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Anemia is associated with monocyte activation in HIV-infected adults on antiretroviral therapy

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Abstract

Background—Anemia has been linked with mortality in HIV infection. The mechanism of anemia in the era of contemporary antiretroviral therapy is not understood. The aim of this study was to describe the association between anemia and markers of immune activation and inflammation in a cohort of HIV-infected adults on stable antiretroviral therapy.

Methods—We performed a cross-sectional study of HIV-infected adults on antiretroviral therapy with HIV-1 RNA < 1000 copies/ml. Soluble and cellular markers of inflammation and immune activation were measured. Relationships between hemoglobin levels, anemia (hemoglobin <13 g/dL for men and <12 g/dL for women) and mild anemia (hemoglobin <14 g/dL for men and <13 g/dL for women) and these markers were explored using multivariable linear regression.

Results—Among the 147 participants, median age was 46 years, 78% were men, 68% were African American and 29% were Caucasian. Median BMI was 26.7 kg/m^2, nadir and current CD4+ T cell counts were 179 and 613 cells/mm^3, respectively, and 78% had HIV-1 RNA <50 copies/ml (range 20–600 copies/ml). Median (IQR) hemoglobin was 14.3 (13.1–15.1) g/dl; 14% were anemic and 33% had at least mild anemia. In multivariable analyses, mild anemia was independently associated with female sex, older age, shorter duration of ART, lower WBC count, higher platelet count, higher sCD14 and a greater number of CD14^{dim}CD16$^+$ cells or “patrolling” monocytes, which remained significant after further adjusting for race and BMI.

Conclusions—Having hemoglobin <14 g/dL for men and <13 g/dL for women was independently associated with monocyte activation (sCD14 and CD14^{dim}CD16$^+$ cells) in HIV-infected adults on stable antiretroviral therapy.

Keywords

HIV; anemia; systemic inflammation; immune activation; antiretroviral therapy; CD14^{dim}CD16$^+$

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INTRODUCTION

Human Immunodeficiency Virus (HIV) is associated with the suppression of multiple hematopoietic lineages, including red blood cells[1]. Anemia is common in HIV and prevalence depends on the sex, age, pregnancy status, injection drug use, and stage of HIV disease[2]. The recently published SILCAAT trial reported that among 1,410 participants with HIV on combination antiretroviral therapy (ART), 313 (22.2%) had anemia[3]. Anemia has been shown to contribute to poorer quality of life, HIV disease progression, and morbidity and mortality in people living with HIV[2, 4]; whereas, resolution of anemia improves quality of life and survival[2]. One study showed that anemia accounts, in part, for the difference in mortality between men and women with HIV[5].

Anemia of chronic disease (or anemia of inflammation) links a chronic inflammatory process to resultant anemia primarily through hepcidin's regulation of iron metabolism by inhibiting intestinal absorption of iron, blocking release of iron from macrophages, and blocking heme delivery to erythroid cells[6]. Hepcidin is induced by IL-6, and is regulated through JAK/STAT-3 signaling[7]. IL-6 is elevated in HIV-infected adults with anemia, and is associated with greater probability of all-cause mortality[2, 8]. Anemia of chronic disease is associated with other markers of inflammation, including IL-1 and TNF-alpha[9]. These inflammatory cytokines may induce apoptosis of red cell precursors, down-regulate the expression of erythropoietin receptors, and ultimately decrease the bone marrow's ability to respond to erythropoietin signaling[9].

It has been suggested that anemia in HIV is due to enhanced inflammation or immune activation; however, few studies have assessed this link in the era of contemporary ART[3, 10, 11]. In this study we describe the association between anemia and markers of immune activation and inflammation in a cohort of HIV-infected adults on stable contemporary ART. Our hypothesis was that anemia would be associated with higher markers of immune activation and inflammation.

