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Systemic inflammation in pregnant women with HIV: relationship with HIV treatment regimen and preterm delivery

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Objective: HIV treatment regimen during pregnancy was associated with preterm delivery (PTD) in the PROMISE 1077 BF trial. Systemic inflammation among pregnant women with HIV could help explain differences in PTD by treatment regimen. We assessed associations between inflammation, treatment regimen, and PTD.

Design/methods: A nested 1 : 1 case–control study ($N = 362$) was conducted within a multicountry randomized trial comparing three HIV regimens in pregnant women: zidovudine alone, or combination antiretroviral therapy (ART) with lopinavir/ritonavir and either zidovudine or tenofovir. Cases were women with PTD (<37 weeks of gestational age). The following inflammatory biomarkers were measured in plasma samples using immunoassays: soluble CD14 (sCD14) and sCD163, intestinal fatty acid-binding protein, interleukin (IL)-6, interferon γ, and tumor necrosis factor α. We fit regression models to assess associations between second trimester biomarkers (measured before ART initiation at 13–23 weeks of gestational age and 4 weeks later), treatment regimen, and PTD. We also assessed whether inflammation was a mediator in the relationship between ART regimen and PTD.

Results: Persistently high interleukin-6 was associated with increased PTD. Compared with zidovudine alone, the difference in biomarker concentration between week 0 and week 4 was significantly higher ($P < 0.05$) for both protease inhibitor-based regimens. However, the estimated proportion of the ART effect on increased PTD mediated by persistently high biomarker levels was 5% or less for all biomarkers.

Conclusion: Persistently high IL-6 during pregnancy was associated with PTD. Although protease inhibitor-based ART was associated with increases in inflammation, factors other than inflammation likely explain the increased PTD in ART-based regimens compared with zidovudine alone.

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AIDS 2024, **38**:1111–1119

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Received: 27 October 2023; revised: 29 January 2024; accepted: 12 February 2024.

DOI:10.1097/QAD.0000000000003877

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Keywords: gut integrity, immune activation, mediation analysis, monocyte activation, prematurity, preterm birth

Introduction

Preterm birth (PTB) is the leading cause of global childhood morbidity and mortality [1]. Data from various settings show that pregnant women with HIV (WHIV) have higher rates of preterm delivery (PTD) than mothers without HIV [2,3]. Although various risk factors such as low $CD4^+$ T-cell count, high viral load, co-infections, and comorbidities likely contribute to this relationship [4–6], higher systemic inflammation is thought to be another contributing factor [7].

Among pregnant WHIV, general inflammatory markers (e.g. acute phase proteins and interleukins) as well as markers of gut dysfunction [e.g. intestinal fatty acid-binding protein (I-FABP)], microbial translocation (e.g. lipopolysaccharide-binding protein) and monocyte activation [e.g. soluble CD14 (sCD14) and sCD163] have been studied in the context of PTD [8–13]. There are conflicting data, with some studies showing an association for specific markers with PTD [8,9] whereas others did not observe an association [10–13]. However, these studies differed by important characteristics [e.g. antiretroviral treatment (ART) regimen and duration, time of assessment during pregnancy, study setting, and sample size]. Larger studies that account for these important factors, especially randomized controlled studies comparing various ART regimens to better understand the relationship of inflammation and PTD, are needed to better understand these discrepant results.

Studies have shown differences in PTD rates by maternal antiretroviral therapy regimen [3,14,15]. For example, data from the multicountry PROMISE (1077BF/FF) trial showed antenatal zidovudine (ZDV) alone was associated with lower PTD than were two protease inhibitor-based ART regimens [14] during the antepartum (1077AP) drug regimen randomization of the trial. Although the biological mechanisms that explain these findings are not clear, we hypothesized that specific maternal ART regimens (i.e. protease inhibitor-based ART regimens vs. ZDV alone) may have a differential impact on inflammation, which may partly explain the increased PTD risk with certain regimens. In other words, we hypothesized that the effect of maternal ART on PTD is partially mediated through inflammation.

To address these research gaps and to test our hypothesis, we conducted a nested case–control study within the IMPAACT 1077BF/1077FF 'PROMISE' trial, which was conducted in 14 sites in East and Southern Africa and 1 site in India. After accounting for timing of sample collection (all in second trimester) and HIV characteristics (ART regimen, duration on ART and $CD4^+$ cell count) in our study design, we assessed the relationship of inflammation at pre-ART and 4-weeks post-ART initiation with PTD. Leveraging the randomized design, we also studied the effect of ART regimen on inflammation levels and the potential mediating role of inflammation in the observed relationship between ART regimen and PTD.

