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MAJOR ARTICLE

Changes in Immune Activation During Pregnancy and the Postpartum Period in Treated HIV Infection

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Background. Pregnant women with HIV (PWWH) have high postpartum morbidity and mortality from infections like tuberculosis. Immunologic changes during pregnancy and postpartum periods may contribute to these risks, particularly the immunoregulatory kynurenine pathway of tryptophan catabolism, which contributes to both HIV and tuberculosis pathogenesis and increases in the early postpartum period.

Methods. Women with HIV initiating antiretroviral therapy (ART) in the Uganda AIDS Rural Treatment Outcomes (UARTO) cohort who were pregnant at enrollment or became pregnant during observation were studied (n = 54). Plasma kynurenine/tryptophan (KT) ratio, soluble CD14 (sCD14), sCD163, sCD27, interferon-inducible protein 10 (IP-10), D-dimer, interleukin-6, and intestinal fatty-acid binding protein levels were assessed through the first year of ART and at 3-month intervals throughout pregnancy and 1 year postpartum. Biomarker changes were assessed with linear mixed models adjusted for ART duration. Hemoglobin concentration changes were used to estimate pregnancy-related changes in plasma volume.

Results. The median pre-ART CD4 count was 134. D-dimer increased through the third trimester before returning to baseline postpartum, while most other biomarkers declined significantly during pregnancy, beyond what would be expected from pregnancy-associated plasma volume expansion. IP-10 and sCD14 remained suppressed for at least 12 months postpartum. KT ratio was the only biomarker that increased above prepregnancy baseline postpartum (mean + 30%; $P < .001$) and remained higher than baseline for ≥9 months ($P \leq .045$ for all time points).

Conclusions. Several immune activation markers decline during pregnancy and remain suppressed postpartum, but the kynurenine pathway of tryptophan catabolism increases above baseline for ≥9 months postpartum. The mechanisms underlying postpartum kynurenine pathway activity are incompletely understood but may contribute to increased tuberculosis risk in this setting.

Keywords. HIV; indoleamine 2,3-dioxygenase-1; inflammation; kynurenine/tryptophan ratio; pregnancy.

Pregnant women with HIV (PWWH) have a higher risk of pregnancy-related or postpartum mortality—particularly from infections—compared with women without HIV in both high- and low-income countries [1–5]. Earlier antiretroviral therapy (ART) initiation and continuation postpartum in PWWH lowers infection rates and mortality, but pregnancy remains an important risk factor for morbidity and mortality even during suppressive ART [3, 5–10]. In people with HIV (PWH) who are not pregnant, innate and adaptive immune activation persist despite suppressive ART and are predictive of morbidity and mortality [11–21], but the degree to which the pregnancy and postpartum periods alter these immunologic pathways remains incompletely defined.

The physiologic state of pregnancy, independent of HIV, leads to many immunoregulatory changes that help maintain the fetal allograft but may increase susceptibility to infections, including tuberculosis (TB) [22–25]. Multiple studies have demonstrated this increased risk of TB in PWWH, particularly postpartum [26, 27]. Women without HIV also have an increased TB risk during pregnancy, and an even greater risk postpartum [28, 29]. How pregnancy- and postpartum-induced biomarker changes affect immune activation pathways known to predict morbidity and mortality in HIV remains incompletely understood [30]. Two studies have reported increases in C-reactive protein (CRP), interleukin-6 (IL-6), and D-dimer in the third trimester followed by declines postpartum, but it was unclear how these changes compared with prepregnancy baseline; neither study controlled for ART duration [31, 32]. Additionally, the kynurenine pathway of tryptophan catabolism, which appears to play an important immunoregulatory role including

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maintaining fetal allograft tolerance, also contributes to HIV and TB pathogenesis by conferring multiple adaptive immune defects, while being one of the strongest predictors of mortality in treated HIV [20, 33–38]. While a study of 20 healthy pregnant women without HIV demonstrated increases in the third trimester through 6 weeks postpartum, no data exist beyond that narrow range or in treated HIV in general [39].

