Sex-Related Differences in Inflammatory and Immune Activation Markers Before and After Combined Antiretroviral Therapy Initiation

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Background: Women progress to death at the same rate as men despite lower plasma HIV RNA (viral load). We investigated sex-specific differences in immune activation and inflammation as a potential explanation.

Methods: Inflammatory and immune activation markers [interferon γ, tumor necrosis factor (TNF) α, IL-6, IL-18, IFN-γ–induced protein 10, C-reactive protein (CRP), lipopolysaccharide, and sCD14] were measured at weeks 0, 24, and 48 after combination antiretroviral therapy (cART) in a random subcohort (n = 215) who achieved virologic suppression in ACTG A5175 (Prospective Evaluation of Antiretrovirals in Resource-Limited Settings). Association between sex and changes in markers post-cART was examined using random effects models. Average marker differences and 95% confidence intervals were estimated using multivariable models.

Results: At baseline, women had lower median $\log_{10}$ viral load (4.93 vs 5.18 copies per milliliter, $P = 0.01$), CRP (2.32 vs 4.62 mg/L, $P = 0.01$), detectable lipopolysaccharide (39% vs 55%, $P = 0.04$), and sCD14 (1.9 vs 2.3 μg/mL, $P = 0.06$) vs men. By week 48, women had higher interferon γ (22.4 vs 14.9 pg/mL, $P = 0.05$), TNF-α (11.5 vs 9.5 pg/mL, $P = 0.02$), and CD4 (373 vs 323 cells per cubic millimeter, $P = 0.02$). In multivariate analysis, women had greater increases in CD4 and TNF-α but less of a decrease in CRP and sCD14 compared with men.

Conclusions: With cART-induced viral suppression, women have less reduction in key markers of inflammation and immune activation compared with men. Future studies should investigate the impact of these sex-specific differences on morbidity and mortality.

Key Words: HIV, inflammation, immune activation, sex, women, antiretroviral treatment

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INTRODUCTION

Fifty percent of people living with HIV are women; yet, sex-specific differences in HIV pathogenesis are poorly understood. Studies of sex-based differences are limited to a few studies. One notable sex difference is that women tend to have lower baseline viral loads (VL) than men before initiation of combination antiretroviral therapy (cART).[1–4] The sex difference in VL persists even with advanced HIV-1–related immunosuppression.[5] Despite this advantage, one study found that women with the same VL as men had 1.6 times the risk of progressing to AIDS.[1]

The exact mechanism behind this phenomenon is still unclear, but some hypothesize that differences in immune activation are responsible.[6] Immune activation is a known risk factor for HIV progression, independent of VL.[6] Interferon (IFN) α, for example, has been associated with immune activation and HIV progression.[7,8] A higher percentage of dendritic cells in women produced IFN-α in response to HIV stimulation compared with men, which was directly correlated with progesterone levels, suggesting a true sex-related mechanism.[9] More robust immune activation in HIV-infected women than in men could explain why women progress to AIDS at the same rate as or faster than men despite lower baseline VLs. Other markers of inflammation and immune activation, such as C-reactive protein (CRP), sCD14, IL-6, and tumor necrosis factor (TNF) α, have been associated with increased progression and mortality in HIV-infected adults.[10–12] Studies on sex-related differences in these markers, however, are lacking.

In this study, we sought to (1) characterize inflammatory and immune activation markers in women vs men at weeks 0, 24, and 48 of cART and (2) compare changes in inflammatory and immune activation markers over time by sex with the hypothesis that women would derive less of a decrease in these markers than men after initiating cART. We used a diverse multinational cohort within the AIDS Clinical Trials Group A5175 Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) trial to assess sex differences in inflammation and immune activation.

METHODS

Study Description

PEARLS (ClinicalTrials.gov NCT00084136) was a phase 4, randomized open-label clinical trial conducted from 2005 to 2010 comparing initial cART regimens in 9 countries: Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, United States, and Zimbabwe.[13] Major enrollment criteria included age ≥18 years, HIV infection, CD4 cell count <300 cells per microliter, and ART naive by self-report and chart review (<7 days of cumulative drug exposure before enrollment). During 2005–2007, 1571 eligible adults were enrolled and randomly assigned to 1 of 3 treatment arms—Arm A: efavirenz + co-formulated zidovudine/lamivudine, n = 519; Arm B: unboosted atazanavir + didanosine + emtricitabine, n = 526; and Arm C; efavirenz + co-formulated emtricitabine–tenofovir disoproxil fumarate, n = 526. Study follow-up was completed in May 2010.

