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MAJOR ARTICLE

Continued Elevation of Interleukin-18 and Interferon-γ After Initiation of Antiretroviral Therapy and Clinical Failure in a Diverse Multicountry Human Immunodeficiency Virus Cohort

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Background. We assessed immune activation after antiretroviral therapy (ART) initiation to understand clinical failure in diverse settings.

Methods. We performed a case-control study in ACTG Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS). Cases were defined as incident World Health Organization Stage 3 or 4 human immunodeficiency virus (HIV) disease or death, analyzed from ART weeks 24 (ART24) to 96. Controls were randomly selected. Interleukin (IL)-6, interferon (IFN)-γ-inducible protein-10, IL-18, tumor necrosis factor-α, IFN-γ, and soluble CD14 (sCD14) were measured pre-ART and at ART24 in plasma. Continued elevation was defined by thresholds set by highest pre-ART quartiles (>Q3). Incident risk ratios (IRRs) for clinical progression were estimated by Poisson regression, adjusting for age, sex, treatment, country, time-updated $CD4^+$ T-cell count, HIV ribonucleic acid (RNA), and prevalent tuberculosis.

Results. Among 99 cases and 234 controls, median baseline $CD4^+$ T-cell count was 181 cells/μL, and HIV RNA was 5.05 $\log_{10}$ cp/mL. Clinical failure was independently associated with continued elevations of IL-18 (IRR, 3.03; 95% confidence interval [CI], 1.27–7.20), sCD14 (IRR, 2.17; 95% CI, 1.02–4.62), and IFN-γ (IRR, 0.08; 95% CI, 0.01–0.61). Among 276 of 333 (83%) who were virologically suppressed at ART24, IFN-γ was associated with protection from failure, but the association with sCD14 was attenuated.

Conclusions. Continued IL-18 and sCD14 elevations were associated with clinical ART failure. Interferon-γ levels may reflect preserved immune function.

Keywords. antiretroviral therapy; HIV; IFN-γ; inflammasome; IL-18; immune activation.

Despite the success of combination antiretroviral therapy (ART), a subset of human immunodeficiency virus (HIV)-infected patients who initiate ART develop early clinical progression to acquired immune deficiency syndrome (AIDS) [1–6]. Clinical progression can occur even among patients who achieve complete viral suppression, termed clinical failure. Whereas clinical failure during ART has been attributed to $CD4^+$ T-cell count, continued viremia, and nonadherence [5], there remains a subset of ART initiators for whom failure is unexplained. In addition to providing insight into mechanisms of clinical failure, biomarkers of failure may allow development of screening algorithms to identify HIV-infected ART initiators who are at higher risk for failure. A screening strategy in ART initiators would be of particular interest in resource-limited settings for those lacking traditional risk factors for clinical failure.

HIV progression has been linked to prolonged immune activation, a multipronged phenotype that involves dysregulation of the immune system [7, 8]. Upon ART initiation, immune activation is only partially attenuated, and not equally among all people [9–12]. We hypothesized that a subset of immune activation biomarkers measurable in ART initiators would not be suppressed by ART and might predict clinical failure. The Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) study was an AIDS Clinical Trials Group

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(ACTG)-sponsored randomized controlled clinical trial of ART among HIV-infected persons in 9 countries in which enrolled participants were observed for 96 weeks or more and HIV-related outcomes were rigorously characterized [6]. We examined whether continued elevation of immune activation biomarkers after ART initiation was associated with clinical failure among PEARLS participants.

