.H. Chan School of Public Health approved the protocol for the SMAART study. The IRB of Icahn School of Medicine at Mount Sinai approved the comparison cohort study. Written informed consent was obtained from the mother for herself and her child.

HIV status, mode of maternal HIV acquisition, and ART regimen

Exposures of interest included HIV status, mode of maternal HIV acquisition (perinatally- vs. non-perinatally-acquired), and last ART regimen received during pregnancy prior to the blood sample with ≥7 days duration. For PWH, participants were classified as having perinatally-acquired HIV if they were born in 1983 or later and mode of maternal HIV acquisition status was reported through interview or chart abstraction or maternal date of HIV diagnosis was within 5 years of maternal date of birth. ART regimen during pregnancy was categorized hierarchically into four categories: INSTI-based ART, PI-based ART, NNRTI-based ART, and ART consisting of ≥3 antiretroviral classes.

Biomarkers of inflammation/immune activation

Plasma (EDTA) concentrations of IL-6, hs-CRP, sTNFR1 (60kDa), sTNFR2 (80kDa), sCD14, and sCD163 were measured using enzyme immunoassay techniques according to manufacturer's directions (hs-CRP: Biomatik, New Castle County, DE, USA; IL-6, sTNFR1, sTNFR2: Invitrogen, Waltham, MA, USA; sCD14: Hycult Biotech, Uden, Netherlands; sCD163: R&D Systems, Minneapolis, MN, USA). Assays were performed in duplicate at the Special Infectious Diseases Laboratory of the Ann & Robert H. Lurie Children's Hospital of Chicago, and the mean of two replicates was used as the measure of interest. Standard dilution procedures were

performed for samples with initial values above or below the limit of detection (LOD) based on instruction guidelines for kits until readings were within the LOD; adjustment by dilution factor was performed.

Pregnancy and infant growth outcomes

Preterm birth was defined as delivery at <37 weeks of gestation. Birth weight was obtained from the clinical chart. Birth length within 14 days and weight and length at 12±4 months of age were measured in triplicate using standard techniques based on CDC recommendations; means were calculated.[23,24] Birth weight and length Z-scores for sex and gestational age and small-for-gestational-age were calculated using Intergrowth-21st fetal growth standards.[25] Postnatal growth at 12-months of age was assessed by weight-for-age, length-for-age, and weight-for-length Z-scores calculated using WHO Growth Standards.[26] In addition, we assessed change in weight-for-age and length-for-age from birth to the 12-month visit.

Covariates

Covariate information was obtained from clinical charts, interviews, and physical examinations collected at visits as per study protocols. We considered a number of potential confounders, focusing on factors associated with both our exposure and outcome of interest in each analysis and occurring earlier than the exposure. For the analysis evaluating the association between HIV status and inflammation/immune activation biomarkers, we considered the following confounders: maternal age at conception (years), education (<high school vs. ≥high school), gravidity, pre-pregnancy/1st-trimester obesity (body mass index (BMI) <30 vs ≥30 kg/m^2), any substance use (tobacco/alcohol/illicit drug) in the 1st-trimester, and any sexually transmitted infection (gonorrhea/chlamydia/trichomoniasis/syphilis/genital herpes) during pregnancy. For sub-group analyses among PWH to assess the association between mode of HIV acquisition and biomarkers, we additionally considered HIV-RNA concentration (<50 vs. ≥50 copies/mL) measured between 3 months before and 1 month after and closest to the time of biomarker assessment as a confounder. For the analysis of the association between ART regimen and inflammatory biomarkers, we additionally considered HIV-RNA concentration[27] and mode of maternal HIV acquisition as confounders.

We considered the following confounders in the analysis assessing associations between inflammatory biomarkers and growth outcomes in CHEU: maternal age at conception, education, pre-pregnancy/1st-trimester obesity, substance use in the 1st-trimester, Type I/II or gestational diabetes, PI use during pregnancy, HIV-RNA concentration, and mode of maternal HIV acquisition.

Statistical analysis

We compared characteristics of pregnancies of PWH included in this study to SMARTT singleton pregnancies that enrolled during the same period but not included in the analysis

because of the lack of plasma specimens or repeat singleton pregnancies. Concentrations of biomarkers were natural log-transformed to approximate a normal distribution. Using Wilcoxon rank-sum tests, we compared the distribution of the inflammation/immune activation biomarkers by HIV status. For models evaluating associations between HIV status, mode of HIV acquisition, and ART regimen with biomarkers, generalized estimating equation (GEE) linear regression models with robust standard errors were fit to estimate the difference in mean log-transformed biomarker concentrations between exposure groups for each biomarker, unadjusted and adjusted for confounders. Separate models were fit for each biomarker.

