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Pre-cART Elevation of CRP and CD4+ T-Cell Immune Activation Associated With HIV Clinical Progression in a Multinational Case–Cohort Study

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Background: Despite the success of combination antiretroviral therapy (cART), a subset of HIV-infected patients who initiate cART develop early clinical progression to AIDS; therefore, some cART initiators are not fully benefitted by cART. Immune activation pre-cART may predict clinical progression in cART initiators.

Methods: A case–cohort study (n = 470) within the multinational Prospective Evaluation of Antiretrovirals in Resource-Limited Settings clinical trial (1571 HIV treatment–naive adults who initiated cART; CD4+ T-cell count <300 cells/mm^3; 9 countries) was conducted. A subcohort of 30 participants per country was randomly selected; additional cases were added from the main cohort. Cases [n = 236 (random subcohort 36; main cohort 200)] had clinical progression (incident WHO stage 3/4 event or death) within 96 weeks after cART initiation. Immune activation biomarkers were quantified pre-cART. Associations between biomarkers and clinical progression were examined using weighted multivariable Cox-proportional hazards models.

Results: Median age was 35 years, 45% were women, 49% black, 31% Asian, and 9% white. Median CD4+ T-cell count was 167 cells per cubic millimeter. In multivariate analysis, highest quartile C-reactive protein concentration [adjusted hazard ratio (aHR), 2.53; 95% confidence interval (CI): 1.02 to 6.28] and CD4+ T-cell activation (aHR, 5.18; 95% CI: 1.09 to 24.47) were associated with primary outcomes, compared with lowest quartiles. sCD14 had a trend toward association with clinical failure (aHR, 2.24; 95% CI: 0.96 to 5.21).

Conclusions: Measuring C-reactive protein and CD4+ T-cell activation may identify patients with CD4+ T-cell counts <300 cells per cubic millimeter at risk for early clinical progression when initiating cART. Additional vigilance and symptom-based screening may be required in this subset of patients even after beginning cART.

Key Words: immune activation, global HIV, cART clinical outcomes, C-reactive protein, T-cell activation

INTRODUCTION

HIV infects more than 30 million people worldwide, but in most people, clinical progression has been controlled with

combination antiretroviral therapy (cART). Some patients, however, remain at risk for early progression and death even after starting cART, suggesting that cART is only partially effective at controlling progression in these patients.[1–5] Furthermore, morbidity and mortality rates among persons from resource-limited settings exceed those in resource-rich settings, most significantly during the first year after cART initiation.[6–9] A subset of treated patients who remains high risk for early clinical HIV progression may require more frequent and closer follow-up than is the current standard of care.[10] Finding baseline factors that identify such high-risk patients across multiple geographic and socioeconomic settings could optimize clinical management strategies, as well as identify common pathogenic mechanisms driving HIV disease progression.[11] A number of factors have been associated with clinical response to cART, including pre-cART clinical stage, CD4$^+$ T-cell count, and HIV viral load. However, the role of baseline inflammation has not been well evaluated, particularly in low- and middle-income settings.[12–16]

Chronic immune activation is a hallmark of HIV infection that has been associated with viral replication, CD4$^+$ T-cell depletion, and other dysfunctions of the immune system.[17] Although the causes of immune activation in HIV infection remain unclear, previous studies have associated baseline elevations of a number of immune activation biomarkers with clinical HIV progression among patients initiating cART, including TNF-α, IL-6, IFN-γ, T-cell activation, sCD14, and microbial translocation (MT) markers.[18–22] However, these studies have only partially accounted for underlying factors that might contribute to immune activation and were conducted in resource-rich communities or in single-site settings. In addition, the findings from these studies have not been consistently replicated.[23] Thus, it is unclear if there are biomarkers that would be universally predictive of clinical response after cART initiation that are independent of geographic location, resource abundance, and diversity of endemic diseases.

Therefore, we conducted a case–cohort analysis of the AIDS Clinical Trials Group (ACTG) Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) A5175 study, a clinical trial of antiretroviral therapy among HIV-infected, cART-naive adults from 9 countries.[24] The diversity of our cohort allowed for assessment of a comprehensive panel of soluble and cellular immune activation biomarkers to identify baseline predictors of HIV clinical progression after cART initiation that are independent of geographic setting.

METHODS

Study Design and Population

The methods and procedures of the ACTG PEARLS A5175 study (ClinicalTrials.gov NCT00084136), a phase IV randomized controlled clinical trial of 3 antiretroviral therapy regimens among 1571 treatment-naive adults from 9 countries (Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, the United States, and Zimbabwe), have been previously described in detail.[24] In brief, the study was conducted

between May 2005 and August 2007. Study inclusion criteria were CD4$^+$ T-cell count <300 cells per cubic millimeter and no recent acute illness (ie, pneumonia, gastroenteritis, or pelvic inflammatory disease) or opportunistic infections. Participants were randomized to receive lamivudine–zidovudine + efavirenz, didanosine + emtricitabine + atazanavir, or tenofovir–emtricitabine + efavirenz, and median follow-up was 142 weeks. Viral resistance testing was not available at baseline. The primary outcome was time from treatment randomization to HIV disease progression. 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.