METHODS

The ACTG PEARLS study (A5175; ClinicaTrials.gov NCT00084136) was a Phase IV randomized controlled trial of 3 ART regimens in 1571 treatment naive adults, performed in Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, the United States, and Zimbabwe [6]. Eligible participants had $CD4^+$ T-cell counts <300 cells/mm^3 and no recent acute illness (ie, pneumonia, gastroenteritis, or pelvic inflammatory disease) or opportunistic infections. Participants were randomized to receive lamivudine/zidovudine/efavirenz (3TC/ZDV/EFV), didanosine/emtricitabine/atazanavir (ddI/FTC/ATV), or tenofovir/emtricitabine/efavirenz (TDF/FTC/EFV). Informed consent, including permission to use biological materials, was obtained from all participants, and the human experimentation guidelines of the US Department of Health and Human Services and local site institutional review boards and ethics committees were followed. We designed a nested case-control study where cases (n = 236) were defined by incident World Health Organization Stage 3 or 4 event or death: among cases, only those who developed the primary outcome after 24 weeks of ART (ART24) and prior to 96 weeks after ART initiation (ART96) were retained for analysis (see Supplementary Digital Content 1 for sample selection). In secondary analyses, only cases and controls who achieved virological suppression by ART24 were examined for continued elevation of biomarkers that predicted clinical failure. A randomly selected subcohort (n = 270, or 30 participants from each of 9 sites) from the full cohort of 1571 was used to estimate the prevalence of continued immune activation.

Biomarkers of immune activation (interleukin [IL]-6, interferon [IFN]-γ-inducible protein-10 [IP-10], IL-18, soluble CD14 [sCD14], tumor necrosis factor [TNF]-α, and IFN-γ) were measured in plasma samples obtained at pre-ART and ART24. Each assay was performed in a single laboratory testing site to avoid laboratory-to-laboratory variation. Enzyme-linked immunosorbent assay (ELISA) kits were used for measurement of sCD14 (R&D Systems, Inc., Minneapolis, MN) and IL-18 (Platinum ELISA; eBiosciences, San Diego, CA). Interferon-γ-inducible protein-10 (CXCL10) was measured using commercially available test kits (electrochemiluminescent bridging immune-assay; Meso-Scale Discovery, Gaithersburg, MD). Soluble IL-6, TNF-α, and IFN-γ were measured using the Luminex multiplex cytokine platform (R&D Systems, Inc., Minneapolis, MN).

Statistical Analyses

All biomarkers were parameterized in quartiles to accommodate right- or left-censored data. Continued elevation of immune activation biomarkers was defined individually for each biomarker: (1) the threshold for elevated levels of a given biomarker was set at >Q3 pre-ART; (2) if at ART24 an elevated biomarker did not decrease to below the threshold set pre-ART, it was considered to have continued elevation in that participant. Univariable analysis was done to assess the effect of baseline covariates on continued immune activation. Variables that were significant in the univariable analysis, and ones that were biologically plausible, were included in the multivariate modeling. Poisson regression analysis was used to estimate incidence risk ratios (IRRs) for persistent immune activation, adjusting for baseline age, sex, treatment, country, $CD4^+$ T-cell count pre-ART and at ART24, $\log_{10}$ HIV ribonucleic acid (RNA) level at baseline, viral suppression at ART24 (HIV RNA level <400 cp/mL), and prevalent tuberculosis (TB). Time-updated $CD4^+$ T-cell counts were included in models to account for immune reconstitution with ART.

RESULTS

Baseline Characteristics

Of 470 evaluable participants in the PEARLS study, 137 persons were excluded from the primary analysis at ART24 after already achieving the primary outcome or after having been lost to follow-up. The case-control study contained 333 persons at baseline (see Supplementary Digital Content 1 for sample selection): 99 (29.7%) cases developed clinical failure, and 234 (70.3%) were controls. The median age overall was 34 years (interquartile range [IQR], 29–40), 160 (48%) were female, and 172 (52%) were black. The percentage of participants from each country ranged from 9% to 16% (Table 1). The median baseline $CD4^+$ T-cell count was 180 cells/μL (IQR, 94–229) and HIV RNA level was 5.05 $\log_{10}$ cp/mL (IQR, 4.54–5.45). Tuberculosis was prevalent in 20% of the cohort at baseline. Cases and controls did not differ by baseline $CD4^+$ T-cell count, HIV-1 RNA level, or ART regimen but did differ by country of origin ($P < .001$), and a higher proportion of cases had TB at baseline (30%) than controls (16%; $P = .004$). Major outcomes that occurred beyond ART24 included opportunistic infections (8.0%), newly diagnosed TB (25.6%), >10% weight loss (10.0%), and cytopenias (14.0%) (see Supplementary Digital Content 2, for a delineation of major outcomes).