To address these knowledge gaps, we leveraged a cohort of Ugandan women initiating their first ART regimen to assess changes in immunologic biomarkers every 3 months from prepregnancy baseline through pregnancy and the first year postpartum. We assessed multiple biomarkers that predict increased morbidity and mortality in treated HIV and reflect discrete but overlapping immunologic pathways, adjusting for ART duration to ensure appropriate attribution of biomarker changes to pregnancy.

METHODS

Study Design and Participants

Women aged ≥18 years initiating ART in the Uganda AIDS Rural Treatment Outcomes (UARTO) cohort who were pregnant at enrollment or became pregnant during observation (confirmed by urine human chorionic gonadotropin [hCG]), resulting in a live birth, were included. The UARTO cohort includes 759 PWH initiating their first ART regimen at Mbarara University of Science and Technology Immune Suppression Syndrome clinic between 2005 and 2014 [36]. Participants were followed every 3 months with questionnaires and biospecimen archiving. Timing of gestation and the postpartum period was estimated based on self-reported delivery dates. ART regimens reflected the local standard of care at the time and included either zidovudine/lamivudine, stavudine/lamivudine, or tenofovir/emtricitabine in combination with either nevirapine or efavirenz. Exclusion criteria included lack of viral suppression (plasma HIV RNA level >400 copies/mL) any time after 6 months of ART or known acute illness.

Biomarker Measurements

Plasma biomarkers were assessed on cryopreserved plasma obtained in the fasting state pre–ART initiation, at months 6 and 12 of ART, and from these time points when available: prepregnancy, first trimester, second trimester, third trimester, and postpartum months 0–3, 3–6, 6–9, and 9–12. As some women were already pregnant at the time of ART initiation, not all women contributed plasma for all time points. In addition to the kynurenine-to-tryptophan (KT) ratio, a ratio representing the activity of the kynurenine pathway's rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO) described above, plasma biomarkers representing discrete but related inflammatory pathways in treated HIV were assessed. D-dimer and IL-6, markers of coagulation and generalized inflammation, respectively, are both predictors of morbidity and mortality in treated HIV [11, 18, 20, 40]. Interferon-inducible protein 10 (IP-10 or CXCL10), an interferon response cytokine, is persistently abnormal despite early HIV treatment and predicts morbidity and mortality in nonpregnant people with HIV [40–42]. Soluble CD14 (sCD14) is a marker of microbial translocation and, in addition to soluble CD163 (sCD163), is a marker of monocyte and macrophage activation and predictive of morbidity and mortality [15, 18, 20, 40, 43–45]. Soluble CDC27 is a T-cell activation marker, part of the tumor necrosis factor superfamily, and a strong predictor of late mortality in treated HIV [12, 20]. Intestinal fatty-acid binding protein (I-FABP) is a marker of intestinal barrier integrity and associated with mortality in HIV [18]. KT ratio was assessed by liquid chromatography–mass spectrometry [36]. All others were assessed via commercial immunoassay kits: IP-10, sCD14, and I-FABP (R&D Systems); sCD27 (eBioscience), sCD163 (Trillium Diagnostics, now IQ products), IL-6 (Meso Scale Diagnostics), and D-dimer (Diagnostico Stago). Approximately 84% of plasma samples were obtained from blood drawn in ACD tubes (the remainder from EDTA-anticoagulated tubes). To account for differences in diluent volume, values obtained from ACD tubes were multiplied by an adjustment factor of 1.276 [20]. Expected dilutional effects with increased plasma volume in pregnancy were estimated by evaluating changes in serum hemoglobin levels [46].

Statistical Analysis

Pregnancy-related changes in biomarkers were assessed via linear mixed models with random intercepts, log-transforming outcome variables to satisfy model assumptions and adjusting for ART duration. This statistical approach accounts for clustering by participant, allowing for the inclusion of more than 1 pregnancy per participant, and also mitigates against biased inferences from missing data at any particular time point. Given the rapid and nonlinear decrease in biomarkers (and increases in CD4 counts) following ART initiation, time on ART was modeled as a linear spline with demarcations (knots) at 0–3, 3–6, 6–12, 12–24, and >24 months, which allowed for the necessary adjustment for background ART-mediated changes in biomarkers so that we could isolate the effect of pregnancy and the postpartum period. Estimated mean relative changes in biomarkers from prepregnancy baseline with 95% CIs are reported, with marginal mean estimates with 95% CIs in the Supplementary Data. Several sensitivity analyses were performed, including (1) excluding women who were pregnant at the time of ART initiation, (2) modeling ART duration with a less complex linear spline (ie, slope before and after 6 months), and (3) modeling ART duration as a linear predictor (without a spline) and excluding observations earlier than month 8 of ART (ie, when most of the major ART-related biomarker reductions occur). All statistical tests were 2-tailed, with an alpha

level of .05 considered statistically significant. All analyses were performed with Stata, version 15.