METHODS

Study design

This is a cross-sectional study to determine prevalence of anemia and explore the relationship between anemia and immune activation and inflammation in HIV-infected adults on stable contemporary ART. Data for this study were collected from the entry visit of the Stopping Atherosclerosis and Treating Unhealthy bone with Rosuvast-atiN (SATURN-HIV) study, a randomized, double blind, placebo-controlled study designed to determine the effect of rosuvastatin on markers of cardiovascular risk, skeletal health, and immune activation in HIV-infected adults on ART. The study is registered on clinicaltrials.gov, Identifier: NCT01218802, and was approved by the Institutional Review Board of the University Hospitals Case Medical Center, Cleveland, OH. All participants signed a written informed consent prior to enrollment.

The eligibility criteria for SATURN-HIV have been described previously[12]. In brief, all participants were ≥18 years of age, without known coronary disease or diabetes, on stable

ART for at least 3 months with cumulative ART duration of at least 6 months, with HIV-1 RNA <1,000 copies/mL and fasting LDL-cholesterol (LDL-C) ≤130mg/dL. Additional entry criteria included evidence of either heightened T-cell activation, identified as proportion of CD8+ T cells that express CD38 and HLA-DR ≥19%, or systemic inflammation, identified as levels of high sensitivity C-reactive protein (hsCRP) ≥2 mg/L. Exclusion criteria were pregnancy or lactation, immunomodulating, hormonal, or anti-inflammatory medications, inflammatory condition besides HIV, hemoglobin level <9 g/dL, or creatinine clearance <50 mL/min as estimated by the Cockcroft- Gault equation. All participants enrolled into the SATURN-HIV study were included in this analysis.

Study evaluations

At the initial screening visit, self-reported demographics and medical history were obtained along with a targeted physical exam including height and weight measurements. Blood was drawn after a 12-hour fast for lipoproteins, percent CD8+ T cell activation and hsCRP. If enrollment criteria were met, participants returned within 30 days for entry evaluations.

At entry, blood was drawn for real time measurement of complete blood count (CBC). A Sysmex XE-5000 Hematology Analyzer (Sysmex America, Inc., Lincolnshire, Illinois, USA) was used for CBC determination. This analyzer counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection enhanced by hydrodynamic focusing. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to SLS-hemoglobin and read photometrically. White blood cell (WBC) count and differential are evaluated using flow cytometry with a semiconductor laser exploiting the differences in cell size, complexity and RNA/DNA content. We utilized two measures of HGB status, anemia and a broader definition of anemia, ie, mild anemia, as our primary outcomes. Anemia was defined using the World Health Organization definition of a HGB level <13 g/dL for men and <12 g/dL for women. Mild anemia was defined as HGB <14 g/dL for men and <13 g/dL for women. Both definitions were utilized in this study in order to capture individuals with less severe anemia which is often how anemia of chronic disease manifests. Subclassifications of anemia include microcytic anemia (mean corpuscular volume or MCV <80), normocytic anemia (80≤ MCV ≥100), and macrocytic anemia (MCV >100).

At the same visit, blood was drawn and processed for measurement of immune activation and inflammation markers. Whole-blood samples were collected into ethylenediaminetetraacetic acid–containing tubes. Peripheral blood mononuclear cell (PBMC) samples were separated by centrifugation with Ficoll-Hypaque and were cryopreserved until analyzed in batches. Plasma was isolated by centrifugation and was frozen at −80°C until analyzed in batches.

Immune activation markers

Monocytes and T-cells were phenotyped from whole blood and PBMCs, respectively, by flow cytometry as previously described[12]. Three monocyte subsets: (1) CD14+CD16+, (2) $CD14^{dim}CD16+$, and (3) CD14+CD16- were each quantified as total number of cells and as a percentage of the overall monocyte population. The monocyte subset gating strategy was

based on methods previously described[13, 14]. In brief, monocyte subsets were identified on expression of CD14 and CD16, based on population and isotype gating strategies. T-cell activation was quantified as the percentage of CD4+ or CD8+ cells that expressed both CD38 and HLA-DR. Soluble CD14 and CD163 were determined using Quantikine ELISA kits (R&D Systems, Minneapolis, MN). The inter-assay variability ranged 0.4–8.6% and 0.7–18.3%, respectively. Above assays were performed at Case Western Reserve University.