Prevalence of Continued Biomarker Elevation After Antiretroviral Therapy Initiation

Biomarkers of immune activation were measured in participants' plasma pre-ART and at ART24. Using the standardized definition (levels >Q3 pre-ART), cutoffs for continued elevation of immune activation biomarkers were set for IL-6 (>48.3 pg/mL), IP-10 (>2856 pg/mL), IL-18 (>774 pg/mL), sCD14 (>6.4 $\log_{10}$ pg/mL), TNF-α (>28.4 pg/mL), and IFN-γ (>48

Table 1. Baseline Characteristics of Study Population

Baseline Characteristics	Overall (n = 333)	Cases (n = 99)	Controls (n = 234)	*P* Value
Female, n (%)	160 (48%)	45 (45%)	115 (49%)	.55
Age, median (IQR)	34 (29–40)	34 (28–38)	35 (29–41)	.20
Race				.013
White	85 (26%)	36 (36%)	49 (21%)	
Black or African Americans	172 (52%)	49 (49%)	123 (53%)	
Asian	36 (11%)	8 (8%)	28 (12%)	
Other	38 (11%)	6 (6%)	32 (14%)	
Country				<.001
Brazil	36 (11%)	7 (7%)	29 (12%)	
Haiti	30 (9%)	5 (5%)	25 (11%)	
India	53 (16%)	33 (33%)	20 (9%)	
Malawi	44 (13%)	20 (20%)	24 (10%)	
Peru	32 (10%)	4 (4%)	28 (12%)	
South Africa	38 (11%)	13 (13%)	25 (11%)	
Thailand	31 (9%)	3 (3%)	28 (12%)	
United States	37 (11%)	8 (8%)	29 (12%)	
Zimbabwe	32 (10%)	6 (6%)	26 (11%)	
$CD4^+$ T cells/μL, median (IQR)	180 (94–229)	182 (108–216)	180 (90–233)	.42
Log_{10} HIV RNA cp/mL, median (IQR)	5.05 (4.54–5.45)	5.05 (4.53–5.43)	5.02 (4.55–5.47)	.91
TB, n (%)	67 (20%)	30 (30%)	37 (16%)	.004
HBsAg, n (%)	15 (5%)	2 (2%)	13 (6%)	.25
HIV RNA < 400 cp/mL (Week 24), n (%)	276 (87%)	74 (77%)	202 (91%)	.002

Abbreviations: HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus; IQR, interquartile range; RNA, ribonucleic acid; TB, tuberculosis.

pg/mL). The prevalence of continued elevation of each biomarker was estimated in the random subcohort using these cutoffs (Table 2). Reductions of elevated biomarker levels after ART initiation did not occur uniformly in all participants: for IP-10 and TNF-α, only 2% (95% confidence interval [CI], .3%–4%) and 3% (95% CI, 1%–6%) of participants, respectively, had continued elevations. Similarly, continued elevation of IL-18 was found in 5% (95% CI, 3%–9%) of participants. In contrast, continued elevations of IL-6 (prevalence 15%; 95% CI, 19%–21%), sCD14 (prevalence 10%; 95% CI, 6%–15%), and IFN-γ (prevalence 18%; 95% CI, 13%–23%) were more common after ART initiation.