Methods

Study design and population

The PROMISE 1077BF study [14] was an open-label, randomized, multisite, multicomponent clinical trial conducted in seven countries (India, Malawi, South Africa, Tanzania, Uganda, Zambia and Zimbabwe). In the antepartum component (PROMISE 1077AP) conducted between 2011–2014, pregnant women were randomized to one of three treatment arms: zidovudine and single-dose nevirapine plus a 1-to-2 week postpartum 'tail' of tenofovir and emtricitabine (ZDV+sdNVP+TRV tail, zidovudine alone) to reduce the risk of transmission, or combination ART with either zidovudine, lamivudine, and lopinavir-ritonavir (3TC-ZDV/LPV-RTV, zidovudine-based ART); or tenofovir, emtricitabine, and lopinavir-ritonavir (FTC-TDF/LPV-RTV, tenofovir-based ART). The primary efficacy outcomes were HIV transmission at 1 week of infant age (range 7–14 days after birth), along with maternal and infant safety (i.e. adverse events and adverse birth outcomes). Eligibility criteria for this study are detailed elsewhere [14], but included pregnancy of at least 13 weeks of gestational age and not in labor, $CD4^+$ cell count at least 350 cells/μl, no prior triple-drug ART, and no serious pregnancy complications. Women with active tuberculosis (TB), receiving TB or hepatitis B virus (HBV) treatment within 30 days of enrollment, with a fetus with serious congenital malformation were excluded from PROMISE [14].

The primary objectives of this study were to determine the association of inflammation during the second trimester of pregnancy with PTD in WHIV and to determine the effect of HIV treatment regimen with changes in maternal inflammation. We conducted a nested 1 : 1 case–control ($N = 362$; 181 cases and 181 controls) study. Cases were defined as all eligible women with a PTD (<37 weeks of gestational age) who were enrolled in PROMISE 1077AP. Controls were women from PROMISE 1077AP without PTD and were

randomly selected after stratifying by country of enrollment and gestational age categories. As this study was focused on second trimester inflammation (i.e. well before labor, a pro-inflammatory state, in the third trimester), women enrolled after 23 weeks' gestation were excluded; the rationale for the 23-week cutoff was because we wanted a second sample from 4 weeks (+/−7 days) after intervention to also be within the second trimester. Additional eligibility criteria for this analysis were that participants were HBV-negative, had a singleton live birth delivery, and had samples collected before and 4 weeks after intervention. Participants from Tanzania were excluded because of challenges with sample shipments out of country.

Ethics statement

Informed consent was obtained from all PROMISE 1077AP participants and US Department of Health and Human Services guidelines for human studies were followed. This study was approved by the institutional review boards of each participating institution.

Data collection

Detailed study procedures for PROMISE 1077AP are explained elsewhere [14]. In brief, after enrollment in PROMISE 1077AP, women were randomized into the three arms and the treatment continued for many participants through the postpartum period. Maternal study visits were at enrollment, week 4, and then every 4 weeks through delivery; and at labor and delivery. Data on sociodemographic information (e.g. age, education level), anthropometrics (i.e. BMI) and HIV data (e.g. WHO clinical stage) and pregnancy history (e.g. parity, history of previous preterm birth) were collected at the initial and relevant follow-up visits. Laboratory values for complete blood count, $CD4^+$ cell count, HIV viral load and hemoglobin were also obtained.

At the labor and delivery visit, infant gestational age was obtained. PTD was defined as a delivery before 37 weeks of gestation. Gestational age at delivery was determined by Ballard examination done by a pediatrician trained on the Ballard [16]; if Ballard score was not available, gestational age was determined by obstetrical estimate (if available) or date of last menstrual period in a hierarchical approach.

Laboratory procedures

Blood samples from study participants were collected in ethylenediamine tetraacetic acid tubes at select visits, including at entry and week 4 postrandomization. Plasma samples were isolated using standardized procedures at each study site and stored at −80 °C until further use. Inflammatory immune markers (i.e. biomarkers) for this study were measured using immunoassays of plasma samples. Using stored PROMISE plasma samples available at the NIAID BRI repository in Rockville MD, MSD multiplex ELISAs (U-Plex, Meso Scale Discovery, Rockville, USA) measured levels in duplicates of interleukin (IL)-6, interferon γ (IFNγ) and tumor necrosis factor α (TNFα). Single-plex ELISAs (Quantikine; R&D Systems, Minneapolis, MN, USA) measured levels, with 20% run in duplicates, of markers for monocyte activation (sCD14 and sCD163) and gut barrier function (I-FABP). The immunoassays were conducted at the Lederman laboratory at Case Western Reserve University.