Inflammation markers

Interleukin-6, soluble tumor necrosis factor receptors-I and –II (sTNF-RI and sTNF–RII), soluble intracellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) and interferon-γ-inducible protein-10 (IP-10) were determined by quantitative sandwich ELISAs (R&D Systems, Minneapolis, MN). Inter-assay variability ranged from 2.02%–15.36%, 3.66%–5.77%, 2.13%–3.79%, 3.43%–7.37%, 4.76%–8.77%, and <5%, respectively. High sensitivity C-reactive protein was determined by particle enhanced immunonephelometric assay on a BNII nephelometer (Siemens, Indianapolis, IN, USA). Inter-assay variability ranged from 3.01%–6.46%. All above biomarker assays were performed at the Laboratory for Clinical Biochemistry Research University of Vermont with the exception of IP-10 which was performed at Case Western Reserve University.

HIV-1 RNA levels and CD4+ T cell counts were obtained as part of routine clinical care.

Statistical methods

Demographic, HIV-related characteristics, and outcome variables are presented overall using median and interquartile range (IQR) for continuous variables and frequency and percent for categorical variables. Hemoglobin level was compared between important categorical variables of interest using Wilcoxon rank sum tests. For continuous variables, Spearman correlation analysis was used to determine associations with HGB. Variables of interest included age, sex, race, smoking, Hepatitis C status, body mass index (BMI) as well as lean body mass, glomerular filtration rate ($eGFR_{cr}$) estimated using the creatinine-based 2009 Chronic Kidney Disease Epidemiology Collaboration equation[15], serum creatinine, current and nadir CD4+ T cell count, HIV-1 RNA level, duration of ART, known duration of HIV infection and markers of inflammation and immune activation described above. Univariable followed by multivariable logistic regression was used to explore factors independently associated with anemia and mild anemia as defined above. Any variable associated with anemia or mild anemia in univariable analysis with $p<0.25$ was considered for inclusion in the multivariable models. Backwards selection was used to generate the final models. When monocyte subset count variables were included in the model, the total monocyte count was incorporated as well. Last, age, sex, race and BMI were added to both final models as known clinically important confounders regardless of statistical significance. Logistic regression was utilized because the variable HGB was not normally distributed.

All statistical tests were two-sided and considered significant with $p<0.05$. Adjustments were not made in this significance level for multiple comparisons. Analyses were performed using SAS v. 9.2 (The SAS Institute, Carey, North Carolina, USA).

RESULTS

In the SATURN-HIV study, 147 adults were enrolled between March 2011– August 2012. All participants enrolled were included in this cross-sectional analysis. Overall, 78% were men, 68% were African American, 29% were Caucasian, and 8% had Hepatitis C co-infection. The median (IQR) age was 46.2 (40.4–52.6) years, BMI was 26.7 (23.5–30.2) kg/m^2, creatinine was 0.94 (0.8–1.1) mg/dl. 63% were current smokers and an additional 16% were smokers in the past. Median (IQR) nadir and current CD4+ T cell counts were 179 (86–298) and 613 (425–853) cells/mm^3, respectively, and 78% had HIV-1 RNA <50 copies/ml (range 20–600 copies/ml). By design, all participants were on ART; 5% were on zidovudine, 1% on stavudine, and 50% on a protease inhibitor (PI). The median (IQR) known duration of HIV infection and ART duration were 11 (6–17) and 5 (3–10) years, respectively. No participants were receiving iron supplements or erythropoietin therapy.

Table I shows the hematologic parameters and markers of immune activation and inflammation for the group. Among the 147 participants, median (range) HGB was 14.3 (9.7–17.4) g/dL; 33% (48/147) had at least mild anemia and14% (20/147) met criteria for anemia. Of those with anemia, 25% (5/20) had microcytic anemia, 65% (13/20) had normocytic anemia, and 10% (2/20) had macrocytic anemia. Hemoglobin was higher in men compared to woman (14.7 (14–15.4) vs 12.7 (11.9–13.2) g/dL; $p<0.0001$) and for Caucasians compared to other races (14.7 (14.1–15.5) vs 14.2 (12.9–15) g/dL; $p=0.004$), but was not different by hepatitis C or smoking status, or whether on a thymidine analogue nucleoside reverse transcriptase inhibitor (NRTI) or a PI. In the correlation analysis (Table II), HGB was positively correlated with lean body mass ($\rho=0.185$; $p=0.025$) and serum creatinine ($\rho=0.223$; $p=0.007$), inversely correlated with platelet count ($\rho=-0.31$; $p=0.0001$), and not correlated with age, $eGFR_{cr}$, current or nadir CD4+ T cell count, HIV-1 RNA level, or HIV or ART duration. Additionally, HGB was not correlated with any markers of immune activation or inflammation although correlation with IL-6 ($\rho=-0.148$; $p=0.073$) and $CD14^{dim}CD16^{+}$ cell count neared significance ($\rho=-0.159$; $p=0.058$).