Table 2. Prevalence of Continued Immune Activation After 24 Weeks of ART (ART24)[a,b]

Marker	N	IQR (pg/mL)	Prevalence	95% CI
IL-6	200	9.1–48.3	15%	(19%–21%)
IP-10	200	601–2856	2%	(.3%–4%)
IL-18	193	163–774	5%	(3%–9%)
sCD14	199	5.7–6.4[c]	10%	(6%–15%)
TNF-α	200	13.7–28.4	3%	(1%–6%)
IFN-γ	200	5.6–48	18%	(13%–23%)

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IFN, interferon; IL, interleukin; IP, IFN-γ-inducible protein; sCD14, soluble CD14; TNF, tumor necrosis factor.

[a] The prevalence of continued immune activation was estimated at ART24 in the random subcohort.

[b] Continued immune activation was defined for each biomarker separately. Based on the distribution of each biomarker at baseline, continued elevation was defined when biomarker levels exceeded the third quartile cutoff pre-ART.

[c] Log_{10} pg/mL values of sCD14 are shown.

Correlates of Continued Biomarker Elevation

Several baseline characteristics were associated with persistent biomarker elevation (Supplementary Digital Content 3). This included country ($P < .05$) and age, where older participants were more likely to have continued elevation of IL-6 ($P = .01$) and IP-10 ($P = .03$), with trends toward significance for TNF-α ($P = .09$) and IFN-γ ($P = .06$). Higher baseline HIV RNA level was also associated with continued elevation of sCD14 ($P = .002$) and IFN-γ ($P = .02$), and it was marginally associated with continued IP-10 ($P = .07$) elevation. Continued elevation of sCD14 was associated with the ART treatment arm, with the highest prevalence occurring among persons who received 3TC/ZDV/EFV and the lowest prevalence among persons who received TDF/FTC/EFV ($P = .001$). In addition, the prevalence of continued elevation of TNF-α was marginally higher among persons who did not achieve virologic suppression at ART24 ($P = .09$), whereas the prevalence of continued elevation of IFN-γ was marginally lower among persons who did not achieve virologic suppression at ART24 ($P = .06$). Baseline TB was not associated with continued biomarker elevation.

Continued Biomarker Elevation and Clinical Failure

Poisson regression models were developed to test whether continued elevation of each biomarker deleteriously contributed to clinical failure. In univariate analyses, continued elevation

Table 3. Univariable and Multivariable Analysis for Clinical Failure at 96 Weeks (ART96)

Marker >Q3 (ART24)	Overall	Case	Control	Univariable Analysis		Multivariable Analysis Model 1[a]		Multivariable Analysis Model 2[b]	
				IRR (95% CI)	*P* Value	IRR (95% CI)	*P* Value	IRR (95% CI)	*P* Value
IL-6	34 of 249 (14%)	6 of 57 (11%)	28 of 192 (15%)	0.65 (.28–1.52)	.33	0.62 (.25–1.52)	.29	0.62 (.25–1.54)	.30
IP-10	5 of 249 (2%)	2 of 57 (4%)	3 of 192 (2%)	2.39 (.58–9.79)	.23	2.27 (.45–11.33)	.32	2.35 (.48–11.78)	.30
IL-18	16 of 242 (7%)	8 of 56 (14%)	8 of 186 (4%)	2.86 (1.35–6.04)	.006	3.03 (1.27–7.20)	.012	2.99 (1.22–7.31)	.02
sCD14	33 of 246 (13%)	13 of 54 (24%)	20 of 192 (10%)	2.42 (1.30–4.51)	.006	2.17 (1.02–4.62)	.045	2.08 (.96–4.49)	.06
TNF-α	9 of 249 (4%)	3 of 57 (5%)	6 of 192 (3%)	1.23 (.38–3.93)	.73	1.05 (.29–3.73)	.94	0.99 (.28–3.59)	>.95
IFN-γ	36 of 249 (14%)	1 of 57 (2%)	35 of 192 (18%)	0.10 (.01–.74)	.02	0.08 (.01–.61)	.02	0.08 (.01–.62)	.02

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; IP, IFN-γ-inducible protein; IRR, incidence rate ratio; sCD14, soluble CD14; TNF, tumor necrosis factor.