A case–cohort[25,26] of 470 participants comprising a randomly selected subcohort (n = 270) was chosen from the parent cohort, to which were added additional cases (n = 200) selected nonrandomly from the parent cohort: (1) the random subcohort component allows estimates disease prevalence and immune activation biomarker distributions among the parent cohort and (2) the addition of cases from the parent cohort allows adequate power to determine the association of individual biomarkers with the primary outcome, defined as incident WHO stage 3 or 4 event or death by 96 weeks after cART initiation. The randomly selected subcohort of 270 parent study participants (30 per country) included 36 individuals meeting our primary outcome (Fig. 1; Table 1). Immune activation biomarkers were quantified among the full case–cohort using pre-cART serum, plasma, and peripheral blood mononuclear cell (PBMC) samples. Serum and plasma were stored in a centralized −80°C repository until the time of testing. Viable pre-cART PBMCs were collected, cryopreserved, and stored in the liquid nitrogen. Three markers of MT were measured, including gram-negative bacterial lipopolysaccharide [LPS], sCD14, and EndoCAb IgM. Five soluble inflammatory cytokines were measured, including C-reactive protein [CRP], IP-10, TNF-α, IL-6, and IFN-γ. Two T-cell activation phenotypes (CD4$^+$/DR+/CD38$^+$ and CD8$^+$/DR+/CD38$^+$) were also measured.

Laboratory Testing

To minimize laboratory-to-laboratory variation and standardize testing across sites, single laboratories performed batch testing for each marker. LPS, sCD14, and EndoCAb IgM were measured in 1 laboratory at Johns Hopkins University. LPS was quantified using an optimized *Limulus* amebocyte lysate assay (Lonza, Walkersville, MD).[27] Commercially available ELISA kits were used for measurement of sCD14 (R&D Systems, Inc., Minneapolis, MN), EndoCAb IgM (Cell Sciences, Canton, MA), and CRP (CRP Quantikine ELISA; R&D Systems, Inc.). The remaining biomarkers were tested in an externally quality-assured ACTG-designated laboratory at the University of California Davis. IP-10 (IFN-γ inducible protein-10, CXCL10) was measured using commercially available testing kits (electrochemiluminescent bridging immuneassay by Meso-Scale Discovery, Gaithersburg, MD). Soluble TNF-α, IL-6, and IFN-γ were measured using the Luminex multiplex cytokine platform (R&D Systems, Inc.). Viable
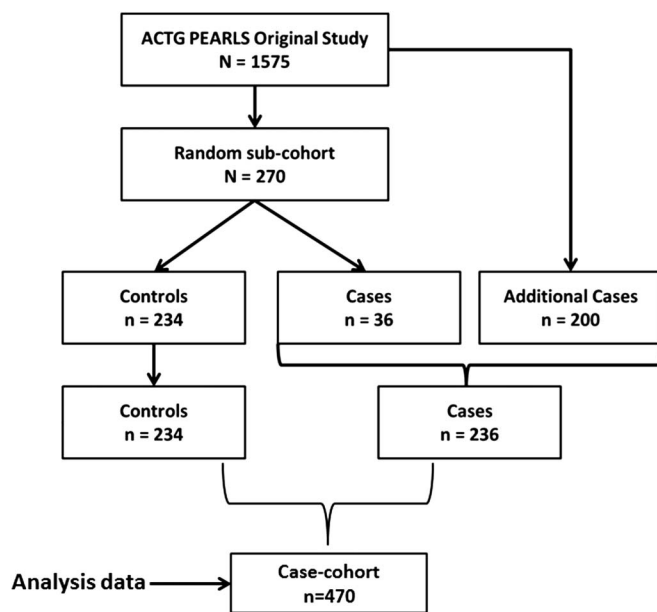
FIGURE 1. Formation of the case–cohort. The case–cohort analysis (n = 470) consisted of the random subcohort [a randomly selected subsample of 30 participants per country (n = 270)] from the original ACTG PEARLS study and any additional cases meeting our case definition from the original study cohort (n = 200). Cases were defined as incident WHO stage 3 or 4 event or death by 96 weeks post-cART initiation (n = 236). Immune activation biomarkers were quantified pre-cART in the random subcohort to estimate baseline immune activation in the original study cohort. Adjusted multivariate models estimated associations between individual pre-cART biomarker values and the primary outcome, incident WHO stage 3 or 4 event, or death by 96 weeks on cART.

pre-cART PBMC samples from 157 participants were available for testing and were stained with conjugated antibodies to CD4, CD8, HLA-DR, and CD38 (BD BioSciences, San Jose, CA). T-cell activation (CD4$^+$ and CD8$^+$) was measured by quantifying the percentages of HLA-DR+/CD38+ cells using flow cytometry.