Statistical analysis

Study population baseline characteristics were summarized by PTD status. Associations between immune markers and PTD were evaluated using multivariable conditional logistic regression models, stratified by enrolment gestational age categories and country. Separate analyses were conducted for each immune marker assessed using three different continuous measures: levels at baseline (i.e. enrolment/week 0/ART initiation), levels at 4 weeks (i.e. 4 weeks postenrollment/post-ART initiation), and change in inflammatory markers between the two time points. Inflammatory marker levels below the lower limit of detection (LLOD) were set to one-half the LLOD and levels above the upper limit of detection (ULOD) were set to twice the recorded measurement.

As prespecified supplemental analyses for PTD outcomes, inflammatory markers were also analyzed as categorical variables. At baseline and 4 weeks, inflammation levels were classified as 'high' inflammation for values in the highest quartile or 'not high' (alternatively, 'low') for values in the lower three quartiles. Enrollment and postenrollment measurements were pooled to determine the quartiles. For analysis involving both time points, we created a categorical variable with four levels: persistently low (i.e. lower three quartiles at both times), persistently high, increasing (i.e. increases from lower three quartiles at baseline to highest quartile at 4 weeks postenrollment), and decreasing. Multivariable models adjusted for the following baseline variables that were selected based on directed acyclic graphs: treatment arm, maternal age, BMI, $CD4^+$ cell count, $CD8^+$ cell count, parity, HIV-1 viral load, hemoglobin levels, education, and history of PTD. Models involving the change in immune markers over 4 weeks also adjusted for immune marker levels at baseline. No adjustments were made for multiple comparisons.

To evaluate the association between treatment regimen and change in inflammatory marker from week 0 to week 4, we fit weighted multivariable parametric regression models for interval-censored data assuming a normally distributed error term. This approach was chosen to account for censoring of measurements below the LLOD and above the ULOD. Models adjusted for baseline inflammation, age, BMI, $CD4^+$ cell count, $CD8^+$ cell count, country, education level, gestational age at entry, HIV viral load, and hemoglobin levels. Inverse-probability weight methods were used to account for the oversampling of cases because of the case–control design. We also conducted a causal

mediation analysis to estimate the indirect effects of maternal ART regimen on PTD via maternal inflammation. The mediator variable was a binary variable indicating persistently high inflammatory marker levels across both time points. Additional details regarding the mediation analysis are included in the Supplement, http://links.lww.com/QAD/D138.

In all multivariable regression analyses, a multiple imputation chained equation procedure was used to impute missing baseline variables (other than biomarkers). All analyses were conducted in SAS version 9.

Results

Study population characteristics

In our nested study of 362 pregnant women from 6 countries, the majority of participants were enrolled from South Africa (43%) and Malawi (35%). Median [quartile 1 (Q1), quartile 3 (Q3)] age of all selected women was 25.5 (21.9, 28.7) years (Table 1). For HIV treatment, 176 (49%) participants were randomly assigned to zidovudine-based ART, 145 (40%) to zidovudine alone, and 41 participants (11%) to tenofovir-based ART. At enrollment, most women were at WHO clinical stage I (97%). Median (Q1, Q3) $CD4^+$ cell count was 530 cells/μl (423, 643) and median HIV-1 RNA was 4.0 $\log_{10}$ copies/ml (3.4, 4.5) (Table 1). Most participants had at least one previous pregnancy (75%), with only 4% having a previous preterm delivery. Additional baseline demographics are summarized in Table 1.

Although most characteristics were similar by cases and controls, there were modest imbalances with respect to HIV treatment and age. The proportion assigned to zidovudine-based triple ART was higher among participants with PTD (56%) compared with participants without PTD (41%), consistent with our previous findings from this study [14]. The median age was higher among participants with PTD (26.3 years) compared with participants without PTD (24.2 years) (Table 1).

Table 1. Study population characteristics by preterm delivery status.