Table III shows the results of the univariable and multivariable logistic regression analyses for anemia. Factors independently associated with anemia include: older age, being a race other than Caucasian and higher platelet count. These variables remain associated with anemia after adjusting for sex and BMI as well.

Table IV shows the results of the univariable and multivariable logistic regression analyses for mild anemia. Factors independently associated with mild anemia include: older age, female sex, shorter duration of ART, lower WBC count, higher platelet count, greater number of $CD14^{dim}CD16^{+}$ cells or “patrolling” monocytes and higher sCD14. These variables remain independently associated with mild anemia after adjusting for race and BMI as well. Last, the odds of mild anemia per quartile of percent $CD14^{dim}CD16^{+}$ cells with the lowest quartile as the reference group are as follows: 2^{nd} quartile OR (95% CI) 1.687 (0.590–4.823), 3^{rd} quartile OR 1.547 (0.534–4.482), and 4th quartile OR 3.02 (1.083–8.416). This exploratory analysis suggests a threshold effect for percent $CD14^{dim}CD16^{+}$ cells on the odds of mild anemia, ie, the odds of mild anemia increases in the highest quartile of percent $CD14^{dim}CD16^{+}$ cells.

DISCUSSION

In this study we have comprehensively assessed the relationships between anemia and markers of immune activation, specifically monocyte and T cell activation, as well as systemic inflammation among HIV-infected adults on stable, contemporary ART. We have shown that having a HGB < 14 g/dL for men or HGB < 13 g/dL for women, i.e. having at least mild anemia, is common even in this population with high CD4+ T cell count. Further, this outcome is associated with markers of monocyte activation, including sCD14 and $CD14^{dim}CD16^{+}$ monocytes or "patrolling monocytes" such that participants with at least mild anemia have higher odds of increased monocyte activation compared to those with normal HGB levels. Further support for the role of monocyte activation in anemia in HIV infection is the demonstrated threshold effect, whereby the odds of having at least mild anemia are greatest in the highest quartile of percentage $CD14^{dim}CD16^{+}$ cells.

It has been shown that even after suppressing HIV with ART, persistent systemic inflammation and activation of the immune system occur[16, 17]. As such, it is not surprising that just as in other chronic inflammatory conditions, i.e. rheumatoid arthritis, diabetes, and inflammatory bowel disease, the prevalence of anemia was high in our study[18–21]. The prevalence of having at least mild anemia was 33%. Although this is slightly higher than in the SILCAAT study, a recent cross-sectional study of HIV-infected adults on ART, the discrepancy is likely due to the different definition used for anemia[3].

Our results are congruent with other studies evaluating the role of persistent inflammation and immune activation in the pathogenesis of anemia in HIV-infected individuals on ART[3, 10, 11]. In the SILCAAT study, the adjusted relative odds of anemia per two-fold higher IL-6 level was 1.22, p=0.007[3]. Measures of immune activation were not evaluated in this study[3]. While IL-6 was not independently predictive of anemia or mild anemia in our study, there was a trend towards significance in the correlation of analysis evaluating the association between HGB level and IL-6. It is possible that more associations were not seen with the inflammation markers tested because this study population was on stable ART. While persistent inflammation is seen in those on ART[16, 17], the magnitude of the effect on Hgb levels is likely to be smaller than that seen with pre-ART levels of inflammation.