Patient Consent

This study received approval of the institutional review boards of Mbarara University, the Uganda National Council of Science and Technology, the University of California, San Francisco, and Partners HealthCare. All participants provided written informed consent.

RESULTS

Patient Demographics and Characteristics

Fifty-four participants contributed 412 biomarker (and 1230 T-cell count) time points. Seven participants contributed observations from more than 1 pregnancy. The median age (interquartile range [IQR]) was 29 (25–33) years, and 30% of women were pregnant at ART initiation (Table 1). The median ART duration before pregnancy (IQR) was 11 (3–24) months. Reflective of the initial rollout of ART in rural Uganda, most participants started ART later in their HIV infection, with a median CD4+ T-cell count (IQR) of 134 (81–216) cells/mm^3.

CD4+ and CD8+ T-Cell Count Changes During Pregnancy and Postpartum

Expected ART-mediated increases in CD4 counts and CD4/CD8 ratios were observed, and subsequent models were adjusted for ART duration as a linear spline (Supplementary Figure 1). As plasma volume expansion during pregnancy might be expected to lower cell counts and plasma cytokine concentrations in the absence of real immunologic changes in the tissues, we first assessed changes in peripheral blood hemoglobin levels as an estimate for the degree to which plasma volume expansion may have occurred during pregnancy [46]. After adjustment for ART duration, blood hemoglobin concentrations tended to increase in the first trimester before declining below prepregnancy values by a mean of 9% in the second trimester and 28% in the third trimester ($P < .001$ for both) (Figure 1; Supplementary Table 1). After adjustment for ART duration, we observed a 9% and 11% decrease in both CD4 and CD8 counts during the second trimester compared with the prepregnancy baseline ($P < .05$ for both, respectively, which was comparable to the reduction in hemoglobin levels at the same time point (Figure 1; Supplementary Table 1 and Supplementary Figure 2). Mean CD4 counts declined from baseline to the third trimester to a far lesser degree (–2%; 95% CI, –13% to 10%) than hemoglobin levels (–28%; 95% CI, –36% to –18%). CD8 counts tended to increase above prepregnancy levels in the first 6–9 months postpartum.

Table 1. Characteristics of 54 Ugandan Women With HIV at ART Initiation

Characteristic	Median (IQR)
Age, y	29 (25–33)
Plasma HIV RNA level, $\log_{10}$ copies/mL	5.0 (4.5–5.5)
CD4 count, cells/mm^3	134 (81–216)
CD8 count, cells/mm^3	639 (447–848)
CD4/CD8 ratio	0.20 (0.13–0.37)
Pregnant at ART initiation, No. (%)	16 (30)[a]
≥2 pregnancy episodes during observation, No. (%)	7 (13)[b]

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range.

[a]First trimester, n = 4; second trimester, n = 5; third trimester, n = 7.

[b]Two pregnancies, n = 6; 3 pregnancies, n = 1.

D-dimer Increased Significantly During Pregnancy

Similarly, we adjusted all plasma biomarker changes during pregnancy for concurrent changes in ART duration using linear splines (Supplementary Figures 3–4). Consistent with prior reports of hypercoagulability during pregnancy, D-dimer levels increased steadily throughout all 3 trimesters, reaching a mean 2.2-fold and 3-fold increase above baseline by the second and third trimesters ($P < .001$ for both) (Figure 2; Supplementary Table 1 and Supplementary Figure 5) [47, 48]. In the early postpartum period, D-dimer levels decreased significantly and approached prepregnancy levels by 3–6 months.