In New Works Concept Sheet 319, a random subcohort of 30 people from each country was selected to have additional analyses performed on their stored samples. Those who did not achieve virologic suppression (HIV-1 RNA <400 copies per milliliter) were excluded. Markers of inflammation [TNF-α, IFN-γ, IL-6, IL-18, IFN-γ–induced protein 10 (IP-10), and CRP] and microbial translocation/immune activation [lipopolysaccharide (LPS) and sCD14] were measured at baseline pre-cART initiation and at weeks 24 and 48 post-cART initiation. The methods for biomarker selection and measurement have been described previously.[14] In brief, we selected markers associated with accelerated HIV disease progression. Baseline marker concentrations were measured using stored plasma or serum collected within 14 days before cART initiation. Single laboratories performed testing in batches for each marker to mitigate variability in methodology. IFN-γ, IL-6, IL-18, and TNF-α were measured in plasma using a Luminex multiplex cytokine platform (R&D Systems, Inc, Minneapolis, MN); IP-10 was measured in plasma using a commercially available multiplex ELISA-based assay (Meso Scale Discovery, Rockville, MD). CRP was measured using ELISA (CRP Quantikine ELISA; R&D Systems), and microbial translocation markers were measured using commercially available ELISA kits for sCD14 (R&D Systems). LPS was measured from plasma samples using a Limulus Amebocyte Lysate assay (LONZA, Walkersville, MD) with previously described modifications.[15]

Analysis

Baseline characteristics of men and women were compared using the nonparametric Mann–Whitney test for continuous variables or Fisher exact test for discrete variables. Plasma LPS was undetectable for most of the study participants; so, we defined the cutoff as detectable vs undetectable. The remaining measured markers were available as continuous data and were analyzed as such.

Effects of sex, as a primary risk factor, on changes in markers pre- and post-cART initiation were examined using random effects models. Average marker differences and 95% confidence intervals by sex were estimated using multivariable models adjusting for age, country, region, income category (country), body mass index (BMI), baseline CD4, $\log_{10}$ VL, hemoglobin (Hb), and randomized cART arm. A 2-way hierarchical cluster analysis (Ward method) of circulating biomarkers by time point was also performed. Comparison of markers between time points was assessed by the nonparametric Friedman test (matched samples), except for LPS, which was compared as frequency of detectable values between the groups. Using 2-sided P values, statistical significance was defined as $P \leq 0.05$. To test the overall trend of variation in concentrations of each marker over time on cART, $\log_{10}$ transformed data were analyzed using Friedman test with a linear trend posttest. Comparison of markers between sexes at each time point after cART initiation was assessed by the Wilcoxon rank-sum test. The statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA), STATA 12.0 (StataCorp., TX), JMP 11.0 (SAS, Cary, NC), and R 3.1.0 (R Development Core Team, Auckland, New Zealand) programs.

RESULTS

We assessed a random sample of 215 individuals who achieved viral suppression by week 24 of cART and had available samples at 0, 24, 48 weeks. As shown in Table 1, there were 105 (48%) women and 110 (51%) men. At entry, women had similar CD4 counts as men (193 vs 168 cells per cubic millimeter, $P = 0.37$) and were more likely to be black ($P < 0.001$). Women also had significantly lower Hb (11.5 vs 13.7 g/dL, $P < 0.001$) and $\log_{10}$ baseline VL (4.93 vs 5.18 copies per milliliter, $P = 0.01$) but did not differ in age, BMI, or cART type (Table 1).

Differences in Biomarkers at Weeks 0, 24, and 48 by Sex

As shown in Figure 1, at week 0 of cART, women had significantly lower CRP levels (2.32 vs 4.62 mg/L, $P = 0.01$) and a lower percentage with detectable LPS (39% vs 55%, $P = 0.04$) compared with men. At week 0, women also had a trend toward lower sCD14 (1.9 vs 2.3 μg/mL, $P = 0.06$) and insignificantly lower levels of TNF-α, IL-6, IL-18, and IP-10 compared with men (see Supplemental Digital Content, http://links.lww.com/QAI/A842).