In analyses of associations between biomarkers and infant growth outcomes in CHEU, we calculated Spearman correlations between the log-transformed biomarkers and continuous growth outcomes and tested associations. Distributions of log-transformed biomarkers were also summarized by infant's small-for-gestational-age and preterm outcomes, using Wilcoxon rank-sum tests for comparisons. To evaluate associations of each biomarker with each growth outcome among CHEU, GEE linear regression models with robust standard errors were fit for continuous growth outcomes, unadjusted and adjusted for confounders. Unadjusted and adjusted modified Poisson regression models with robust variance were fit for the binary small-for-gestational-age and preterm birth outcomes. Models were conducted on complete cases and no imputation was performed. All hypothesis tests were 2-sided. Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

Characteristics

A total of 188 pregnant PWH and 76 pregnant HIV-seronegative persons met criteria to be included in the study (**Supplemental Figure 1**). Characteristics are in **Table 1**. Pregnant PWH were older (mean age 29 vs. 24 years), and more frequently identified as Black non-Hispanic (60 vs. 41%) than pregnant persons without HIV. More pregnant PWH had less than a high school education and an annual household income <$20,000. Median gravidity was higher in pregnant PWH, and a higher proportion of PWH were obese pre-pregnancy or during the 1st-trimester. A higher proportion of PWH reported any substance use in the 1st-trimester (21 vs 1%) and any sexually transmitted infection during pregnancy (31 vs 3%) compared to persons without HIV. We compared the 188 PWH included to the 343 participants who were eligible for the study but not included, and characteristics were similar (data not shown).

Among PWH, 39 had perinatally-acquired HIV and 149 had non-perinatally-acquired HIV. Overall, 32% were on an INSTI-based, 31% on PI-based and 27% on an NNRTI-based regimen; 6% were on an ART regimen consisting of ≥3 antiretroviral classes. The median (IQR) days on regimen are provided as follows: INSTI-based, 94(57-176); PI-based, 102(57-141); NNRTI-based, 145(95-182); ≥3 classes, 156(68-178). 67% had an HIV-RNA level <50 copies/mL at the

time of biomarker assessment. Median CD4 count was higher in the non-perinatally-acquired HIV group (605 vs. 355 cells/mm^3) as was the proportion with HIV-RNA <50 copies/mL (72% vs 49%) as compared to the perinatally-acquired HIV group (data not shown).

Biomarker concentrations by HIV status

Distributions of raw biomarker concentrations and log-transformed concentrations by HIV status are shown in **Table 2**. After log-transformation, distributions approximated a normal distribution, and mean log concentration levels were higher in pregnancy among PWH than persons without HIV for IL-6, sTNFR1, SCD14, and SCD163, while they were lower for sTNFR2 among PWH than persons without HIV. Concentrations of hs-CRP were similar between the groups.

Unadjusted and adjusted associations of HIV status with biomarkers are presented in **Figure 1A**. PWH had higher mean IL-6 (adjusted difference 0.64; 95%CI: 0.19, 1.09), sTNFR1 (adjusted difference 0.57; 95%CI: 0.33, 0.81), sCD14 (adjusted difference 0.32; 95%CI: 0.22, 0.41), and sCD163 (adjusted difference 0.25; 95%CI: 0.12, 0.39) and lower mean sTNFR2 (adjusted difference -0.70; 95%CI: -1.21, -0.18), compared to HIV-seronegative persons. No detectable differences between groups were observed in hs-CRP concentrations.

Biomarker concentrations by mode of maternal HIV acquisition

Unadjusted and adjusted associations of mode of maternal HIV acquisition status with biomarkers are presented in **Figure 1B**. Among PWH, sCD163 was higher in those with perinatally-acquired HIV vs. non-perinatally-acquired HIV (adjusted difference 0.18; 95%CI: 0.01, 0.36). Similarly, the estimated adjusted difference in mean sCD14 was higher in the perinatally-acquired HIV group (adjusted difference 0.13; 95%CI: -0.03, 0.29). No clear adjusted differences in other mean biomarker concentrations were observed by mode of maternal HIV acquisition.