Most Inflammatory Biomarkers Declined During Pregnancy

As expected, most inflammatory biomarkers declined during ART, with the greatest changes occurring in the first few months of viral suppression (Supplementary Figures 3–4). Of all plasma biomarkers, IP-10 levels declined to the greatest degree during pregnancy—by a mean of 30%–40% in the 3 trimesters ($P < .001$ for all)—far exceeding concurrent hemoglobin declines (Figure 2; Supplementary Table 1 and Supplementary Figure 5). Plasma sCD14 levels also declined by a mean of 17%–18% in the first 2 trimesters ($P < .001$ for both), respectively, exceeding associated reductions in hemoglobin. Both sCD27 and sCD163 declined significantly in the first trimester, though more modestly than other biomarkers. IL-6 and I-FABP also declined by a mean of 29% and 23% ($P < .03$ for both) in the first trimester, but then increased toward prepregnancy baseline by the third trimester. Similarly, KT ratio, which should be unaffected by the degree of plasma volume expansion in pregnancy (as it reflects the ratio of 2 metabolite concentrations), declined by a mean of 10%–12% in the first 2 trimesters ($P < .03$ for both) before increasing back to the baseline in the third trimester (Figure 2; Supplementary Figures 5–6).

Several Biomarkers Remained Suppressed Postpartum

Multiple biomarkers were suppressed during pregnancy and remained below prepregnancy values during the postpartum period, while hemoglobin levels returned to the prepregnancy baseline (Figures 1 and 2; Supplementary Table 1 and Supplementary Figure 5). IP-10 and to a lesser degree sCD14 remained significantly below baseline for at least 12 months postpartum. Postpartum IL-6 levels fluctuated postpartum, but tended to remain lower than prepregnancy baseline.