Statistical Analyses

To determine whether the random subcohort was representative of the full parent cohort, Wilcoxon rank-sum and χ^2 tests were used to compare continuous and categorical covariates, respectively. All biomarkers were parameterized in quartiles to accommodate right- or left-censored data, except for LPS, which, despite using an optimized assay,[27] was only detected in samples from 137/367 (37%) participants and was subsequently dichotomized as detectable vs. undetectable (Table 2). Kruskal–Wallis nonparametric tests were used to compare pre-cART biomarker values among baseline demographic and biologic parameters, and inverse probability weighting was used to account for the case–cohort study design in identifying baseline covariates associated with each biomarker. Univariate and multivariate Cox-proportional hazards models weighted for case–cohort sampling were used to identify

TABLE 1. Baseline Characteristics of the Case–Cohort by Study Group*

Characteristics	Overall (n = 470)	Cases† (n = 236)	Controls (n = 234)	P
Age, median (IQR), yrs	35 (29–40)	35 (28–40)	35 (29–41)	
Female	215 (45)	100 (42)	115 (49)	0.22
DAIDS race				0.003
White	48 (9)	20 (8)	28 (12)	
Black	231 (49)	107 (45)	124 (53)	
Asian	146 (31)	97 (41)	49 (21)	
Hispanic	0 (0)	0 (0)	0 (0)	
Other	45 (10)	12 (5)	33 (14)	
Country				0.005
Brazil	45 (10)	16 (7)	29 (12)	
Haiti	38 (8)	13 (6)	25 (11)	
India	107 (23)	87 (37)	20 (9)	
Malawi	62 (13)	38 (16)	24 (10)	
Peru	36 (8)	8 (3)	28 (12)	
South Africa	58 (12)	33 (14)	25 (11)	
Thailand	37 (8)	9 (4)	28 (12)	
United States	49 (10)	20 (8)	29 (12)	
Zimbabwe	38 (8)	12 (5)	26 (11)	
BMI, kg/m^2				0.0001
Underweight (<18.5)	58 (12)	38 (16)	20 (9)	
Normal (18.5 to <25)	315 (67)	163 (69)	152 (65)	
Overweight (25 to <30)	74 (16)	27 (11)	47 (20)	
Obese (≥30)	23 (5)	8 (3)	15 (6)	
CD4$^+$ T-cell count, cells/mm^3				0.004
<50	83 (18)	46 (19)	37 (16)	
50–99	59 (13)	33 (14)	26 (11)	
100–199	162 (34)	85 (36)	77 (33)	
200–249	97 (21)	48 (20)	49 (21)	
250–299	69 (15)	24 (10)	45 (19)	
HIV RNA, copies/mL				0.06
<400	4 (1)	1 (0.4)	3 (1)	
400 to <4000	16 (3)	6 (3)	10 (4)	
4000 to <40,000	98 (21)	48 (20)	50 (21)	
40,000 to <400,000	254 (54)	123 (52)	131 (56)	
≥400,000	98 (21)	58 (25)	40 (17)	
TB prevalence, %	112 (24)	75 (32)	37 (16)	0.001
HBsAg prevalence, %	24 (5)	11 (5)	13 (5)	0.97
Treatment regimen				0.81
3TC-ZDV + EFV	171 (36)	90 (38)	81 (35)	
DDI + FTC + ATV	149 (32)	71 (30)	78 (33)	
TDF-FTC + EFV	150 (32)	75 (32)	75 (32)	

*Data presented as n (%) unless otherwise indicated.

†Cases defined as any incident WHO stage 3 or 4 event or death by 96 weeks post-cART initiation.

3TC, lamivudine; ATV, atazanavir; DAIDS, Division of AIDS; DDI, didanosine; EFV, efavirenz; FTC, emtricitabine; HBsAg, hepatitis B surface antigen; TDF, tenofovir; ZDV, zidovudine.

pre-cART biomarker values (grouped by quartile) that predicted the primary outcome. Multivariate models were stratified for country and treatment group and adjusted for baseline age, sex, body mass index (BMI), CD4$^+$ T-cell

TABLE 2. Pre-cART Immune Activation Biomarker Values and Number Tested (N) in the Random Subcohort (n = 270) and by Study Group in the Case–Cohort (n = 470)