Characteristic	Preterm delivery (<37 weeks)		
	Yes ($N=181$)	No ($N=181$)	Total ($N=362$)
Country			
India	8 (4%)	8 (4%)	16 (4%)
Malawi	64 (35%)	64 (35%)	128 (35%)
South Africa	78 (43%)	78 (43%)	156 (43%)
Uganda	18 (10%)	18 (10%)	36 (10%)
Zambia	8 (4%)	8 (4%)	16 (4%)
Zimbabwe	5 (3%)	5 (3%)	10 (3%)
Randomization arm			
Triple ARV (3TC-ZDV/LPV-RTV)	102 (56%)	74 (41%)	176 (49%)
Triple ARV (FTC-TDF/LPV-RTV)	22 (12%)	19 (10%)	41 (11%)
ZDV+sdNVP+TRV tail	57 (31%)	88 (49%)	145 (40%)
WHO HIV stage			
Clinical stage I	176 (97%)	175 (97%)	351 (97%)
Clinical stage II	3 (2%)	6 (3%)	9 (2%)
Clinical stage III	2 (1%)	0 (0%)	2 (1%)
$CD4^+$ cell count (cells/μl)	521 (425, 664)	543 (423, 623)	530 (423, 643)
$CD8^+$ cell count (cells/μl)	827 (608, 1,098)	854 (639, 1,083)	839 (622, 1,087)
HIV viral load ($\log_{10}$-copies/ml)	4.0 (3.3, 4.6)	3.9 (3.4, 4.4)	4.0 (3.4, 4.5)
Age (years)	26.3 (22.0, 29.3)	24.2 (21.9, 28.3)	25.5 (21.9, 28.7)
BMI (kg/m^2)	25.2 (22.7, 28.3)	24.9 (22.8, 29.2)	25.1 (22.7, 28.7)
Gestational age (weeks)	19.1 (16.9, 21.3)	19.1 (17.0, 21.3)	19.1 (17.0, 21.3)
Parity	1 (1, 2)	1 (0, 2)	1 (0, 2)
0	45 (25%)	46 (25%)	91 (25%)
1 or more	136 (75%)	135 (75%)	271 (75%)
Hemoglobin (g/dl)	11.1 (10.2, 11.8)	11.2 (10.5, 11.8)	11.1 (10.4, 11.8)
Education			
Primary school or less	59 (61%)	67 (59%)	126 (60%)
Secondary school or above	37 (39%)	46 (41%)	83 (40%)
Missing	85	68	153
History of PTD			
Yes	9 (5%)	7 (4%)	16 (4%)
No	134 (74%)	133 (73%)	267 (74%)
Unknown	38 (21%)	41 (23%)	79 (22%)

Study population characteristics are shown overall and by preterm delivery (PTD) status, defined as delivery at less than 37 weeks of gestational age. Numbers (*N*) and percentage (%) are shown for categorical variables, whereas median and interquartile range (Q1, Q3) are shown for continuous variables. $CD8^+$ cell count measurements were missing for 146 participants (73 PTD, 73 non-PTD). ART, antiretroviral therapy; 3TC-ZDV/LPV-RTV, zidovudine, lamivudine, and lopinavir-ritonavir (zidovudine-based ART); FTC-TDF/LPV-RTV tenofovir, emtricitabine, and lopinavir-ritonavir (tenofovir-based ART); ZDV+sdNVP+TRV tail, tenofovir and emtricitabine (zidovudine alone).

Association of maternal inflammation with preterm delivery

Inflammatory marker levels at baseline and week 4 are summarized in Supplementary Table 1, http://links.lww.com/QAD/D138. In the primary analyses on the association of immune marker concentrations with PTD, there were no statistically significant associations with PTD for any of the inflammatory markers at baseline, 4 weeks, or change from baseline to 4 weeks (Table 2), but some notable odds ratios were observed. These included those for baseline TNFα [adjusted odds ratio [OR]: 2.15 per 1 $\log_{10}$ unit increase; 95% CI 0.87–5.28] and IL-6 (OR: 1.61; 95% CI 0.71–3.64), and week 4 TNFα (OR: 2.29; 95% CI 0.86–6.11) and IL-6 (OR: 1.85; 95% CI 0.77–4.44) (Table 2).

In supplementary analyses of the association of immune marker categories with PTD using conditional logistic regression, there were similar findings of increased odds of PTD with several immune markers at baseline or week 4 (Supplementary Table 2, http://links.lww.com/QAD/D138). However, these associations were not statistically significant. In the categorical analysis involving both time points of baseline and week 4, odds of PTD for participants with persistently high levels of IL-6 resulted in a statistically significant 2.47-fold increase in the odds of PTD compared with those with persistently low levels of IL-6 (95% CI 1.16–5.29; Table 3). Of note, 17.4% of those with PTD had persistently high IL-6 while only 7.9% of those without PTD had persistently high IL-6. Although persistently high levels of the other markers (except for sCD14) were also associated with increased odds of PTD, these relationships were not statistically significant (Table 3).

Treatment regimen and inflammation

Median immune marker concentrations were higher at week 4 compared with week 0 for all six markers among participants on tenofovir-based ART and four markers (I-FABP, IFNγ, IL-6, and sCD14) among participants on zidovudine-based ART (Supplementary Table 3, http://links.lww.com/QAD/D138). For participants on zidovudine alone, median immune marker concentrations were lower at week 4 for all markers (Supplementary Table 3, http://links.lww.com/QAD/D138).