Biomarker concentrations by ART regimens

Unadjusted and adjusted associations of ART regimens with biomarkers are presented in **Figure 1C**. Compared to PWH on INSTI-based ART, sCD163 was higher in PWH on PI-based ART (adjusted difference 0.19; 95%CI: 0.02, 0.35). Similarly, sCD163 was higher for those on NNRTI-based (adjusted difference 0.12; 95%CI: -0.06, 0.30) and those receiving ≥3 classes of ART (adjusted difference 0.24; 95%CI: -0.07, 0.55) vs. INSTI-based ART. The adjusted mean difference in sCD14 was 0.30 (95%CI: 0.02, 0.59), 0.11 (95%CI: -0.04, 0.27), and 0.03 (95%CI: -0.14, 0.21) in those receiving ≥3 classes of ART, PI-based, and NNRTI-based ART, respectively, compared to INSTI-based ART.

Biomarker concentration and birth and growth outcomes

The prevalence of preterm birth (16 vs. 5%) and small-for-gestational-age (12 vs. 9%) was higher among CHEU than HIV-unexposed children (**Table 1**). CHEU had lower length-for-age z-score at 12-months of age than HIV-unexposed children (-0.01±1.25 vs. 0.84±1.42).

Associations of biomarkers with birth anthropometrics and growth at 12-months of age in CHEU are presented in **Supplemental Table 1** and **Figure 2**, respectively. In adjusted analyses, for each one-unit increase in log hs-CRP there was a 0.19 SD increase in birth length-for-age (95%CI: 0.02, 0.37).

In adjusted analyses for growth outcomes at 12-months of age (**Figure 2**), there was a 0.15 SD lower mean length-for-age at 12-months of age for each one unit increase in log IL-6. A one-unit increase in log IL-6 was associated with a mean 0.20 SD decrease in weight-for-age change and a mean 0.22 SD decrease in length-for-age change from birth to 12-months of age. For each one unit increase in log sTNFR2 there was a 0.20 SD lower mean weight-for-age and 0.21 lower length-for-age at 12-months of age. A one-unit increase in log sTNFR2 was associated with a mean 0.20 SD decrease in weight-for-age change from birth to 12-months of age. A one unit increase in log hs-CRP was associated with a mean 0.19 SD decrease in length-for-age change from birth to 12-months of age. Finally, inverse associations between sCD14 and weight-for-age and length-for-age at 12-months were observed (one unit increase in sCD14 associated with a mean 0.54 and 0.50 SD decrease, respectively). For the other biomarkers (sTNFR1 and sCD163), there was no clear association between the biomarkers and 12-month growth outcomes with wide confidence intervals.

DISCUSSION

In this study of pregnant PWH and their infants in the United States, maternal HIV status was associated with a distinct profile of inflammation/immune activation in the 2nd-trimester of pregnancy. Specifically, pregnant PWH had higher concentrations of IL-6, sTNFR1, sCD14, and sCD163, and lower concentrations of sTNFR2 compared to pregnant HIV-seronegative persons. In addition, immune activation was found to be more pronounced in those with perinatally-acquired HIV and those on PI-based ART.

Our finding of elevated sCD14 among pregnant PWH was also observed in studies of pregnant PWH in India and Spain; elevated IL-6 was also reported in the India study.[11,28] However, the higher concentrations of sCD163 in pregnant PWH we observed in our study was not seen in either study. sCD14 and sCD163, biomarkers of monocyte-macrophage activation,[18,19] have been shown to increase in response to immune activation, microbial translocation, and intestinal dysbiosis in the context of HIV, and found to independently predict mortality in ART-treated populations with HIV.[29] In addition, both sCD14 and sCD163 have been found to be associated with preterm birth in PWH;[28,30] this was not observed in our study. Of note, prevalence of

preterm birth among pregnant PWH was lower in our study (16%) compared to the other studies (24-25%).[28,30]

Similar findings have been established in non-pregnant PWH,[27,31,32] and increases in these biomarkers, including IL-6 and sCD14, have been found to independently predict mortality in ART-treated PWH. There are several well-established biological mechanisms whereby persistent inflammation occurs, including failed T-cell homeostasis due to collagen deposition, excess levels of pathogens such as cytomegalovirus, destruction of mucosal surfaces, and gut microbial translocation.[33–36]