In contrast, at week 24, women no longer had significant differences in any of the markers measured, including CD4 (309 vs 292 cells per cubic millimeter, $P = 0.21$). Compared with men, TNF-α levels in women were higher (12.5 pg/mL in women vs 10.6 pg/mL in men, $P = 0.07$) and sCD14 (1.5 μg/mL vs 1.9 μg/mL, $P = 0.06$) and percent detectable LPS were lower (44% vs 57%, $P = 0.08$), though none of these differences reached statistical significance (see Supplemental Digital Content, http://links.lww.com/QAI/A842).

By week 48, women had developed a statistically significant CD4 advantage compared with men (373 vs 323 cells per cubic millimeter, $P = 0.02$). Despite this, women had higher median levels of TNF-α (11.5 vs 9.5 pg/mL, $P = 0.02$) and IFN-γ (22.4 vs 14.9 pg/mL, $P = 0.05$). Differences were not detected in IL-6, IL-18, IP-10, CRP, and percent detectable LPS (see Supplemental Digital Content, http://links.lww.com/QAI/A842). Levels of sCD14, however, had equalized between men and women by week 48. Figure 1 summarizes the changes in cytokine levels across all 3 time points for the full cohort (Figs. 1A, B) and also stratified by sex (Figs. 1C, D).

TABLE 1. Baseline Characteristics of the Random Subcohort, by Sex

		Random Subcohort (n = 215)		
	Overall (n = 215)	Male (n = 110)	Female (n = 105)	*P*
Median baseline age (IQR), yrs	35 (30–41)	36 (31–41)	34 (29–41)	0.25
Country, n (%)				
Brazil	26 (12)	21 (19)	5 (5)	<0.001
Haiti	23 (11)	12 (11)	11 (10)	
India	24 (11)	13 (12)	11 (10)	
Malawi	21 (10)	4 (4)	17 (16)	
Peru	27 (13)	17 (15)	10 (10)	
South Africa	26 (12)	9 (8)	17 (16)	
Thailand	29 (13)	11 (10)	18 (17)	
United States	19 (9)	14 (13)	5 (5)	
Zimbabwe	20 (9)	9 (8)	11 (10)	
Race, n (%)				
White	24 (11)	22 (20)	2 (2)	<0.001
Black	105 (49)	43 (39)	62 (60)	
Asian	53 (25)	24 (22)	29 (28)	
Other	32 (15)	21 (19)	11 (11)	
Treatment regimen, n (%)				
3TC/ZDV/EFV	78 (36)	39 (35)	39 (37)	0.74
ATV/FTC/DDI	70 (33)	34 (31)	36 (34)	
FTC/TDF/EFV	67 (31)	37 (34)	30 (29)	
Median baseline CD4 cell counts (IQR) (1/mm^3)	178 (86–230)	168 (86–229)	193 (109–230)	0.37
Baseline CD4 cell counts, n (%)				
CD4 cell count ≥ 200 (1/mm^3)	89 (41)	40 (36)	49 (47)	0.32
CD4 cell count 100–199 (1/mm^3)	67 (31)	37 (34)	30 (29)	
CD4 cell count < 100 (1/mm^3)	59 (27)	33 (30)	26 (25)	
Median baseline log VL (IQR)	5.07 (4.56–5.47)	5.18 (4.76–5.53)	4.93 (4.46–5.41)	0.01
Median baseline Hb	12.5 (11.1–13.8)	13.7 (12.4–14.5)	11.5 (10.5–12.6)	<0.001
Median baseline BMI	22 (20–25)	22 (20–24)	23 (20–26)	0.24

3TC, lamivudine; ATV, atazanavir; DDI, didanosine; EFV, efavirenz; FTC, emtricitabine; IQR, interquartile range; TDF, tenofovir; ZDV, zidovudine.