Maternal ART regimen was associated with changes in immune marker concentrations for five of the six immune markers (I-FABP, IFNγ, IL-6, TNFα, and sCD14; Fig. 1). Compared with zidovudine alone, the adjusted mean difference in concentration between week 0 and week 4 was significantly higher in the tenofovir-based ART arm for concentrations of I-FABP (0.25 $\log_{10}$ pg/ml; 95% CI 0.19–0.31), IFNγ (0.13 $\log_{10}$ fg/ml; 95% CI 0.06–0.21), TNFα (0.04 $\log_{10}$ fg/ml; 95% CI 0.01–0.06), and sCD14 (0.09 $\log_{10}$ ng/ml; 95% CI 0.07–0.11) (Fig. 1). Compared with zidovudine alone, those on zidovudine-based ART had a higher adjusted mean difference in concentrations between week 0 and week 4 for I-FABP (0.11 $\log_{10}$ pg/ml; 95% CI 0.07–0.14), IFNγ (0.12 $\log_{10}$ fg/ml; 95% CI 0.08–0.17), IL-6 (0.05 $\log_{10}$ fg/ml; 95% CI 0.02–0.07), and sCD14 (0.08 $\log_{10}$ ng/ml; 95% CI 0.07–0.09) (Fig. 1).

In formal causal mediation analyses, the estimated proportion of the ART effect on increased PTD mediated by persistently high biomarker levels was 5% or lower for all biomarkers (Supplementary Table 4, http://links.lww.com/QAD/D138). This suggests that there is insufficient evidence that any of the immune markers, including persistently high IL-6, mediates the relationship between maternal ART regimen and PTD.

Discussion

In this multicountry study of pregnant WHIV initiating HIV treatment, we examined the association of second trimester maternal inflammation with PTD. Among various systemic inflammation and gut function markers tested as continuous and categorical variables, we observed that only persistently high IL-6 at treatment

Table 2. Adjusted odds ratios for preterm delivery by immune marker concentration.

	Week 0		Week 4 post-ART initiation		Change (week 4-week 0)	
Immune marker	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
I-FABP	0.96 (0.40–2.31)	0.92	1.06 (0.50–2.24)	0.88	1.03 (0.45–2.37)	0.95
IFNγ	1.08 (0.58–2.02)	0.81	1.56 (0.86–2.82)	0.15	1.68 (0.88–3.21)	0.11
IL-6	1.61 (0.71–3.64)	0.25	1.85 (0.77–4.44)	0.17	1.75 (0.62–4.98)	0.29
TNFα	2.15 (0.87–5.28)	0.097	2.29 (0.86–6.11)	0.098	1.94 (0.39–9.73)	0.42
sCD14	0.96 (0.14–6.46)	0.96	1.50 (0.25–8.84)	0.65	2.70 (0.19–38.11)	0.46
sCD163	0.90 (0.29–2.85)	0.86	1.87 (0.49–7.15)	0.36	4.40 (0.63–30.91)	0.14

Odds ratios (ORs) greater than 1 indicate higher odds of PTD for a 1-$\log_{10}$-unit increase in immune marker concentration. Units for all cytokines are $\log_{10}$-fg/ml, for monocyte activation biomarkers are $\log_{10}$-ng/ml, and for I-FABP are $\log_{10}$-pg/ml. Multivariable conditional logistic models stratified by enrollment gestational age and country and adjusted for treatment, age, BMI, parity, previous preterm delivery, hemoglobin, viral load, $CD8^+$ cell count, education level, and $CD4^+$ cell count. A multiple imputation procedure was used to impute missing values for education level and $CD8^+$ cell count.

Table 3. Adjusted odds ratios for preterm delivery by categorized immune marker levels at week 0 and week 4.

Immune Marker	Categories	OR (95% CI)	*P* value
I-FABP	Persistently low	Ref.	
	Increasing	1.49 (0.81–2.77)	0.20
	Decreasing	0.73 (0.33–1.62)	0.44
	Persistently high	1.05 (0.50–2.21)	0.89
IFNγ	Persistently low	Ref.	
	Increasing	0.83 (0.42–1.63)	0.59
	Decreasing	0.77 (0.39–1.54)	0.46
	Persistently high	1.72 (0.79–3.72)	0.17
IL-6	Persistently low	Ref.	
	Increasing	0.96 (0.45–2.05)	0.91
	Decreasing	0.96 (0.49–1.89)	0.91
	Persistently high	2.47 (1.16–5.29)	0.020
TNFα	Persistently low	Ref.	0.63
	Increasing	0.78 (0.28–2.16)	
	Decreasing	2.32 (0.92–5.88)	0.076
	Persistently high	1.32 (0.69–2.50)	0.40
sCD14	Persistently low	Ref.	
	Increasing	1.16 (0.62–2.17)	0.65
	Decreasing	1.38 (0.46–4.19)	0.57
	Persistently high	0.94 (0.48–1.83)	0.85
sCD163	Persistently low	Ref.	
	Increasing	0.90 (0.29–2.78)	0.85
	Decreasing	1.18 (0.46–3.02)	0.73
	Persistently high	1.45 (0.79–2.67)	0.23