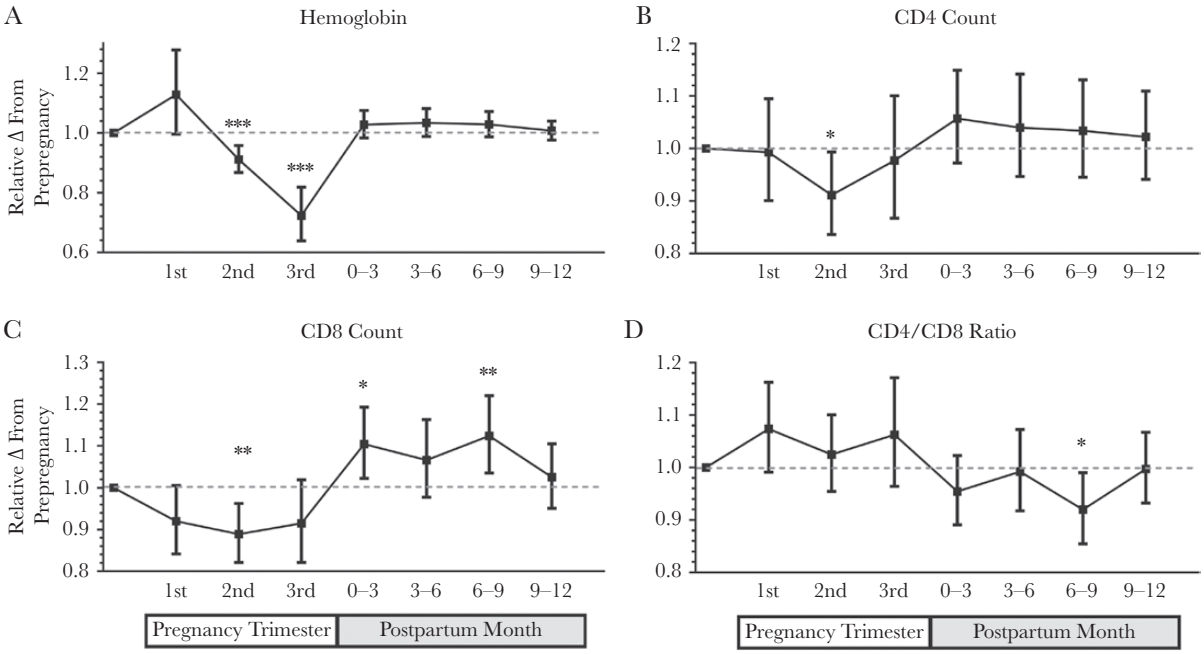

Figure 1. Relative changes in hemoglobin levels and T-cell counts throughout pregnancy and postpartum. Changes in peripheral blood hemoglobin (A), CD4+ (B), and CD8+ (C) T-cell counts and CD4/CD8 ratio (D) are presented as mean fold changes relative to prepregnancy baseline with 95% CIs. Changes were assessed with linear mixed models adjusted for time on ART modeled as a linear spline (0–3, 3–6, 6–12, 12–24, and >24 months). Abbreviation: ART, antiretroviral therapy.

KT Ratio Was the Only Plasma Biomarker to Increase Significantly Postpartum

After initial declines in the first 2 trimesters and a return to baseline in the third trimester, KT ratio increased by 35% in the first 3 months postpartum ($P < .001$) and remained elevated above the prepregnancy baseline for 6–9 months ($P \leq .045$ for all). This pattern coincided with the relative increase in CD8 counts postpartum.

Sensitivity Analyses

All major inferences related to biomarker changes during pregnancy and postpartum were robust to several sensitivity analyses. These included (1) models restricted to women who had incident pregnancies after initiating ART, (2) modeling ART duration in splines with 2 (instead of 5) segments (before and after month 6 of ART), and (3) models restricted to observations after more than 8 months of ART (beyond the major ART-mediated changes).

DISCUSSION

While numerous studies have demonstrated increased morbidity and mortality largely from infections during pregnancy and postpartum in PWWH (and those without HIV), none have comprehensively assessed the immunologic changes that occur in this setting that might contribute to these risks [1–6]. Leveraging a cohort of ART-treated PWH from Uganda, we assessed how biomarkers of immune activation change in PWWH during pregnancy and postpartum. We made several observations. First, plasma levels of most of the measured inflammatory biomarkers declined significantly during early pregnancy, beyond what might be expected from pregnancy-related plasma volume expansion [46]. Second, D-dimer increased substantially during pregnancy and returned to baseline postpartum. Third, some inflammatory biomarkers remained durably suppressed for at least 9–12 months postpartum. Perhaps most importantly, KT ratio, a biomarker of the immunoregulatory kynurenine pathway of tryptophan catabolism, was the only biomarker to increase significantly postpartum, remaining elevated above prepregnancy baseline for at least 6–9 months postpartum. While the clinical implications of these findings remain unclear, they raise testable hypotheses to explain the increased risk of infectious complications postpartum.

Consistent with well-described immunoregulatory changes during pregnancy in women without HIV, most inflammatory biomarkers declined during pregnancy in treated PWWH [49, 50]. IP-10, sCD14, sCD163, and sCD27 all declined significantly throughout pregnancy, while KT ratio, I-FABP, and IL-6 transiently declined during early pregnancy before returning to baseline by parturition. CD4 and CD8 counts, however, appeared fairly stable throughout pregnancy, with the exception of modest declines in the second trimester. As these T-cell declines in the second trimester were comparable in magnitude to concurrent hemoglobin declines, it is likely that the declines are related to the well-characterized