[a] Model 1 was adjusted for age, sex, country, random treatment assignment, baseline BMI, time-updated $CD4^+$ T-cell count at ART24, baseline $\log_{10}$ HIV RNA level, and baseline TB.

[b] Model 2 was adjusted for age, sex, country, random treatment assignment, baseline BMI, time-updated $CD4^+$ T-cell count at ART24, baseline $\log_{10}$ HIV RNA level, baseline TB, and viral suppression at ART24 (HIV RNA <400 cp/mL).

of IL-18, sCD14, and IFN-γ each separately predicted clinical failure (Table 3). Continued elevation of IL-6, IP-10, and TNF-α appeared to have no association with clinical failure. A multivariable analysis was performed, adjusting for age, sex, country, random treatment assignment group, baseline BMI, time-updated $CD4^+$ T-cell count (at ART24), $\log_{10}$ HIV RNA level, and prevalent TB. Continued IL-18 elevation remained strongly associated with clinical failure (IRR, 3.03; 95% CI, 1.27–7.20; $P = .012$), as did sCD14 (IRR, 2.17; 95% CI, 1.02–4.62; $P = .045$). In contrast, continued elevation of IFN-γ was associated with a lower likelihood of developing clinical failure (IRR, 0.08; 95% CI, .01–.61; $P = .02$).

Not all ART initiators achieved virologic suppression at ART24, which could contribute to and confound an analysis of immune activation. Therefore, multivariate models were developed that adjusted for virologic suppression at ART24 in addition to the previous covariates. The new models continued to show associations between clinical progression and continued elevation of IL-18 (IRR, 2.99; 95% CI, 1.22–7.31; $P = .02$) and sCD14 (IRR 2.08; 95% CI, .96–4.49; $P = .06$). Likewise, the association between continued IFN-γ elevation and a decreased likelihood of developing clinical failure was maintained after adjustment for virologic suppression (IRR, 0.08; 95% CI, .01–.62; $P = .02$). As an added measure to avoid potential confounding from incomplete virologic suppression, a secondary analysis was performed that was restricted to only cases and controls who were virologically suppressed at ART24 (n = 276; cases = 74, controls = 202), although this decreased the power to observe associations (Table 4). Continued elevation of IL-18 was associated with a 2.84 IRR (95% CI, .97–8.29; $P = .06$) of clinical failure, whereas continued elevation of IFN-γ was associated with a 0.08 IRR (95% CI, .01–.63; $P = .02$) of clinical failure. More importantly, IL-18 and IFN-γ levels were not closely correlated with each other at any time point (data not shown). The association of clinical failure with continued elevation of sCD14 was attenuated: 2.11 IRR (95% CI, .82–5.43; $P = .12$).

Kaplan–Meier survival analyses were performed among 333 persons in the primary analysis group with or without continued elevations of IL-18, sCD14, and IFN-γ (Figure 1). At ART96, persons with continued elevations of IL-18 had significantly more

Table 4. Univariable and Multivariate Models of Clinical Failure at ART96 Among Persons Who Were Virologically Suppressed[a]

Marker >Q3 (ART24)	Overall	Case	Control	Univariable Analysis		Multivariable Analysis	
				IRR (95% CI)	*P* Value	IRR (95% CI)	*P* Value
IL-6	30 (14%)	5 (11%)	25 (14%)	0.72 (.28–1.81)	.48	0.59 (.22–1.60)	.30
IP-10	4 (2%)	2 (6%)	2 (1%)	2.93 (.71–12.1)	.14	2.83 (.53–15.1)	.22
IL-18	12 (6%)	5 (12%)	7 (4%)	2.68 (1.05–6.82)	.04	2.84 (.97–8.29)	.06
sCD14	26 (12%)	9 (22%)	17 (10%)	2.51 (1.20–5.26)	.02	2.11 (.82–5.43)	.12
TNF-α	6 (3%)	1 (3%)	5 (3%)	0.62 (.09–4.51)	.64	0.60 (.07–5.05)	.64
IFN-γ	35 (16%)	1 (2%)	34 (20%)	0.12 (.02–0.87)	.04	0.08 (.01–.63)	.02