We observed alterations in TNFα-signaling as evidenced by higher concentrations of sTNFR1 and lower concentrations of sTNFR2 in pregnant PWH vs. HIV-seronegative persons. The membrane-bound counterpart of sTNFR1 is found on most tissues and placental cells during pregnancy, leading to pro-inflammatory and programmed cell death pathways.[17] The membrane-bound counterpart of sTNFR2 is expressed on regulatory T-cells and associated with immune modulation and lymphocyte stability, expansion, and function.[17,37] In animal models TNFR2 exerts a protective and regenerative function in several tissue cell types, while knockout TNFR2 results in reduced proliferation of these tissue cells.[38,39] The higher sTNR1 and lower sTNFR2 levels observed in our pregnant PWH raises the notion that there is a propensity for pro-inflammatory imbalances in cell death and survival as well as diminished cell regeneration and maturation associated with HIV and pregnancy. To our knowledge no studies have measured sTNFR1 and sTNFR2 concentrations in pregnant PWH.

Our study is one of the first to report on inflammation/immune activation in pregnant PWH with perinatally-acquired vs. non-perinatally-acquired HIV. We found that immune activation, as reflected by sCD163 concentrations in the 2nd-trimester of pregnancy, may be more pronounced in PWH with perinatally-acquired HIV. Greater immune activation and immune cell exhaustion in PWH with perinatally-acquired HIV vs. non-perinatally-acquired HIV has also been reported outside the context of pregnancy.[40,41] It is possible that increases in sCD163 in PWH with perinatally vs. non-perinatally-acquired HIV are partially driven by differences in levels of viremia, since sCD163 concentrations were similar between groups when we restricted the analysis to virally-suppressed individuals (data not shown). Nonetheless, little is known about whether this increased immune activation observed in our PWH with perinatally-acquired HIV during pregnancy influences health outcomes of their children. One study reported higher infectious morbidity in infants born to PWH with perinatally-acquired vs. non-perinatally-acquired HIV, but another did not find this association.[42,43]

We observed that immune activation may be greater among pregnant PWH on PI-based ART vs. those on INSTI-based ART. A study in non-pregnant PWH found that switching from a PI-based to an INSTI-based regimen was associated with a decline in sCD14.[44] Of note, controversy remains over whether INSTI-based ART regimens have a more favorable inflammatory profile

than PI-based regimens.[45–47] Regardless, our finding may have clinical implications and support the use of INSTI-based ART for pregnant PWH.

In analyses of growth outcomes among CHEU, we found that higher maternal concentrations of IL-6, sTNFR2, and hs-CRP in the 2nd-trimester of pregnancy were associated with poorer child growth outcomes at 12-months of age. A similar association between higher maternal IL-1β concentration and infant growth deficits (lower length-for-age and weight-for-age) was observed in a study in India.[11] In populations without HIV, higher maternal IL-6 concentration during pregnancy has been found to be associated with lower birthweight.[48] Changes in insulin sensitivity (known to be associated with IL-6) and immune activation (sTNFR2) may work synergistically in the *in-utero* environment to affect fetal "re-programming," thereby influencing basic metabolic outcomes such as postnatal growth.[17] Additional follow up is needed to assess whether poorer growth persists beyond 12-months. Worldwide there are almost 15 million CHEU and this population will continue to expand.[1] Poor growth is not a benign condition and has considerable long-term health burden globally.[49]

Our study has several limitations. First, our study did not measure markers of inflammation/immune activation in cord blood or the infants; therefore we do not know how maternal levels affect levels in the fetus/newborn. Second, we measured inflammation at a cross-sectional timepoint in the 2nd-trimester. Thus, we do not know how biomarkers may have changed across the duration of pregnancy. A study of women initiating ART in Uganda with longitudinal measurements of biomarkers observed that most inflammatory biomarkers declined during pregnancy in treated PWH.[50] We selected a low-risk group of pregnant HIV-seronegative persons from a single geographic location rather than a comparison group matched on risk factors. As a result, differences in some baseline characteristics (e.g. sexually transmitted infections, substance use) between groups may limit generalizability as well as potentially cause us to overestimate our results. There may be some residual confounding due to variables not fully assessed in both cohorts (e.g. history of preterm birth, antibiotic use). Finally, our study was not powered to assess potential mediators or effect measure modifiers of the association between biomarkers of inflammation/immune activation and child growth; these should be considered in future studies.