Biomarker*	Random Subcohort† (n = 270)	Case–Cohort‡		
		Case§ (n = 236)	Control (n = 234)	P‖
Detectable LPS¶, n (%)	92 (41)	55 (32)	82 (42)	0.06
sCD14, pg/mL	2.1×10^6 (5.53×10^5 to 2.7×10^6)	2.1×10^6 (3.49×10^5 to 2.9×10^6)	2.0×10^6 (5.11×10^5 to 2.7×10^6)	0.81
EndoCAb IgM, MMU/mL	48.08 (29.7 to 71.5)	44.28 (27.3 to 67.7)	49.43 (29.7 to 69.7)	0.30
IFNγ, pg/mL	17.47 (6.12 to 49.65)	15.88 (4.99 to 41.45)	17 (6.04 to 55.14)	0.78
IL-6, pg/mL	23.76 (8.89 to 51.04)	27.47 (11.02 to 49.62)	24.01 (8.89 to 50.34)	0.45
TNFα, pg/mL	19.13 (13.19 to 26.67)	20.4 (14.11 to 33.79)	19.13 (13.35 to 26.67)	0.60
IP-10, pg/mL	1228.67 (584.53 to 2665.64)	2079.11 (701.73 to 4043.64)	1203.81 (584.53 to 2665.64)	0.02
CRP, mg/L	3.36 (1.37 to 10.27)	6.3 (1.85 to 20.58)	3.03 (1.23 to 9.29)	0.001
CD8+/DR+/38+, %CD8	45.73 (34.76 to 56.01)	46.97 (37.45 to 60.17)	45.79 (34.76 to 56.01)	0.70
CD4+/DR+/38+, %CD4	22.60 (15.28 to 34.78)	28.63 (14.72 to 42.28)	22.29 (15.28 to 33.82)	0.33

*Biomarker values presented as median (IQR) unless otherwise indicated.
†Between 241 and 255 samples were available for testing of soluble markers; 102 viable PBMC samples were available for testing of cellular markers.
‡Between 378 and 417 samples were available for testing of soluble markers; 155 and 157 viable PBMC samples were available for testing of cellular markers.
§Case defined as incident WHO stage 3 or 4 event or death by 96 weeks post-cART initiation.
‖P values shown are for comparisons of cases and controls.
¶LPS measurement was dichotomized as detectable vs. undetectable due to low detection rate among samples (137/367) despite using an optimized assay.[27]
LPS, Gram-negative bacterial LPS; N, number of samples tested.

count, and hemoglobin. Because baseline HIV RNA was largely collinear with CD4+ T-cell count and hemoglobin, adjustment for baseline HIV RNA level did not substantially contribute to a model in which the latter 2 covariates were included. Albumin was collinear with hemoglobin, and so was not added to the models. A separate model also adjusted for baseline tuberculosis (TB). Receiver operating characteristic (ROC) curve analysis was performed to estimate the C-statistic and the corresponding 95% confidence interval (CI) for CRP at each cutoff, before and after adjusting for covariates.

RESULTS

Baseline Characteristics

The random subcohort was not statistically different from the parent cohort by baseline demographics, clinical status, and laboratory results, except for a slightly lower baseline creatinine clearance although all participants had CrCl ≥60 cc/min (CrCl <60 cc/min was an exclusion criterion for the parent study).[24] The case–cohort had a median age [interquartile range (IQR)] of 35 years (29–40) and was composed of 215 (45%) women and 231 (49%) black, 146 (31%) Asian, and 48 (9%) white individuals (Table 1). The median BMI (IQR) was 21.8 kg/m² (19.6–24.4), with 97 (21%) overweight or obese and 58 (12%) undernourished or underweight participants. On enrollment, the median CD4+ T-cell count (IQR) was 167 cells per cubic millimeter (79–219), and the median plasma HIV RNA level (IQR) was 5.11 log₁₀ copies per milliliter (4.60–5.53). TB coinfection was common (24%), and the prevalence of hepatitis B virus coinfection was 5%.

In the parent trial of 1571, 236 (15%) participants were cases who developed the composite primary outcome of WHO stage 3 or 4 diagnosis or death by 96 weeks after cART

initiation. The most frequent incident events were TB (27.1%), death (16.9%), cytopenia (15.7%), serious bacterial infection (11.4%), and weight loss (10.6%) (see Supplemental Digital Content Figure 1, http://links.lww.com/QAI/A696). Notably, cases from Haiti, India, and Thailand were disproportionately more likely to experience weight loss than cases from other countries and TB was one of the top 3 incident diagnoses among cases from all countries, except the United States (data not shown). Cases differed from controls by race, country, BMI, and baseline CD4+ T-cell count ($P <$ 0.01),[24,28] and cases were more likely to have a history of past or current TB ($P = 0.001$) (Table 1).

Pre-cART Immune Activation

All available blood, plasma, and PBMC samples were used to quantify biomarkers. Notably, some of the blood and plasma samples from India could not be exported, and participants from Thailand and India did not have archived PBMC samples. Compared with participants lacking PBMC samples, those with available samples for T-cell activation measurement had similar baseline age, gender, BMI, hemoglobin, and CD4+ T-cell count but a significantly lower baseline median HIV RNA level (4.99 vs. 5.18 log₁₀ copies/mL, $P = 0.04$). Table 2 shows the median pre-cART biomarker values grouped by quartile in the random subcohort and by study group in the full case–cohort. Except for CRP and IP-10, pre-cART biomarker values did not differ significantly between cases and controls. Compared with controls, cases had significantly higher median concentrations of CRP (6.3 vs. 3.03 mg/mL, $P = 0.02$) and IP-10 (2079.11 vs. 1203.81 pg/mL, $P = 0.001$). Individual pre-cART immune activation biomarkers were associated with a number of baseline study population characteristics. Notably, except for CRP ($P = 0.10$) and CD8+ T-cell activation ($P = 0.11$; data not

shown), biomarkers varied significantly by country ($P < 0.001$; see Supplemental Digital Content Figure 2, http://links.lww.com/QAI/A696).