The role of monocyte activation in HIV infection has recently received much attention due to observations linking monocyte activation markers to adverse outcomes[13, 22–27]. In an earlier cross-sectional study, anemia was shown to be associated with monocyte activation through the measure of neopterin, which is produced by monocytes/macrophages upon stimulation with the cytokine interferon-gamma[10]. In this study, low HGB level was associated with increased interferon-gamma, neopterin and beta 2-microglobulin[10]. Six months after initiating ART, improved HGB level was associated with a decrease in neopterin levels supporting the role of monocyte activation in anemia in HIV infection[11]. Soluble CD14, a marker of monocyte response to lipopolysaccharide, has been an important measure that has been linked to subclinical atherosclerosis[24] and even mortality in HIV[27]. In our study, higher sCD14 was associated with increased odds of having at least mild anemia. Further supporting the role of monocyte activation in anemia in HIV, higher $CD14^{dim}CD16+$ cells or "patrolling" monocytes were also associated.

There are some limitations to our study. First, we did not measure hepcidin levels, reticulocyte counts, or complete iron studies. As such, there may be additional factors not considered contributing to anemia mainly in those with non-normocytic anemia. It is possible this is the reason associations were not seen between the more restrictive criteria for anemia and monocyte activation. In these cases, perhaps the underlying cause of the anemia, e.g. iron deficiency, was a more significant contributor. Further, associations between Hgb and the inflammation makers tested may have been diluted by the presence of individuals with anemia other than anemia of chronic disease. Importantly, those with non-normocytic anemia were a minority in this study. Another limitation is that this population was preselected for having heightened inflammation or immune activation at baseline due to their preexisting disease status. As this is a hypothesis generating study, our results should be confirmed prior to generalizing to all HIV-infected adults on ART. We have not adjusted for multiple comparisons, and this may increase the risk of false associations; however, we did not want to miss associations that could inform future investigation in this exploratory study. Last, this was a cross-sectional study and causality cannot be determined.

In conclusion, in HIV-1 infected adults on stable, contemporary ART, the prevalence of anemia was high and having HGB < 14 g/dL for men and HGB < 13 g/dL for women was associated with monocyte activation measured by sCD14 and number of CD14dimCD16+ cells even after controlling for factors known to be linked with anemia. Longitudinal studies are needed to confirm the associations between monocyte activation and anemia in HIV infection.

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Table I

Hematologic Parameters and Markers of Immune Activation and Inflammation

Variable	Median (IQR)
Hemoglobin, g/dL	14.3 (13.1–15.1)
Hematocrit, %	42.7 (39.3–44.7)
Mean Corpuscular Volume, fL	91 (88–96)
White Blood Cell Count, x10^9/L	5.6 (4.6–7.3)
Platelet Count, x10^9/L	233 (192–284)
CD14+CD16+, %	23.3 (18.28–34.85)
CD14^{dim}CD16+, %	11.26 (7.8–14.94)
sCD163, ng/mL	650 (482–872)
sCD14, ng/mL	2140 (1736–2479)
CD4+CD38+HLA-DR+, %	5.13 (3.58–6.62)
CD8+ CD38+HLA-DR+, %	12.2 (8.71–17.5)
hsCRP, µg/mL	1.67 (0.77–4.91)
IL-6, pg/mL	2.76 (1.9–4.71)
sTNF-RI, pg/mL	1581 (1268–2330)
sTNF-RII, pg/mL	2329 (1677–2850)
IP-10, pg/mL	219 (155–350)

hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; sTNFR-I, soluble tumor necrosis factor α-receptor I; sTNF-RII, soluble tumor necrosis factor α-receptor II; IP-10, interferon γ inducible protein-1

Table II

Spearman Correlation Analysis between Hemoglobin and Demographics, HIV-Related Factors and Markers of Immune Activation and Inflammation