In conclusion, our study found that HIV status during pregnancy was associated with higher concentrations of markers of systemic inflammation/immune activation in a cohort of pregnant individuals in the United States. Among CHEU in this study, higher concentrations of certain maternal inflammatory biomarkers, were associated with poor growth in the first year of life. Future research is warranted to better understand the role of ART in systemic inflammation/immune activation during pregnancy and whether modulating inflammation in pregnancy may improve maternal and infant health outcomes for pregnant PWH and their children.

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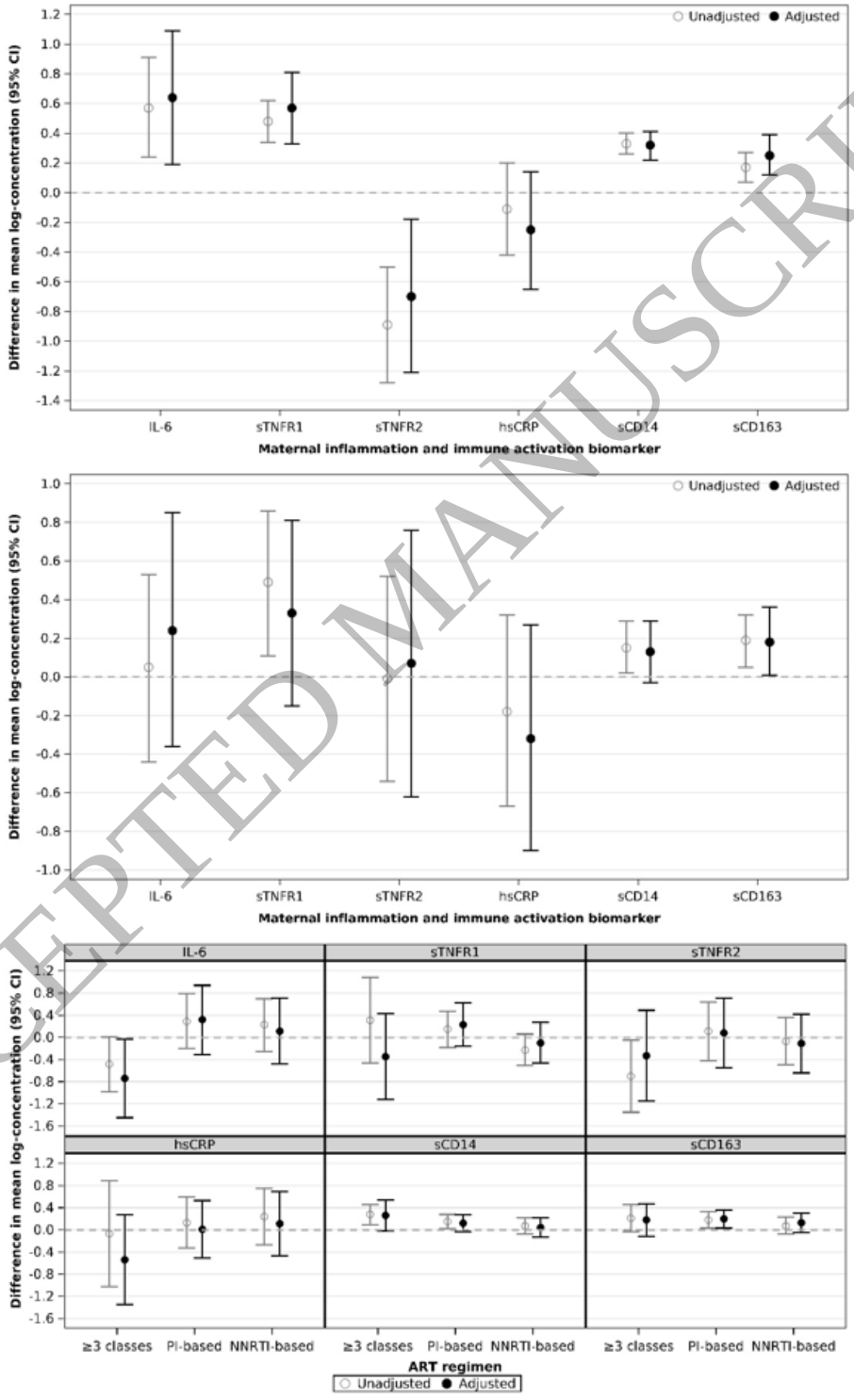
FIGURE LEGENDS