Predictors of HIV Clinical Progression on cART

Cox-proportional hazards models stratified for country and treatment group were used to estimate the hazards of the primary outcome (incident WHO stage 3 or 4 disease or death within 96 weeks after cART initiation), for each pre-cART biomarker level grouped by quartile relative to the lowest quartile. In univariate analysis, elevated baseline levels of several biomarkers (highest quartile relative to lowest quartile) were associated with clinical progression after cART initiation, including sCD14 [hazard ratio (HR), 2.20; 95% CI: 1.01 to 4.79], IP-10 (HR, 2.81; 95% CI: 1.27 to 6.24), CRP (HR, 2.19; 95% CI: 1.11 to 4.34), CD4$^+$/DR+/CD38$^+$ (HR, 4.39; 95% CI: 1.22 to 15.69), and CD8$^+$/DR+/CD38$^+$ (HR, 6.32; 95% CI: 1.50 to 26.67) (Table 3). In multivariate analyses adjusting for baseline sex, age, BMI, CD4$^+$ T-cell count, and hemoglobin, only highest quartile CRP [adjusted hazard ratio (aHR), 2.53; 95% CI: 1.02 to 6.28] and CD4$^+$/DR+/CD38$^+$ T-cell activation (aHR, 5.18; 95% CI: 1.09 to 24.47) remained associated with the primary outcome, compared with the first quartile of both markers (Table 3 and Supplemental Digital Content Figure 3, http://links.lww.com/QAI/A696). The highest quartile of sCD14 showed a trend toward an association with clinical progression (aHR, 2.24; 95% CI: 0.96 to 5.21), as did the highest quartile of CD8$^+$/DR+/CD38$^+$ T-cell activation (aHR, 4.28; 95% CI: 0.88 to 20.76). Adjustment for baseline HIV RNA level did not substantially alter the models (Table 3 footnote). To account for the high pre-cART TB prevalence, separate multivariate models were developed that adjusted for TB at study entry and showed similar findings: there was a trend toward association that does not reach statistical significance for the highest quartile CRP (aHR, 2.43; 95% CI: 0.98 to 6.04; $P = 0.06$) and clinical progression, whereas CD4$^+$/DR+/CD38$^+$ T-cell activation maintained its association with clinical progression (aHR, 5.56; 95% CI: 1.04 to 29.76; $P = 0.045$) (see Supplemental Digital Content Table, http://links.lww.com/QAI/A696). In addition, when we set a strict definition of incident TB as occurring >4 weeks after entry to avoid misclassifying persons with subclinical prevalent TB as having incident TB, we still note that persons with the highest quartile CRP values at baseline have an increased HR of 1.63 ($P < 0.05$) of developing a WHO stage 3 or 4 outcome.

We explored ROC curve analysis to assess the predictive power of pre-cART CRP concentration as a marker for clinical HIV progression post-cART initiation (ie, incident WHO stage 3 or 4 disease or death by 96 weeks on cART). Highest quartile CRP concentration (>10 mg/L) had a specificity of 77% but low sensitivity (40%). Lowering the cutoff to CRP concentration >5 mg/L had the best predictive power but overall low sensitivity (55%) and specificity (62%). In unadjusted ROC curve analysis, the area under the curve (AUC) for CRP greater than 5 mg/L was 0.60. After adjusting for baseline age, sex, country, BMI, hemoglobin, CD4$^+$ T-cell count, plasma HIV RNA, and baseline TB, the area under the

curve was 0.71, irrespective of the cutoff used (CRP >5 mg/L or CRP >10 mg/L; Fig. 2), indicating that CRP levels pre-cART might have fair discriminating power to distinguish HIV-infected persons who are likely to clinically progress after cART initiation from persons who are unlikely to progress.

DISCUSSION

To our knowledge, this is the first study to assess and identify baseline biomarkers associated with early clinical HIV progression after cART initiation across multiple resource and clinical settings. After measuring a comprehensive panel of immune activation biomarkers, our analysis indicates that the highest quartiles of baseline pre-cART CRP concentration and CD4$^+$ T-cell activation predict subsequent progression to WHO stage 3 or 4 disease or death within 96 weeks on cART after adjusting for baseline age, sex, BMI, and CD4$^+$ T-cell count. Notably, the associations were independent of country-to-country differences in baseline biomarker levels and cART regimen. Conversely, while a trend toward significant association was observed for sCD14 and CD8$^+$ T-cell activation, we could not confirm an association between early HIV progression on cART and biomarkers that have been previously described in relation to HIV disease progression, namely in LPS, EndoCAb IgM, IL-6, TNF-α, and IP-10.[17,29–31] In resource-limited settings where follow-up intervals can be prolonged, our findings may help clinicians identify high-risk cART-naive patients who require additional vigilance for OIs and AIDS progression even after cART initiation.