ORs greater than 1 indicate higher odds of PTD for each category vs. persistently low inflammation. Multivariable conditional logistic models stratified by enrolled country and gestational age and adjusted for treatment, age, BMI, parity, previous preterm delivery, hemoglobin, viral load, $CD8^+$ cell count, education level, and $CD4^+$ cell count. A multiple imputation procedure was used to impute missing values for education level and $CD8^+$ cell count. Categories are: ' Persistently low' if concentration levels at week 0 and week 4 were in the lower three quartiles, 'Increasing' if the concentration level went from the lower three quartiles at week 0 to the uppermost quartile at week 4, 'Decreasing' if the concentration level went from the uppermost quartile at week 0 to the lower three quartiles at week 4, and ' Persistently High' if the concentration level stayed in the highest quartile at week 0 and week 4.

initiation and 4 weeks postinitiation was strongly associated with increased odds of PTD. We also observed differences in inflammation levels by ART regimen, with higher levels of posttreatment in the maternal ART (tenofovir-based or zidovudine-based) arms as compared with zidovudine only. However, this increased inflammation did not explain our earlier observed relationship between maternal ART regimen and PTD in formal mediation analyses. Overall, our results suggest that select inflammatory markers (i.e. persistently high IL-6) in the second trimester are associated with PTD, and maternal ART regimen are linked to increases in levels of various inflammatory markers; however, inflammation does not mediate the effect of ART regimen on PTD.

In our study, only persistently high IL-6 was associated with PTD. Other studies in nonpregnant adults with HIV also suggest an important role for IL-6, where higher levels at time of early HIV infection was one of the strongest predictors of adverse clinical outcomes [17]. Relevant to pregnancy, we also observed an association of IL-6 (third trimester) with PTD in another study conducted in women with and without HIV from India [10], consistent with data from other studies in the general populations without HIV [18]. Biologically, it is known that IL-6 is involved in pro-inflammatory parturition processes, including by recruiting leukocytes that lead to delivery, and it is hypothesized that high levels earlier in pregnancy might result in PTD [18]. Other studies in pregnant WHIV, however, did not observe an association of IL-6 with PTD [11,13]. Although the reason for this inconsistency is not clear, it is likely because of differences in study design. Our study population, obtained from a randomized trial, was different from the two cohort studies that did not detect an association with IL-6. Important differences from these studies included our study population of ART-naive pregnant WHIV who initiated ART during the second trimester of pregnancy, and samples were all obtained in the second trimester prior to and exactly 4 weeks after ART initiation. These differences, along with our analytical approaches (e.g. a categorical analysis using persistently high levels as a variable was statistically significant whereas continuous variables had nonsignificant associations) could potentially explain the discrepancy in findings.

Consistent with other recent studies in people with HIV on ART, we did not observe a statistically significant association of inflammatory markers other than IL-6 (acute phase proteins, other cytokines and gut inflammation markers) and PTD [8,10,12]. This is in contrast to two earlier studies from Spain and India, which showed that WHIV with higher levels of intestinal barrier dysfunction, microbial translocation and monocyte activation had higher rates of PTD [8,9]. However, these were small studies; and women in the study in India were not on ART. Recent larger studies have not seen this