Figure 1. Estimated mean differences in log-transformed biomarkers during pregnancy, by exposures

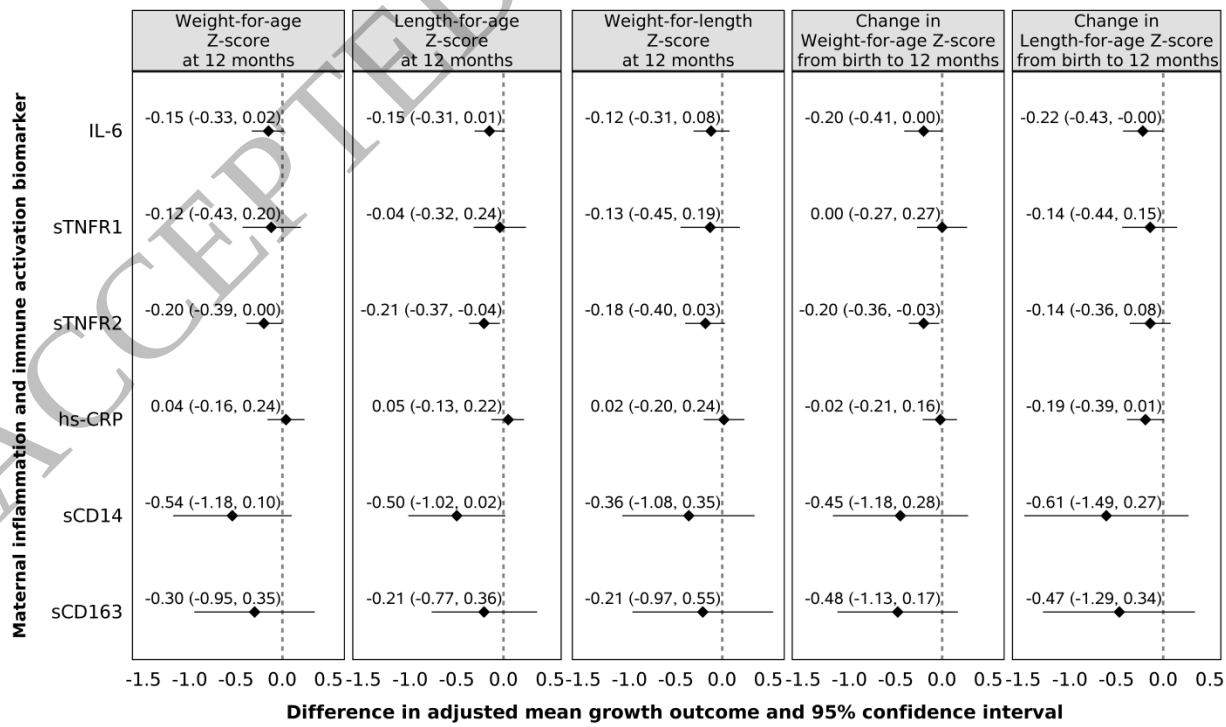


Difference in adjusted mean log-transformed biomarker concentration by exposure group. For analyses of the associations between HIV status, mode of HIV acquisition, and ART regimen with biomarkers, generalized linear regression models with robust standard errors were fit to estimate the difference in mean log-transformed biomarker concentrations between exposure groups for each biomarker, unadjusted and adjusted for maternal age at conception, education, pre-pregnancy/1st trimester obesity, any substance use in the 1st trimester, and any sexually transmitted infection. For analyses to assess the association between mode of HIV acquisition and biomarkers we additionally adjusted for HIV RNA concentration. For analyses to assess the association between ART regimen and biomarkers, we additionally adjusted for HIV RNA concentration and mode of maternal HIV acquisition.