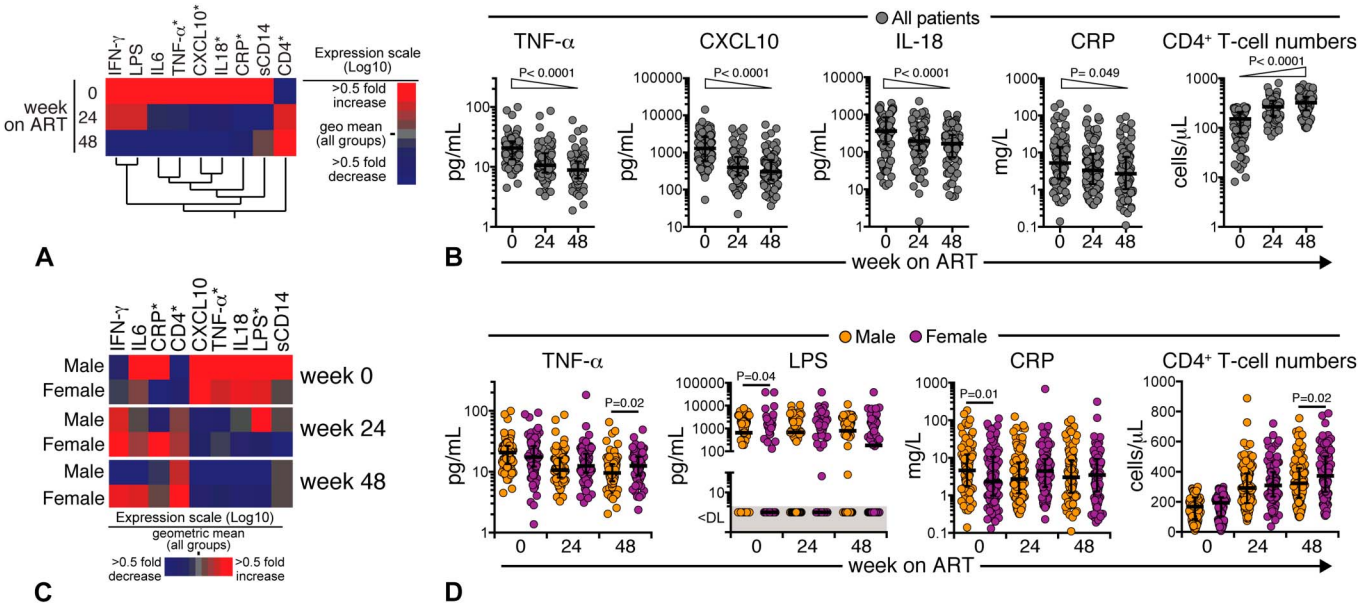

FIGURE 1. Plasma concentrations of biomarkers of inflammation in HIV-infected patients on ART initiation. Levels of indicated biomarkers were assessed in HIV-infected patients before and after ART initiation (n = 215). A, A heat map was designed to depict the overall pattern of expression of markers of inflammation and CD4 counts in patients at pre-ART and at weeks 24 and 48 after ART initiation. Patients at different study time points were listed in rows, and each biomarker was placed in a different column. Expression scale for each biomarker represents $\log_{10}$ fold change from the geometric mean of the entire study population at each time point. A 2-way hierarchical cluster analysis (Ward method) of circulating biomarkers by time point was performed. Asterisks indicate markers that exhibited statistically significant differences between the time points assessed by the nonparametric Friedman test (matched samples), except for LPS, which in this study was compared as frequency of detectable values between the groups. B, Scatter plots of the markers highlighted in (A). To test the overall trend of variation in concentrations of each marker over time on ART, $\log_{10}$ transformed data were analyzed using Friedman test with a linear trend posttest (*P* values are from the linear trend analysis ad hoc test). C, The data were reanalyzed to compare differences between male (n = 110) and female (n = 105) patients. Asterisks highlight markers that displayed statistically different comparisons between male and female patients at a certain time point on ART initiation assessed by the Wilcoxon rank-sum test. D, Scatter plots of the markers highlighted in (C). Data were compared using the Wilcoxon rank-sum test. In (B) and (C), lines represent median values and interquartile ranges (IQRs) (except for LPS scatter plot, which does not show IQR because there were many values below the detection limit of the assay).