The response to HIV treatment can vary considerably and AIDS-related events can still develop in the early months after cART initiation.[10,32] Indeed, AIDS-related events are still the leading cause of early mortality among persons living with HIV on cART in low- and middle-income settings, even among those with virologic suppression.[15,33] Consequently, current treatment guidelines offer no provision for patients who start cART but who will ultimately clinically progress. A baseline prognostic marker would be of particular importance in such settings, where tests for HIV RNA level and CD4$^+$ T-cell counts tests may be prohibitively expensive.[6,11]

Immune activation has been previously described in HIV-infected persons, but has been defined using differing biomarker panels, and most previous studies have been conducted at a single site or in resource-rich countries. In resource-limited settings, high CRP levels among untreated HIV-infected women who were pregnant has been previously associated with progression to WHO stage 4 disease or death in the mothers and in their children.[34] In addition, elevated CRP levels were associated with clinical progression in an HIV-infected cART-naive population of Ugandans.[35] More recently, the SMART and INSIGHT studies have associated elevated pre-cART CRP levels with death, opportunistic infection, and AIDS progression among patients in resource-rich countries.[36,37] To our knowledge, however, these are the first data to show that pre-cART CRP levels are associated with clinical progression in HIV-infected persons initiating cART in settings with mixed resource

TABLE 3. Univariate and Multivariate Analyses Showing Associations Between Pre-cART Immune Activation Biomarker Levels Grouped by Quartile and Incident WHO Stage 3 or 4 Disease or Death*

Biomarker	Univariate Analysis†			Multivariate Analysis‡§		
	HR	95% CI	P	HR	95% CI	P
CRP (mg/L); n = 413						
Q1 (<1.43)	1	—	—	1	—	—
Q2 (1.43 to <3.67)	0.90	0.41 to 1.99	0.79	0.89	0.35 to 2.27	0.80
Q3 (3.67 to <10.50)	1.32	0.67 to 2.58	0.42	1.58	0.70 to 3.60	0.27
Q4 (≥10.50)	2.19	1.11 to 4.34	0.03	2.53	1.02 to 6.28§	0.045
sCD14 (pg/mL); n = 400						
Q1 (<0.58 × 10^6)	1	—	—	1	—	—
Q2 (0.58 × 10^6 to <2.20 × 10^6)	1.64	0.73 to 3.68	0.23	2.31	0.96 to 5.56	0.06
Q3 (2.20 × 10^6 to <2.80 × 10^6)	1.29	0.58 to 2.87	0.53	1.14	0.46 to 2.84	0.78
Q4 (≥2.80 × 10^6)	2.20	1.01 to 4.79	0.047	2.24	0.96 to 5.21§	0.06
LPS‖; n = 367						
Undetectable	—	—	—	1	—	—
Detectable	1.64	0.89 to 3.0	0.11	1.27	0.65 to 2.48	0.49
EndoCAb IgM (MMU/mL); n = 417						
Q1 (<29.33)	1	—	—	1	—	—
Q2 (29.33 to <46.62)	1.07	0.52 to 2.19	0.85	1.06	0.50 to 2.28	0.87
Q3 (46.62 to <69.85)	1.12	0.56 to 2.21	0.75	1.07	0.51 to 2.24	0.86
Q4 (≥69.85)	1.41	0.73 to 2.74	0.31	1.41	0.69 to 2.90	0.34
IL-6 (pg/mL); n = 356						
Q1 (<8.90)	1	—	—	1	—	—
Q2 (8.90 to <23.77)	1.17	0.57 to 2.42	0.68	0.84	0.36 to 1.93	0.68
Q3 (23.77 to <49.98)	1.54	0.78 to 3.05	0.21	1.20	0.53 to 2.74	0.66
Q4 (≥49.98)	0.78	0.36 to 1.71	0.53	0.60	0.25 to 1.45	0.26
TNF-α (pg/mL); n = 372						
Q1 (<13.42)	1	—	—	1	—	—
Q2 (13.42 to <19.11)	1.03	0.47 to 2.24	0.94	0.92	0.37 to 2.28	0.86
Q3 (19.11 to <26.68)	0.67	0.33 to 1.37	0.27	0.44	0.17 to 1.11	0.08
Q4 (≥26.68)	1.41	0.68 to 2.93	0.36	1.20	0.53 to 2.71	0.66
IP-10 (pg/mL); n = 378						
Q1 (<585.07)	1	—	—	1	—	—
Q2 (585.07 to <1297.57)	1.15	0.548 to 2.46	0.72	1.15	0.50 to 2.62	0.74
Q3 (1297.57 to <2842.54)	1.33	0.63 to 2.82	0.45	1.17	0.47 to 2.90	0.74
Q4 (≥2842.54)	2.81	1.27 to 6.24	0.01	2.19	0.86 to 5.56	0.10
IFN-γ (pg/mL); n = 351						
Q1 (<5.53)	1	—	—	1	—	—
Q2 (5.53 to <15.85)	0.93	0.44 to 1.97	0.85	1.15	0.44 to 3.02	0.78
Q3 (15.85 to <38.43)	0.70	0.33 to 1.47	0.34	0.97	0.39 to 2.39	0.94
Q4 (≥38.43)	1.37	0.62 to 3.01	0.44	1.94	0.72 to 5.24	0.19
CD4$^+$/DR+/CD38$^+$ (%CD4); n = 155						
Q1 (<14.93)	1	—	—	1	—	—
Q2 (14.93 to <22.22)	0.63	0.20 to 1.96	0.42	0.38	0.09 to 1.62	0.19
Q3 (22.22 to <33.15)	0.86	0.23 to 3.19	0.82	0.76	0.18 to 3.17	0.71
Q4 (≥33.15)	4.39	1.22 to 15.79	0.02	5.18	1.09 to 24.47	0.04
CD8$^+$/DR+/CD38$^+$ (%CD8); n = 157						
Q1 (<34.75)	1	—	—	1	—	—
Q2 (34.75 to <44.94)	1.76	0.52 to 5.94	0.36	0.97	0.19 to 4.86	0.97
Q3 (44.94 to <55.29)	1.59	0.49 to 5.18	0.44	0.80	0.16 to 4.13	0.79
Q4 (≥55.29)	6.32	1.50 to 26.67	0.01	4.28	0.88 to 20.76	0.07