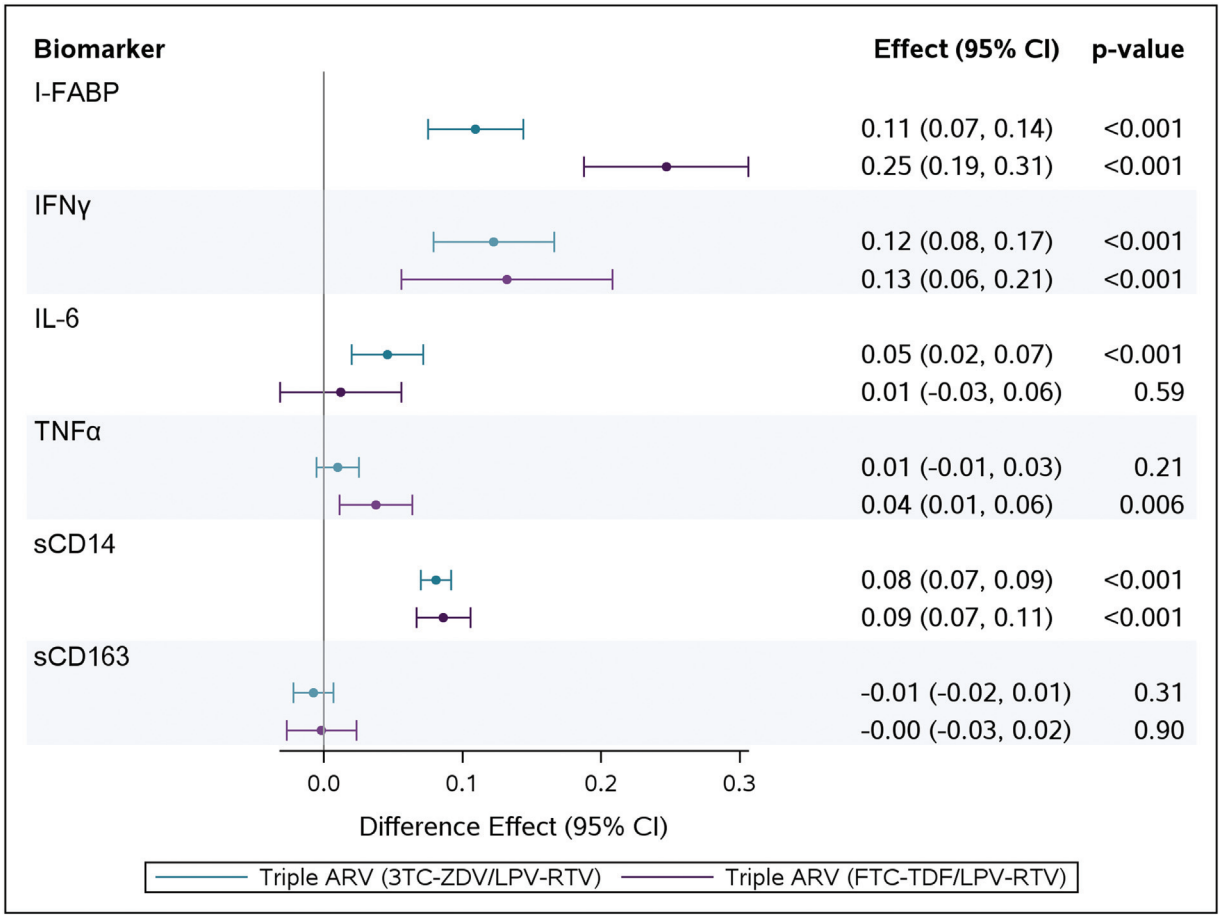


Fig. 1. Forest plot for treatment effect on change in immune marker concentrations. All estimates use ZDV+sdNVP+TRV tail as reference treatment group. Models additionally adjusted for baseline inflammation, age, BMI, gestational age at entry, country, education level, $CD4^+$ and $CD8^+$ cell counts, hemoglobin, and HIV-1 RNA. Units for all cytokines are log_{10}-fg/ml, for monocyte activation biomarkers are log_{10}-ng/ml, and for I-FABP are log_{10}-pg/ml. 3TC-ZDV/LPV-RTV, zidovudine, lamivudine, and lopinavir-ritonavir (zidovudine-based ART); FTC-TDF/LPV-RTV, tenofovir, emtricitabine, and lopinavir-ritonavir (tenofovir-based ART); ZDV+sdNVP+TRV tail, tenofovir and emtricitabine (zidovudine alone).

association [8,10,12], in line with our findings in this study. As the women in these more recent studies were on ART, one possible explanation could be that the partial resolution of gut dysfunction with ART might be sufficient to reduce risks related to PTD. Alternatively, as described with IL-6 above, the discrepancy between gut markers and PTD could be related to differences in other factors such as study design, timing of sample collection during pregnancy or ART/HIV factors. It should also be noted that several of these immune markers did have an association with increased PTD in our study. Notably, we observed a greater than two-fold higher odds of PTD for participants with high TNF-alpha levels at both week 0 and week 4. It is possible that there is a true association between other immune markers and PTD, but our study had insufficient power to detect it.

We also observed higher inflammation after treatment initiation for the tenofovir-based and zidovudine-based ART arms. In nonpregnant ART-naive adults, a reduction in soluble markers IL-6 and sCD14 was observed within 4 weeks of tenofovir/raltegravir/emtricitabine ART initiation, with further decreases over the first year [19]. Over longer periods (e.g. 24 and 48 weeks) post-ART initiation, reduction of multiple soluble inflammatory markers have also been generally observed compared with baseline in nonpregnant adults, although some studies did not show a significant difference for specific soluble markers or at specific time points (e.g. 24 vs. 48 weeks) [19–21].