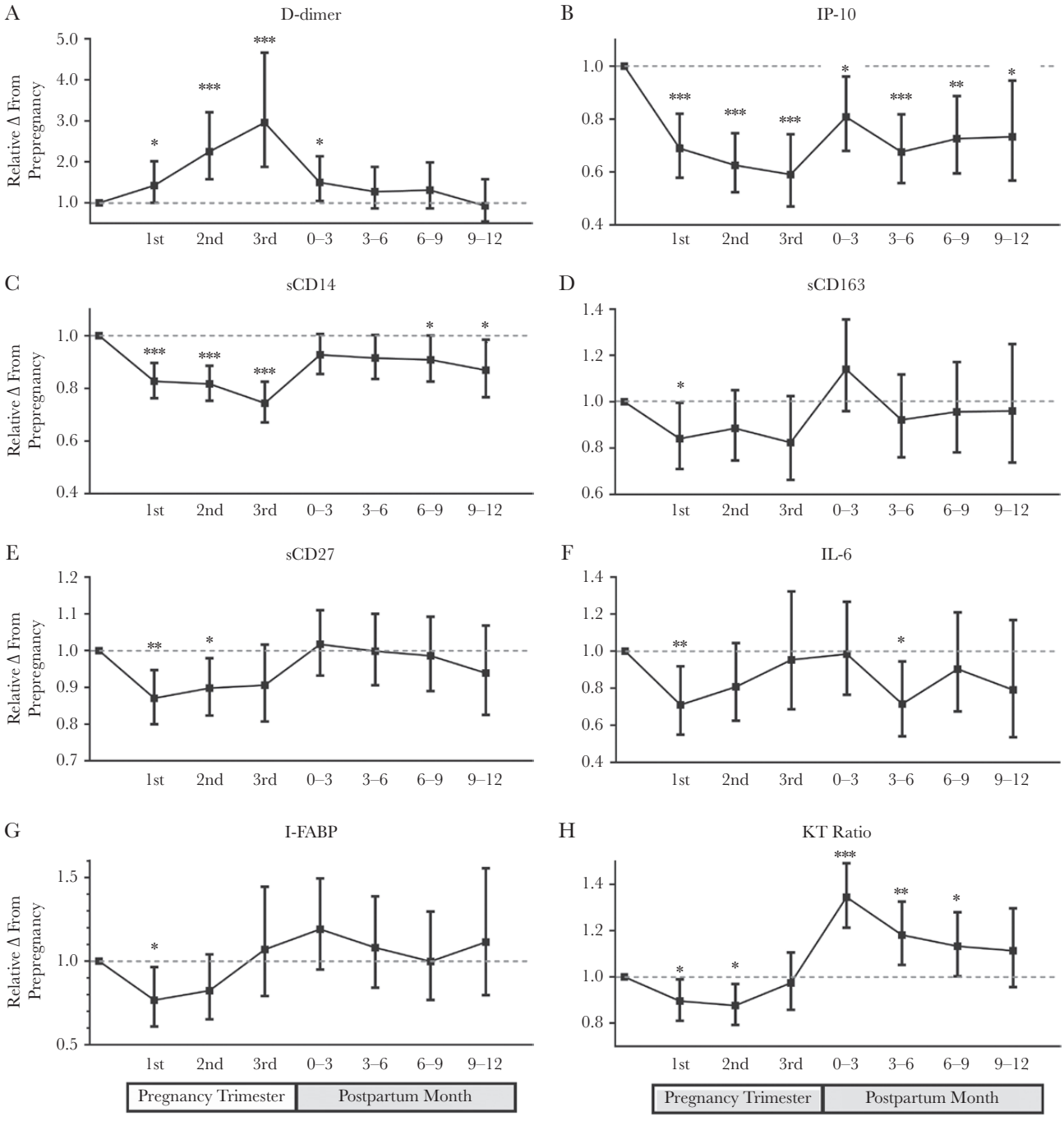

Figure 2. Relative changes in biomarkers throughout pregnancy and postpartum. Changes in plasma D-dimer (A), IP-10 (B), sCD14 (C), sCD163 (D), sCD27 (E), IL-6 (F), I-FABP (G), and KT ratio (H) are presented as mean fold changes relative to prepregnancy baseline with 95% CIs. Changes were assessed with linear mixed models adjusted for time on ART modeled as a linear spline (0–3, 3–6, 6–12, 12–24, and >24 months). Abbreviations: ART, antiretroviral therapy; I-FABP, intestinal fatty-acid binding protein; IL, interleukin; IP-10, interferon-inducible protein 10; KT, kynurenine/tryptophan; sCD14, soluble CD14; sCD27, soluble CD27; sCD163, soluble CD163.

dilutional effects of pregnancy-mediated volume expansion [46]. Nevertheless, most of the plasma biomarker declines we observed occurred before significant volume expansion, suggesting that the declines are unlikely to be explained by dilution. As CD4 count, I-FABP, and IL-6 all appeared to increase back toward prepregnancy baseline in the third trimester despite concurrent volume expansion, these increases might be even greater when accounting for dilution. Collectively, these observations support the notion of relative immunosuppression during pregnancy, which partially reverses in the third

trimester [30]. The magnitude of these relative changes in immune activation among PWWH compared with those seen in pregnant women without HIV is currently unknown, particularly given that microbial translocation—which is significantly greater in treated HIV compared with the HIV-negative population—may be a key effect modifier in the relationship between immune activation and stage of pregnancy [41, 49].