*Data presented as HR and 95% CI, comparing each quartile with the first quartile for the specified marker. The quartile breakpoints for each marker are listed below the marker. The HRs of quartiles 2, 3, and 4 are shown in comparison to quartile 1, which was used as reference. The indicated quartile comprises the interval from the next lowest quartile to labeled quartile.

†Univariate models were stratified by country and treatment group as was prespecified.

‡Multivariate models, stratified for country and treatment group and adjusted for gender, age, BMI, baseline CD4$^+$ T-cell count, and hemoglobin, are shown for each marker.

§Separate multivariate models were developed that included baseline HIV RNA level, but were no more informative than models that included baseline CD4$^+$ T-cell count and hemoglobin [CRP Q4 HR = 2.55, 95% CI: 1.04 to 6.23, P = 0.04; sCD14 Q4 HR = 2.38, 95% CI: 1.01 to 5.57, P = 0.04].

‖Because most samples did not have detectable LPS, samples with detectable LPS were compared with those without detectable LPS in a bivariate model.

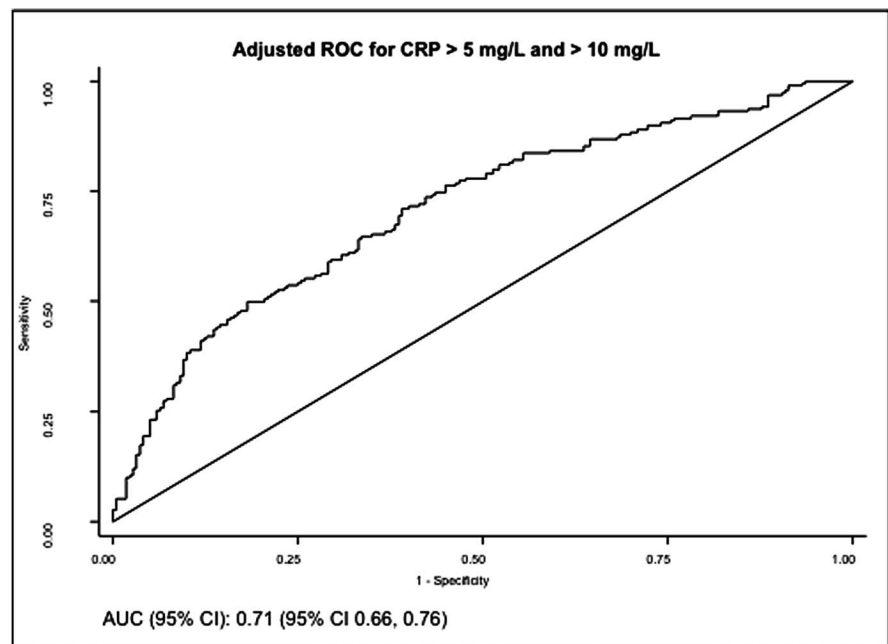LPS, gram-negative bacterial LPS; n, number of samples tested.

FIGURE 2. Pre-cART CRP as a prediction tool for clinical failure. ROC analysis was performed to evaluate if elevated levels of CRP pre-cART can be used to predict clinical failure post-cART initiation. The CRP cutoff was varied using observed CRP levels. After adjusting for baseline age, sex, country, BMI, hemoglobin, CD4+ T-cell count, plasma HIV RNA, and baseline TB, the area under the curve (AUC) was 0.71, irrespective of the cutoff used (CRP >5 mg/L or CRP >10 mg/L).

levels. In contrast, evidence regarding CD4+ T-cell activation has been less consistent. Apart from the present analysis, only 1 other study has associated baseline CD4+ T-cell activation with outcomes among patients initiating cART,[38] whereas several studies have found no such association.[37,39,40] Thus, our analysis supports and expands previous CRP findings in resource-rich settings now to diverse resource-limited settings while also adding evidence linking CD4+ T-cell activation to cART outcomes.