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IFN, interferon; IL, interleukin; IP, IFN-γ-inducible protein; IRR, incidence rate ratio; sCD14, soluble CD14; TNF, tumor necrosis factor.

[a] The multivariable model was adjusted for age, sex, country, random treatment assignment, baseline BMI, time-updated $CD4^+$ T-cell count at ART24, baseline $\log_{10}$ HIV RNA level, and baseline TB.

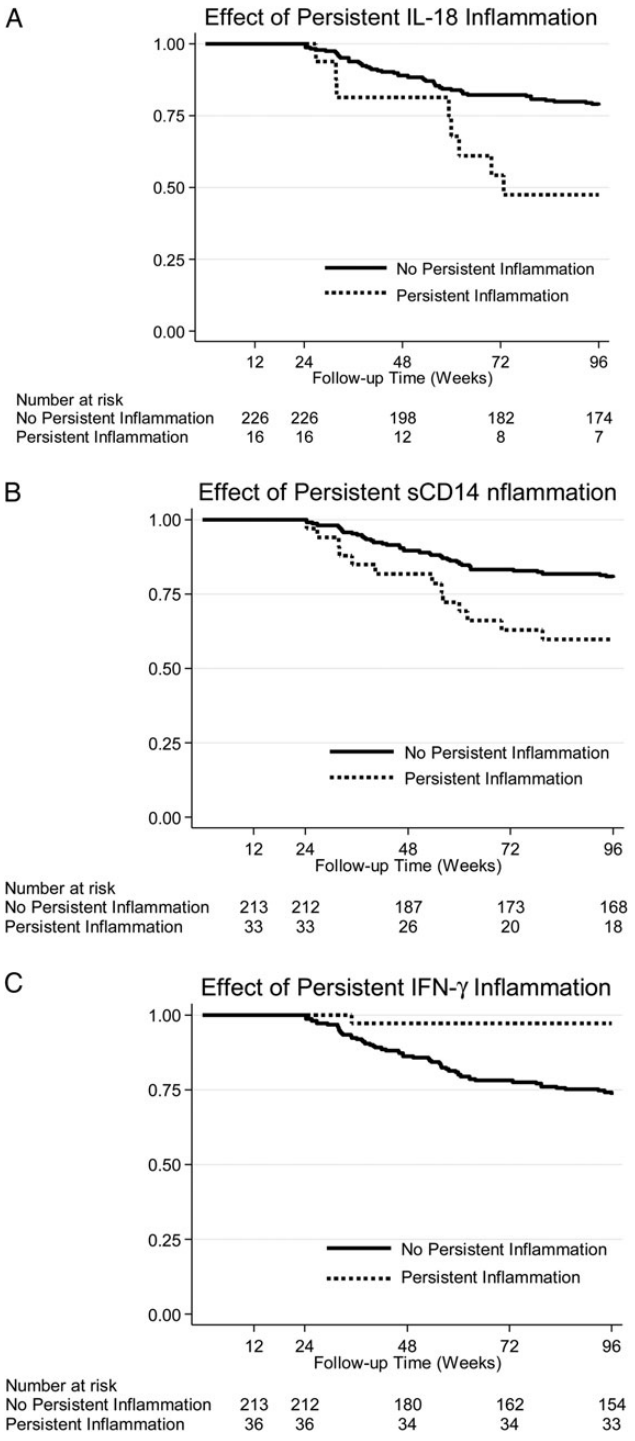

Figure 1. Kaplan–Meier survival in persons who have persistent immune activation. Kaplan-Meier curves are shown for (A). Interleukin (IL)-18 ($P = .004$). (B) Soluble CD14 (sCD14) ($P = .006$), and (C) interferon (IFN)-γ ($P = .003$), beginning after ART24 to reflect censoring of individuals who achieved outcomes before 24 weeks.