Like prior studies in women without HIV, we confirmed that D-dimer levels increase steadily throughout pregnancy, remain slightly elevated early postpartum, and then decrease to baseline thereafter. Compared with the HIV-negative population, however, PWWH likely have a higher baseline D-dimer, reach a higher peak, and decrease back to a prepregnancy baseline more slowly [47, 48]. A study in pregnant women without HIV demonstrated an elevated risk of venous thromboembolism beginning in the third trimester through the third postpartum month [51]. These findings are fairly compatible with the only prior study of D-dimer in PWWH, where levels increased to delivery and then returned to baseline several weeks postpartum [31, 32]. In 1 study in PWWH, 6-month postpartum D-dimer levels predicted subsequent morbidity [31]. As our study indicates that D-dimer levels have returned to prepregnancy baseline by 6 months postpartum, it remains unclear whether pregnancy-mediated increases in D-dimer are associated with increased morbidity and mortality risk beyond the well-described early thromboembolic complications [11, 17, 20, 40].

Our study also demonstrates that several biomarkers remain suppressed for at least 12 months postpartum. IP-10 and sCD14, both predictors of morbidity and mortality in treated HIV, declined within the first trimester of pregnancy and remained below baseline for at least 1 year postpartum [15, 18, 42]. While few studies have investigated postpartum changes in inflammatory biomarkers, 1 prior study found significant differences in several biomarkers among women who exclusively breastfed for 5 months vs formula-fed their infants [31]. The sustained suppression of inflammation we observed may be hormonally driven in our cohort as well; while most women likely did not breastfeed for 12 months (or exclusively for 6 months) from a qualitative study performed in a subset of the UARTO cohort, the degree of breastfeeding that may be required in prolonging these immunologic changes is unknown [52, 53]. These biomarkers, however, are mechanistically linked to the kynurenine pathway of tryptophan catabolism, which increased significantly postpartum. IP-10, like IDO, is induced by type I and II interferons. Similarly, kynurenine pathway–mediated suppression of Th17 cells may also contribute to microbial translocation, increasing sCD14 directly [54, 55], while lipopolysaccharides augment IDO activity [33]. These findings suggest a postpartum "de-linking" of these pathways.

The changes in KT ratio throughout pregnancy and postpartum represent the most significant finding in this study. We observed that the KT ratio decreased in the first and second trimesters compared with prepregnancy and then increased significantly early postpartum, with sustained elevation for at least 6–9 months. A study of HIV-uninfected pregnant women observed increases in KT ratio in the third trimester through 6 weeks postpartum with similar relative increases but with lower absolute KT ratio values compared with the present study. Ours is the first study in humans to demonstrate reductions in kynurenine pathway activity during early pregnancy and the duration of its elevation through at least 9 months postpartum [39]. Pregnancy-related changes in kynurenine pathway activity may be mediated by changes in tryptophan 2,3-dioxygenase (TDO; found in the liver and placenta), IDO (found in mucosal, lymphoid, and placental tissues), gut bacteria, or a combination [56, 57]. The reduction in KT ratio we observed in early pregnancy in humans parallels the estrogen- and progesterone-driven inhibition of liver TDO activity observed in pregnant rats and IDO-1 expression in humans at the materno-fetal interface [56–60]. Nevertheless, rat liver TDO activity does not increase postpartum despite nursing [58], raising the possibility that other enzymes (eg, IDO or microbiome-derived enzymes) are responsible for the postpartum increases in KT ratio we observed in PWWH. As ~90% of total tryptophan (as assessed in our study) is albumin-bound and unavailable to kynurenine pathway enzymes, fluctuations in albumin and free fatty acids (which displace tryptophan from albumin) might plausibly lead to an over- or underestimation of kynurenine pathway activity in vivo [61]. While serum triglycerides are known to increase in the third trimester, they tend to return to prepregnancy levels by 6 weeks postpartum [62]. Thus, changes in albumin or triglycerides are unlikely to explain the sustained increased KT ratio for 6–9 months postpartum that we observed.