Longitudinal Changes in Biomarkers by Sex

Women experienced an increase in percent detectable LPS between weeks 0 and 48, which trended toward significance (39% at week 0 vs 51% at week 48, $P = 0.08$) (see Supplemental Digital Content, http://links.lww.com/QAI/A842). In the multivariate random effects model adjusted for age, country, region, income category (country), cART regimen, Hb, BMI, and HIV VL, there were statistically significant sex-related differences over time with respect to CD4 count, TNF-α, sCD14, and CRP (Table 2). Women were more likely to have more of an increase over time in CD4 count and TNF-α levels but less likely to have a decrease in sCD14 and CRP levels over time compared with men.

DISCUSSION

Before cART initiation, women had a more favorable immune profile with higher CD4 counts and lower VL, CRP, detectable LPS, and sCD14 than men. By week 48, however, women had higher levels of IFN-γ and TNF-α compared with men, despite a higher CD4 count. Furthermore, men experienced a greater decrease in sCD14 and CRP levels over time, suggesting that women experience less of a cART-related reduction in inflammation and immune activation.

The median sCD14 among women in this study increased between weeks 24 and 48. Although it is impossible to know if these levels would have continued to rise, sCD14 is an important marker for monocyte/macrophage activation and has been linked to microbial translocation.[16] In the SMART study, increased sCD14 was associated with increased all-cause mortality.[12] The specific data on HIV progression are less definitive,[17–19] but increased sCD14 has been linked to hepatitis C virus progression in HIV-coinfected patients[20] and faster progression of carotid intima medial thickness in those with HIV.[21]

In many HIV studies, increases in sCD14 often occur in conjunction with increases in CRP and IL-6 levels. In our study, IL-6 levels in men steadily decreased after cART initiation in contrast to women, in whom IL-6 steadily increased. Similarly, CRP levels were lower at baseline but higher by week 48 in women vs men. In ACTG A5095, high-sensitivity CRP did not differ by gender at baseline but higher levels were seen at week 96 in women compared with men (6 vs 1.6 mg/L, $P = 0.001$), with an estimated shift in high-sensitivity CRP by gender of 2.5 mg/L.[22] CRP has been studied

TABLE 2. Longitudinal Analysis of Inflammatory Markers to Assess the Effect of Sex Using a Random Effects Model

	Univariable Model		Multivariable Model	
Biomarker	**Slope (95% CI)***	***P***	**Slope (95% CI)***	***P***
CD4†	12.66 (−8.30 to 33.63)	0.23	34.10 (9.01 to 59.18)	0.008
Log_{10} VL‡	−0.21 (−0.39 to −0.03)	0.03	−0.27 (−0.49 to −0.05)	0.02
IFN-γ§	−0.12 (−9.90 to 9.64)	0.97	3.57 (−8.09 to 15.23)	0.54
TNF-α§	1.37 (−1.38 to 4.13)	0.33	3.25 (−0.10 to 6.61)	0.05
IL-6§	−9.83 (−29.06 to 9.39)	0.31	−1.48 (−26.81 to 12.83)	0.90
IP-10§	25.92 (−241.16 to 293.00)	0.84	−103.68 (−442.67 to 235.30)	0.54
IL-18§	−44.38 (−114.80 to 26.04)	0.21	−21.76 (−110.14 to 66.60)	0.62
sCD14§	−219,304.2 (−405,530.9 to −33,077.4)	0.02	−172,507.9 (−270,215 to −74,800)	0.001
Detectable LPS‖, (odds ratio)	0.18 (0.03 to 0.97)	0.04	0.23 (0.03 to 1.45)	0.11
CRP§	−0.43 (−6.62 to 5.76)	0.89	−6.28 (−11.91 to −0.65)	0.03

*Parameters are mean differences (females − males).

†Longitudinal CD4 was modeled using random effects with random intercept and slope with exchangeable correlation for variance of random effects. The multivariable model was adjusted for age, country, treatment, BMI, and log_{10} VL.

‡Longitudinal markers were modeled using random effects with random intercept and slope with exchangeable correlation for variance of random effects. The multivariable model was adjusted for age, country, treatment, BMI, CD4 counts, and Hb.