occurrences of clinical failure compared with persons without continued IL-18 elevations ($P = .004$), and there was a similar trend for sCD14 ($P = .006$). In parallel, persons with continued elevations of IFN-γ levels had significantly less clinical failure at ART96 than persons who had lower IFN-γ levels at baseline, 24 weeks, or at both time points ($P = .003$).

DISCUSSION

In a diverse multicountry cohort of persons predominantly from low- and middle-income countries, we found that HIV-infected ART initiators with high levels of IL-18 and sCD14 pre-ART that failed to normalize during ART were more likely to develop clinical failure than persons who did not have continued high levels of these biomarkers. Continued IFN-γ elevation was strongly associated with protection from clinical failure. Repeated measurements of IL-18, sCD14, and IFN-γ may be useful in identifying ART-naive persons for whom ART initiation alone may not be sufficient to prevent HIV complications.

IL-18 and sCD14 are measures of monocyte activation. IL-18 is released upon stimulation of the inflammasome, a multicomponent intracellular protein complex involved in innate immune signaling [13]. Human immunodeficiency virus virions are potent inflammasome triggers in circulating monocytes [14]. Inflammasome activation primes defenses against intracellular pathogens, and persistently elevated serum IL-18 levels have been associated with unfavorable outcomes among HIV-infected [15, 16] and HIV-uninfected persons [17–19]. Whereas in the present study we did not ascertain non-AIDS outcomes, the strong association of continued elevation of IL-18 with clinical failure suggests that ART does not fully turn off inflammasome signaling. Interleukin-18 elevations pre-ART have been associated with TB immune reconstitution inflammatory syndrome [20], although our findings were not driven by that outcome. Monocyte activation with sCD14 release has been increasingly recognized as a key component of the immune activation phenotype that drives AIDS pathogenesis. Indeed, Hasegawa et al [21] found that monocyte turnover in simian immunodeficiency virus (SIV)-infected macaques was the strongest predictor of mortality even after considering SIV RNA and $CD4^+$ T-cell depletion. However, it is still unclear how monocyte activation contributed to the diverse outcomes in the present study and what triggers continued monocyte activation in the absence of detectable plasma viremia, although microbial translocation and type 1 IFN signaling have both been proposed.

Continued IFN-γ elevation was protective against clinical failure despite its association with pre-ART HIV RNA. Although IFN-γ is a critical component of pathogen-specific immunity, we measured bulk levels in circulation and not in association with any particular pathogen. The source of plasma IFN-γ was unclear; $CD8^+$ T cells, natural killer (NK) cells, and to some degree $CD4^+$ T cells are robust producers of IFN-γ. To explain why IFN-γ may have been beneficial, we speculate that IFN-γ levels reflect retained functionality of $CD8^+$ T cells that are specific for opportunistic infections despite HIV-associated immunosuppression before ART initiation. Natural killer cells have been reported to be pathologically activated in HIV, a

phenotype that is only partially reversed by ART [22, 23]. Whereas NK cell activation has been assumed to have negative consequences in HIV, a more complex relationship between NK cell activation and HIV progression is conceivable, especially during ART. It is compelling to consider that IFN-γ itself is protective in ART initiators. In the case of TB, one of the leading diagnoses that contributed to clinical failure, genetic deficiencies of IFN-γ or dysfunctional IFN-γ signaling can lead to severe disease [24]. Similar genetic differences in IFN-γ signaling may have contributed to our results. However, exogenous IFN-γ administration to HIV-infected persons has yielded mixed results [25]. In addition, IFN-γ levels in our study were far in excess than what has been reported among healthy controls [26, 27]. Therefore, the implications of continued IFN-γ elevation will need to be explored further in prospective studies.