The clinical significance of postpartum increases in kynurenine pathway activity is only partially understood but, in the context of HIV, is likely to be highest with regard to TB risk. Indeed, TB risk is known to be increased for at least 9 months postpartum in women with and without HIV, strikingly paralleling the prolonged postpartum kynurenine pathway elevations we observed [26–29]. Furthermore, the kynurenine pathway has been directly linked to TB pathogenesis. In animal models, increased IDO expression (and higher KT ratio) correlates with higher mycobacterial burden, and IDO inhibition increases TB killing, decreases disease progression, and reorganizes granulomas, allowing lymphocyte trafficking to infected macrophages [33, 35, 38]. KT ratio also strongly predicts incident and prevalent active and latent TB [35, 37, 38]. KT ratio also strongly predicts mortality in PWH, particularly in settings where TB is prevalent [18, 20, 36, 40]. The APPRISE trial demonstrated isoniazid preventive therapy (IPT) in PWWH to be particularly beneficial in those with low CD4+ T-cell counts [63]. As higher KT ratios have been associated with slower CD4+ T-cell recovery, the benefit of IPT in this setting could be in part a surrogate for those with increased kynurenine pathway

activity and thus heightened TB risk. Given the public health importance of TB in PWWH, IDO activity may be a plausible putative driver in this setting and a viable therapeutic target for TB prevention efforts [63–65]. Postpartum kynurenine pathway activity has also been previously linked to postpartum depression, as tryptophan depletion may decrease serotonin synthesis in the brain [66, 67]. While we observed a link between KT ratio and depressive symptoms in UARTO previously [68], we did not observe a further increase in depressive symptoms postpartum among PWWH [69].

Our study had several limitations. First, our relatively small sample size may have limited our ability to detect clinically significant changes in some biomarkers. Additionally, we did not measure gestational age by last menstrual period, but rather by reported delivery dates, which could induce bias based on differential carriage to term. Third, we were unable to adjust for other confounders beyond ART duration that are known to impact markers of inflammation. Fourth, we did not measure free (ie, protein-unbound) tryptophan or its mediators. Finally, the study was too small to evaluate the association between biomarker changes and clinical outcomes, and we specifically excluded women with acute illnesses to avoid confounding by these factors (ie, acute infections are likely to increase inflammatory markers), so the clinical relevance of our findings remains unclear.

In summary, we found that most immune activation markers decline during pregnancy and several stay suppressed for up to 1 year postpartum, but only the kynurenine pathway of tryptophan catabolism increases in the postpartum period, remaining elevated above prepregnancy baseline for at least 9 months. Peripartum and postpartum kynurenine pathway induction could plausibly explain the increased risk of infection-related complications, particularly TB, in PWWH and even pregnant women without HIV. These data further support the kynurenine pathway as a relevant interventional target in future studies.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. All authors contributed to the interpretation of the data and the editing of the manuscript. S.R.S. performed data analysis and wrote the first draft of the manuscript. P.W.H. conceived of and designed the study, obtained funding, coordinated the study team, assisted with biospecimen quality assurance procedures, performed the primary data analysis, and mentored S.R.S. on the analysis, interpretation, and writing of the manuscript. H.B. organized the selection and shipment of plasma samples and contributed to the interpretation of the data. Y.B., J.E.H., A.K., and D.R.B. oversaw the operations of the UARTO cohort from which participant specimens were sampled, including the recruitment and retention of participants, clinical and questionnaire data, and specimen biobanking. D.R.B. obtained funding for the UARTO cohort. J.N.M. oversaw data management and quality assurance procedures for clinical, questionnaire, and biospecimens in the UARTO cohort, helped design the study instruments, and oversaw specimen shipping and distribution. J.K., L.T.M., and A.K. oversaw the reproductive health questionnaires and testing procedures and helped characterize the timing of pregnancy for the participants. T.H.B., Y.H., R.P.T., D.M., and M.M.L. oversaw biomarker measurements and quality assurance procedures in their laboratories.

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