Acronyms - *PWH: people with HIV; HIV-: HIV-seronegative; ART: antiretroviral therapy; SD: standard deviation; IL-6: interleukin-6; sTNFR1: soluble TNF-alpha receptor 1; sTNFR2: soluble TNF-alpha receptor 2; hs-CRP: high-sensitivity C-reactive protein; sCD14: soluble CD14; sCD163: soluble CD163; ART: antiretroviral therapy; INSTI: Integrase strand transfer inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor*

Number of participants included in adjusted analysis *- A) HIV status, N=151; B) Mode of HIV acquisition, N=151; ART regimen, N=147.*

Figure 2. Adjusted associations of log-transformed maternal inflammation and immune activation biomarker concentrations with growth outcomes at 12-months of age in HIV-exposed uninfected (HEU) infants



Difference in adjusted mean 1) growth z-score at 12 months of age; or 2) change in growth z-score from birth to 12 months of age, per one unit increase in a specific log-transformed biomarker concentration. Separate generalized linear regression model with robust standard error was fit for each biomarker, adjusting for maternal age at conception, education, pre-pregnancy/1st trimester obesity, any substance use in the 1st trimester, Type I/II or gestational diabetes, PI use during pregnancy, HIV RNA viral load, and mode of maternal HIV acquisition.

Acronyms *- HEU: HIV-exposed and uninfected; 95% CI: 95% confidence interval; IL-6: interleukin-6; sTNFR1: soluble TNF-alpha receptor 1; sTNFR2: soluble TNF-alpha receptor 2; hs-CRP: high-sensitivity C-reactive protein; sCD14: soluble CD14; sCD163: soluble CD163*

Number of participants included in adjusted analysis *- Weight-for-age Z-score at 12 months, N=106; Height-for-age Z-score at 12 months, N=105; Weight-for-length Z-score at 12 months, N=105; Change in weight-for-age Z-score from birth to 12 months, N=105; Change in height-for-age Z-score from birth to 12 months, N=94.*

Table 1. Characteristics of pregnant people with HIV (PWH) vs. HIV-seronegative (HIV-) people and children who are HIV-exposed and uninfected (CHEU) and HIV-unexposed children

		HIV Status	
Maternal characteristics		**PLHIV (N=188)**	**HIV- (N=76)**
Age at conception, years	Mean (SD)	29.06 (5.50)	24.01 (5.13)
Race/Ethnicity, N (%)	Black non-Hispanic	113 (60%)	31 (41%)
	White/Other non-Hispanic	18 (10%)	5 (7%)
	Hispanic	57 (30%)	38 (50%)
Education	< high school	50 (27%)	13 (17%)
	≥ high school	132 (70%)	63 (83%)
Annual household income	< 10K	82 (44%)	9 (12%)
	10 - 20K	33 (18%)	11 (14%)
	> 20 - 50K	49 (26%)	24 (32%)
	> 50K	10 (5%)	0 (0%)
Gravidity	Median (Q1, Q3)	3 (2, 4)	2 (1, 3)
Pre-pregnancy/1st trimester BMI (kg/m²)	Mean (SD)	30.70 (9.95)	26.37 (6.27)

Pre-pregnancy/1st trimester obesity (BMI ≥ 30 kg/m²)	Yes	79/168 (42%)	17/76 (22%)
Any tobacco, alcohol or illicit drug use in the 1st trimester	Yes	39/183 (21%)	1/76 (1%)
Any syphilis, trichomoniasis, gonorrhea, chlamydia, or genital herpes during pregnancy	Yes	58/184 (31%)	2/76 (3%)
Type I/II diabetes diagnosis or gestational diabetes	Yes	20/185 (11%)	0/76 (0%)
HIV characteristics (PLHIV only)			
Mode of HIV acquisition	Perinatally-acquired HIV	39 (21%)	--
	Non-perinatally acquired HIV	149 (79%)	
Antiretroviral regimen	ART with ≥ 3 antiretroviral classes	12 (6%)	--
	INSTI-based ART	60 (32%)	--
	PI-based ART	59 (31%)	--
	NNRTI-based ART	50 (27%)	--
	Other	2 (1%)	--
	No ART	4 (2%)	--
CD4 count (cells/mm³)	Median (Q1, Q3)	565 (339, 779)	--
CD4 count (cells/mm³)	≥ 350	137 (73%)	--
	200 - 349	32 (17%)	--
	< 200	16 (9%)	--
HIV RNA (copies/mL)	< 50	126 (67%)	--
	50 - 200	20 (11%)	--
	> 200 - 400	10 (5%)	--
	> 400	23 (12%)	--