§Longitudinal markers were modeled using random effects with random intercept and slope with exchangeable correlation for variance of random effects. The multivariable model was adjusted for age, country, treatment, BMI, CD4 counts, log_{10} VL, and Hb.

‖Longitudinal detectable LPS was modeled using a random effects logistic regression model to determine odds ratios. The multivariable model was adjusted for age, country, treatment, BMI, CD4 counts, log_{10} VL, and Hb.

CI, confidence intervals.

extensively in HIV populations with reports of increased HIV progression,[23,24] HIV treatment failure,[25] increased cardiovascular disease,[26] tuberculosis,[14] and increased risk of maternal mortality.[23]

There were also notable changes in other biomarkers. TNF-α is a pro-inflammatory cytokine that is associated with immune activation.[27] Increased levels of TNF-α before cART initiation have been associated with HIV progression.[28] Furthermore, people who progressed to AIDS 1 year after starting cART experienced a smaller decrease in the soluble TNF-α receptor compared with those who did not,[29] suggesting that cART-induced decreases in TNF-α are protective against HIV progression. Elevated levels of TNF-α in postpartum women in Botswana were also found to be predictive of major adverse clinical events, including AIDS-defining illnesses and death.[30] Therefore, the higher TNF-α levels seen in women compared with men by week 48 could have important long-term clinical implications.

The same could be said of IFN-γ, though the role of this cytokine in HIV progression is less clear. IFN-γ is primarily produced by CD4 cells and natural killer cells. Increased levels, then, may simply reflect an increase in CD4 count. In fact, some studies have shown that increased IFN-γ is associated with favorable outcomes, such as decreased disease progression[31,32] and decreased mother-to-child HIV transmission.[33] In early infection, though, higher IFN-γ has been implicated in setting a higher viral set point.[34,35] Increased IP-10 has also been associated with HIV progression.[36] By week 48, women in our study had significantly higher levels of IFN-γ and minimally higher levels of IP-10.

Differences in sex distribution at the study sites could contribute to sex differences in the markers measured. We analyzed the data by country, region, and income category to account for differences in sex distribution; the sex-related associations remained significant. A limitation of our study was that the number of non-HIV morbidities that occurred in the PEARLS study was small. Furthermore, the study was not designed to detect long-term non–HIV-related morbidities, such as cardiovascular events, limiting our ability to detect differences in these outcomes between men and women. Data from a large registry study, however, showed that HIV-infected women had higher relative increases in myocardial infarctions compared with HIV-uninfected women and also HIV-infected men.[37,38] Other studies have reported increased risk of ischemic stroke and coronary artery plaques in HIV-infected women.[39,40] Our data may provide a starting point for linking inflammation and translocation with these non–HIV-related outcomes even in the setting of suppressed viremia. We were unable to measure levels of other important cytokines, including IL-7, which is important in T-cell proliferation and known to be higher in women than men.[41] We also do not have data on the women's menstrual cycle or use of hormonal contraception, which can cause fluctuations in inflammatory cytokines.[42–44] Future studies that include women should routinely collect these data. Coinfections with cytomegalovirus or parasites in either men or women can also affect the inflammatory and immune activation pathways. If present, however, these types of infections should have occurred with comparable frequency in both sexes.[45–47]

Our study provides a unique and informative insight into the differences in inflammation and immune activation after cART initiation in men vs women. Few other studies have examined longitudinal changes, instead of focusing on how baseline inflammation and immune activation levels predict long-term outcomes. Longitudinal analysis allows a more comprehensive understanding of these complex immune changes. A further strength of our analysis is the adequate representation of women in the parent PEARLS

study. Even today, a few nonprevention of mother-to-child transmission studies include an appropriate sampling of HIV-infected women. In fact, most of the studies cited above had a much higher proportion of men than women. We are starting to appreciate the impact of sex on the safety and efficacy of cART.[48,49] But, similar to lessons learned from studying the independent impact of race on HIV outcomes,[50] a dedicated effort to specifically study the impact of sex on HIV outcomes must also be a priority. Future prospective studies should further investigate sex-specific differences in immune activation and inflammation pathways, including mechanism, and whether the different responses in inflammatory markers by sex have a significant impact on all-cause morbidity and mortality.

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