Previous studies during ART have largely found that immune activation decreases with virologic control, although a minority of treated patients have normalized immune activation markers. As an example, $CD4^+$ and $CD8^+$ T-cell activation appeared to reduce with ART by 0.06% and 0.1%, respectively [28]. Likewise, sCD14, which when measured before ART initiation has been linked with mortality, did not decline to levels found in HIV-uninfected persons during ART [8, 29]. The microbial translocation marker lipopolysaccharide shared the same pattern of decrease without normalization [29]. IL-6, one of the strongest predictors of mortality and AIDS in untreated HIV-infected patients and in patients initiating ART, did not change appreciably during ART [30–32], a finding underscored by us here and previously [33]. Although several immune activation markers have been characterized pre-ART, few studies have examined how continued immune activation during ART affects treatment outcome, particularly in resource-constrained countries, from where the majority of study population was recruited. Tien et al [34] reported that persistently elevated fibrinogen and C-reactive protein in ART-treated HIV-infected persons had an increased risk of mortality. To our knowledge, ours is the first study to identify IL-18 and IFN-γ as immune activation markers that could potentially be followed during ART to predict early outcomes.

There are some limitations to our study. A universal definition of persistent immune activation that encompasses all markers does not exist; participants for whom continued elevation of one marker was found did not necessarily have continued elevation of all markers, reflecting the complexity of stimuli that result in immune activation. By defining continued immune activation for each biomarker separately, we answered the question that is most relevant to a treating physician; namely, whether measurement of a given marker will indicate which patients will require added vigilance after ART initiation. A related point is that we used empiric cutoffs to determine elevations of each biomarker because there are no known cutoffs defining abnormal elevations for the biomarkers that we used. By using the same empiric definition for continued elevations to each biomarker, we have normalized the analysis of each biomarker to allow comparisons between them, although it is possible that we consequently reduced our power to identify other associations of importance. A second challenge was in interpreting biomarker levels across multiple countries, where exposures to prevalent infections are likely to be different. By adjusting for country, we have partially accounted for different exposures. It will be important to confirm our findings in separate cohorts and in separate geographical areas. A third limitation is in interpreting serum IFN-γ levels. If, as we speculate, serum IFN-γ is produced by $CD8^+$ T cells, then in future studies IFN-γ production by pathogen-specific $CD8^+$ T cells should be quantified to test whether elevated IFN-γ levels are the result of retained or restored pathogen-specific immunity. Future studies should also include examination of cellular immune activation markers longitudinally during ART, although these were not available in the present study. A fourth limitation is that IFN-γ, IL-18, and IP-10 are typically linked mechanistically: IP-10 is typically induced by IFN-γ stimulation, whereas IL-18 induces IFN-γ in T-cell subsets. Intriguingly, we found reciprocal associations of continued IL-18 elevation and IFN-γ elevation with clinical failure. Further research is required to understand the complexity of these signaling networks.

CONCLUSIONS

In summary, we found that among HIV-infected ART initiators, elevation of IL-18 and sCD14 pre-ART and at ART24 predicted clinical failure, whereas IFN-γ elevation appeared to be favorable. There is clinical value in identifying markers that predict failure after starting ART. Point-of-care tests are being developed for biomarkers that can be readily deployed in resource-limited settings. Therefore, because the present dogma to "test and treat" may be insufficient for some people with HIV, an expanded algorithm of "test, treat, and stratify" into risk groups by measuring IL-18, sCD14, and IFN-γ may be a more comprehensive approach to avoid the pitfalls of delayed clinical progression that can occur even among persons who are virologically suppressed. In addition, dampening of residual immune activation, such as has been proposed pharmacologically for the inflammasome [35, 36], may reduce the likelihood of clinical progression.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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