Infant characteristics		**CHEU**	**HIV-unexposed**

			children
Sex	Male	97 (52%)	36 (47%)
	Female	91 (48%)	40 (53%)
Preterm birth (< 37 weeks gestation)	Yes	30/186 (16%)	4/76 (5%)
Small-for-gestational age	Yes	22/182 (12%)	7/75 (9%)
Birth weight z-score	N	182	75
	Mean (SD)	-0.02 (1.02)	0.05 (1.03)
Birth length z-score	N	162	64
	Mean (SD)	0.39 (1.47)	0.73 (1.24)
Weight-for-age z-score at 12-months of age	N	123	33
	Mean (SD)	0.60 (1.31)	0.53 (1.35)
Length-for-age z-score at 12-months of age	N	122	32
	Mean (SD)	-0.01 (1.25)	0.84 (1.42)
Weight-for-length z-score at 12-months of age	N	122	31
	Mean (SD)	0.79 (1.42)	0.28 (1.23)

Missing values - There were missing values for the following variables: race/ethnicity (N=3), education (N=6), annual household income (N=46), gravidity (N=9), pre-pregnancy/1st trimester BMI (N=20), Any tobacco, alcohol or illicit drug use in the 1st trimester (n=6), Any syphilis, trichomoniasis, gonorrhea, chlamydia, or genital herpes during pregnancy (N=4), Type I/II diabetes diagnosis or gestational diabetes (N=3), Antiretroviral regimen (N=1), CD4 count (N=3), HIV RNA (N=9).

Acronyms - PWH: people with HIV; HIV-: HIV-seronegative; CHEU: children who are HIV-exposed and uninfected; SD: standard deviation; BMI: body mass index; ART: antiretroviral therapy; INSTI: Integrase strand transfer inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor

Table 2. Inflammation and immune activation biomarkers in pregnant people with HIV (PWH) vs. HIV-seronegative (HIV-) people

Biomarker	HIV Status		
	PWH (N=188)	HIV- (N=76)	P-Value*

IL-6 (pg/mL)	Median (Q1, Q3)	1.33 (0.65, 4.45)	0.71 (0.46, 1.66)	<0.001
	Min, Max	0.17, 109.17	0.13, 43.59	
Log IL-6	Mean (SD)	0.54 (1.30)	-0.03 (1.25)	
	Min, Max	-1.75, 4.69	-2.07, 3.78	
sTNFR1 (ng/mL)	Median (Q1, Q3)	1.84 (1.46, 2.71)	1.58 (1.20, 1.86)	<0.001
	Min, Max	0.70, 38.12	0.87, 2.65	
Log sTNFR1	Mean (SD)	0.88 (0.88)	0.40 (0.29)	
	Min, Max	-0.35, 3.64	-0.15, 0.97	
sTNFR2 (ng/mL)	Median (Q1, Q3)	5.80 (2.88, 14.64)	17.45 (5.70, 45.06)	<0.001
	Min, Max	1.47, 929.0	2.10, 598.5	
Log sTNFR2	Mean (SD)	2.06 (1.34)	2.95 (1.50)	
	Min, Max	0.39, 6.83	0.74, 6.39	
hs-CRP (mg/L)	Median (Q1, Q3)	4.76 (1.65, 13.45)	5.23 (2.78, 11.70)	0.49
	Min, Max	0.18, 63.04	0.24, 36.95	
Log hs-CRP	Mean (SD)	1.60 (1.33)	1.71 (1.09)	
	Min, Max	-1.74, 4.14	-1.41, 3.61	
sCD14 (ng/mL)	Median (Q1, Q3)	1,951 (1,635, 2,457)	1,519 (1,353, 1,680)	<0.001
	Min, Max	833.3, 5,814	685.6, 2,281	
Log sCD14	Mean (SD)	7.64 (0.39)	7.31 (0.20)	
	Min, Max	6.73, 8.67	6.53, 7.73	
sCD163 (ng/mL)	Median (Q1, Q3)	528.4 (386.7, 697.7)	433.9 (327.5, 595.7)	0.001
	Min, Max	154.5, 1,525	198.7, 1,217	
Log sCD163	Mean (SD)	6.24 (0.41)	6.08 (0.38)	
	Min, Max	5.04, 7.33	5.29, 7.10	

*Wilcoxon rank-sum Test

Abbreviations - PWH: people with HIV; HIV-: HIV-seronegative; SD: standard deviation; IL-6: interleukin-6; sTNFR1: soluble TNF-alpha receptor 1; sTNFR2: soluble TNF-alpha receptor 2; hs-CRP: high-sensitivity C-reactive protein; sCD14: soluble CD14; sCD163: soluble CD163