CRP assays are relatively inexpensive and could be used in resource-limited and resource-rich settings. Our finding that elevated baseline CRP concentration greater than 10 mg/L is independently associated with poor clinical response to cART may provide clinicians in multiple settings with a more generalizable approach to risk-stratify patients before cART initiation. Furthermore, despite its lack of specificity, CRP (an acute phase reactant produced by the liver in response to general inflammatory stimuli) has remarkable predictive value in varied conditions from cardiovascular disease to osteomyelitis.[41,42] For example, among healthy persons, a CRP concentration greater than 3 mg/L is an independent risk factor for cardiovascular risk.[43] In our study, cases had a median baseline CRP of 6 mg/L compared with that of 3 mg/L in controls, supporting its potential clinical utility to distinguish HIV-infected persons who may progress after cART initiation. However, larger cohort studies will be needed to determine appropriate breakpoints before using CRP concentration as a clinical marker among cART initiators.

Interestingly, we did not confirm a significant association between the remaining immune activation biomarkers and early HIV progression on cART. While CD8+ T-cell activation has been associated with HIV disease progression, it has not been consistently associated with cART treatment outcomes.[38–40,44–46] Similarly, elevated baseline IL-6 levels, which have long been associated with HIV progression, has been an inconsistent predictor of outcomes among cART initiators.[36,37,47,48] IP-10, a chemokine that is strongly predictive of hepatitis C virus treatment outcomes, has been associated with HIV outcomes in some studies.[31,49] We did find a trend for an association between sCD14 and clinical progression that confirms previous studies. Finally, the MT markers LPS and EndoCAb IgM have been associated with mortality and disease progression among cART initiators in some, but not all, studies.[21,36,40,50] Therefore, there may be a distinction between markers that predict disease progression in untreated patients, and markers that are predictive among HIV-infected patients receiving cART.

This study did not provide evidence for MT in this cohort as defined by detectable LPS levels despite using an optimized assay that accounts for serum interference.[27] It is possible that prevalent diarrheal disease in some endemic settings may alter the associations of MT and HIV. However, sCD14 and CD8+ T-cell activation had a trend toward association with incident WHO stage 3 or 4 disease or death on cART, which is consistent with and extends previous findings to multiple geographic settings. Overall, we speculate that a combination of factors, including socioeconomics, malnutrition, and prevalent acute infections (eg, malaria, diarrheal disease), may have contributed to the generalized immune activation state of patients but that these factors may not have been directly involved in the causal pathways that led to HIV disease progression.

Strengths of this study include the diversity of settings and the comprehensive panel of immune activation biomarkers assessed. However, this study has some potential limitations. Our study was limited to participants with CD4+ T-cell

counts <300 cells per cubic millimeter; given current guidelines, future investigations will need to determine whether our findings are applicable to HIV-infected persons initiating cART at higher CD4$^+$ T-cell counts. There were a large number of prevalent and incident cases of TB, a leading cause of morbidity and mortality among HIV-infected persons in resource-limited settings. Therefore, it is possible that baseline subclinical TB may have resulted in augmented immune activation, thus confounding the analysis. Notably, however, non-TB diagnoses comprised more than 70% of the incident diagnoses among cases; therefore, our findings are likely to be generalizable to HIV-infected populations, irrespective of TB risk. Moreover, our analysis accounted for the effect of prevalent TB at baseline, which did not substantially affect the overall strength of the predictive models. Notably, the entry criteria for the PEARLS study included a relatively healthy population. For example, fewer than 20% of cases and controls in the subcohort were underweight. Whereas on the one hand, the relative health of the study population suggests that these findings will need confirmation, on the other hand, we propose that an immune activation biomarker is particularly useful in identifying people where the risk of clinical progression is not obvious. Another challenge was the limited availability of viable PBMC samples for T-cell activation marker testing, especially from India and Thailand. Participants with available PBMC samples were similar to those without samples among baseline characteristics, except for lower median baseline HIV RNA level, which would be expected to underestimate the association between baseline CD4$^+$ T-cell activation and HIV disease progression. Nevertheless, our findings will need to be confirmed in Asian populations to broaden the generalizability of CD4$^+$ T-cell activation.

In summary, our analysis identified 2 biomarkers of immune activation that seem to independently predict early clinical HIV disease progression in multiple countries among HIV-infected adults initiating cART. While measurement of baseline CD4$^+$/DR+/CD38$^+$ T-cell activation may provide predictive information about the clinical response to cART, the utility of this biomarker may be limited due to its high cost and complexity in measuring it. CRP assays are low-cost, relatively easy to perform, and may be available in some resource-limited settings. Pre-cART risk stratification using CRP measurements may prove worthwhile to identify patients for whom added vigilance, such as more frequent clinical monitoring or disease-specific screening after treatment initiation, may be warranted, similar to the cardiovascular disease paradigm.

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