Data on changes in inflammation is limited in ART-naive pregnant women initiating ART [22], and it is unclear whether the differences between pregnant women and nonpregnant adults is due to pregnancy-related factors or HIV-related factors. Pregnancy is characterized by immune changes, and particularly the second trimester of pregnancy is considered to be an 'anti-inflammatory

state' [23]. It is possible that the interaction of pregnancy and more specifically second trimester-related immune profile with HIV or ART initiation results in increases in inflammation, at least in the short-term, in contrast to nonpregnant adults who have reductions. There is data to suggest that levels of some markers, including IL-6 and I-FABP, increase over time during pregnancy in WHIV [22]. Alternatively, or in addition, the discrepant results between the populations could also be due to factors such as the specific ART regimen, timing of assessment (i.e. within 4 weeks of ART initiation) and study population characteristics. Related to ART regimen, it is possible that use of lopinavir/ritonavir-based regimen might partly explain the increased inflammation (e.g. potentially through its lipid-raising effects [24]), as a study in nonpregnant adults with HIV showed an increase in soluble markers including TNFα after initiating lopinavir/ritonavir-based treatment [25]. Further studies in pregnant women initiating ART could help us better understand these results. We also note that I-FABP levels were higher in the TDF-based ART regimen compared with zidovudine-based ART, a finding that has previously been reported in an HIV prevention trial [26], although future studies are needed to confirm this and understand the potential mechanisms.

Our findings of persistently elevated IL-6 being associated with PTD and also being higher in ART-based regimens raised the possibility that IL-6 could be a potential mediator in the observed relationship in the parent PROMISE trial between ART-based regiments and increased PTD as compared with zidovudine alone. However, formal mediation analyses suggested that inflammatory markers (including IL-6) had only minimal mediating effects. This suggests that increased inflammation does not explain the increased PTD with ART-based regimens. We do want to point out that these findings do not preclude IL-6 or other inflammatory markers having a mediating role for other adverse perinatal outcomes (e.g. low birth weight) or for preterm birth at other time points in pregnancy.

Our study has some limitations, including potential limited generalizability to other periods of pregnancy (e.g. third trimester), a limited set of inflammatory markers assessed only in plasma samples (i.e. no data on biomarker production by specific immune cells), potential unmeasured confounders, and wide confidence intervals in select mediation analyses, and no data on the effect of treatment on inflammation at times beyond 4 weeks post-ART initiation. Despite these limitations, our study also has a number of strengths including nesting within a multicountry randomized controlled trial where HIV treatment regimen was randomized, standardization of timing of sample collection in second trimester, longitudinal data, a large sample size of PTD, and assessment of inflammatory markers relevant in pregnancy and HIV.

In conclusion, we observed that persistently elevated IL-6, but not other markers, during the second trimester of pregnancy was associated with PTD in a population of WHIV initiating ART. Although initiation of ART regimens resulted in increases in inflammation, our results indicate that this increased inflammation is not a mediator between the observed relationship of ART regimens and increased PTD. This suggests the importance of factors other than inflammation in terms of risk of PTD, and should be addressed in future studies.

Acknowledgements

The authors thank the study participants for their time and contributions. We also thank the PROMISE study team and the sites, the IMPAACT leadership and the support from pharmaceuticals and NIH.

Data availability agreement: the data is not publicly available because of the restrictions in the study's informed consent documents and in the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network's approved human subject protection plan. However, data are available to all interested researchers upon request to the IMPAACT Statistical and Data Management Center's data access committee (e-mail address: sdac.data@fstrf.org or sdac.data@sdac.harvard.edu) with the agreement of the IMPAACT Network.

Funding: overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID) with co-funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (NIMH), all components of the National Institutes of Health (NIH), under Award Numbers UM1AI068632-15 (IMPAACT LOC), UM1AI068616-15 (IMPAACT SDMC) and UM1AI106716-15 (IMPAACT LC), and by NICHD contract number HHSN275201800001I. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The study products were provided free of charge by Abbott, Gilead Sciences, Boehringer Ingelheim, and GlaxoSmithKline.

Author's contributions: R.S. conceived of the research question, obtained funding, led this study's implementation, helped guide the analysis and wrote the primary version of the manuscript. M.J.G., R.K., and S.B. helped with the study design and conducted the data analysis for this project. They also contributed to the interpretation of study findings and manuscript writing. M.L. and D.M. led the laboratory analysis and contributed to the study design, implementation, interpretation, and manuscript

review. S.D., D.M., A.V., L.C., M.O., and T.E.T. were key leading investigators of the parent PROMISE study and contributed to manuscript review and interpretation. A.G., J.S.C., and M.G.F. helped obtain funding, contributed to the study's design, implementation, interpretation, and manuscript review, and were also key leading investigators of the parent PROMISE study. All authors read and approved the final version of the manuscript. All authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE) and were fully responsible for all aspects of manuscript development.

Conflicts of interest

The authors have no relevant conflicts of interest to declare.

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