Variable	Correlation Coefficient	P
Age, years	−0.104	0.21
Body Mass Index, kg/m^2	−0.134	0.11
Lean Body Mass, kg	0.185	0.03
eGFRcr, mL/min/1.73m^2	−0.072	0.39
Creatinine, mg/dL	0.223	<0.01
White Blood Cell Count, x1000 cells/mm^3	0.106	0.2
Platelet Count, x1000 cells/mm^3	−0.31	<0.01
CD4+ T Cell Count, cells/mm^3	−0.094	0.26
Nadir CD4+ T Cell Count, cells/mm^3	−0.103	0.22
HIV-1 RNA Level, copies/mL	−0.047	0.57
Antiretroviral Duration, months	0.14	0.11
HIV Duration, months	0.121	0.14
CD14+CD16+, %	0.033	0.7
CD14+CD16+ Cell Count, cells	−0.034	0.69
CD14^{dim}CD16+, %	−0.134	0.11
CD14^{dim}CD16+ Cell Count, cells	−0.159	0.06
sCD163, ng/mL	0.07	0.4
sCD14, ng/mL	−0.135	0.1
CD4+CD38+HLA-DR+, %	−0.056	0.5
CD8+ CD38+HLA-DR+, %	0.076	0.37
hsCRP, μg/mL	−0.126	0.13
IL-6, pg/mL	−0.148	0.07
sTNF-RI, pg/mL	0.135	0.1
sTNF-RII, pg/mL	−0.044	0.59
IP-10, pg/mL	0.01	0.9

hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; sTNF-RI, soluble tumor necrosis factor α-receptor I; sTNF-RII, soluble tumor necrosis factor α-receptor II; IP-10, interferon γ inducible protein-1

Table III

Univariable and Multivariable Logistic Regression Models for Anemia (Hemoglobin < 13 g/dL for men; Hemoglobin < 12 g/dL for women)

Variable[1]	Univariable Models			Multivariable Model[2]		
	Parameter estimate (SE)	OR (95% CI)	P	Parameter estimate (SE)[2]	aOR (95% CI)	P
Age, year	$4.13x10^{-2}$ ($2.61x10^{-2}$)	1.042 (0.99–1.097)	0.11	$6x10^{-2}$ ($3.03x10^{-2}$)	1.062 (1.001–1.127)	<0.05
Sex, 1=male	−1.31 (0.51)	0.27 (0.1–0.727)	<0.01			
Race, 1=Caucasian	−2.24 (1.04)	0.107 (0.014–0.823)	0.03	−2.48 (1.08)	0.084 (0.01–0.693)	0.02
Hepatitis C, 1=yes	0.84 (0.72)	2.314 (0.569–9.402)	0.24			
Platelet Count, $x10^9$/L	$9.91x10^{-3}$ ($3.50x10^{-3}$)	1.01 (1.003–1.017)	<0.01	$1.19x10^{-2}$ ($4.31x10^{-3}$)	1.012 (1.003–1.021)	<0.01
Total Monocyte Cell Count, cells[3]	$-1x10^{-5}$ ($2x10^{-5}$)	1 (1-1)	0.53			
$CD14^{dim}CD16^+$ Cell Count, cells	$2.27x10^{-4}$ ($1.44x10^{-4}$)	1 (1–1.001)	0.11			
sCD163, ng/mL	$1.06x10^{-3}$ ($5.66x10^{-4}$)	1.001 (1–1.002)	0.06			
IL-6, pg/mL	$2.78x10^{-2}$ ($1.83x10^{-2}$)	1.028 (0.992–1.066)	0.13			

[1] All variables with p<0.25 in univariable analysis are shown in the table. Variables tested but not shown include: smoking status, body mass index, lean body mass, $_eGFR_{Cr}$, creatinine, protease inhibitor status, thymidine analogue status, HIV-1 RNA <50 copies/mL, HIV-1 RNA level (as continuous variable), current and nadir $CD4^+$ T cell count, HIV duration, ART duration, white blood cell count, $\%CD14^+CD16^+$, $CD14^+CD16^+$ cell count, $\%CD14^{dim}CD16^+$, sCD14, IP-10, $\%CD4^+DR^+38^+$, $\%CD8^+DR^+38^+$, hsCRP, sTNF-RI, sTNF-RII.

[2] All variables with p<0.25 in univariable analysis were considered in the final model selection. Backward elimination was used for final model selection.

[3] Total monocyte count included in all models including $CD4^{dim}CD16^+$ cell count.

SE, standard error; CI, confidence interval; sCD163, soluble CD163; IL-6, interleukin-6

Table IV

Univariable and Multivariable Logistic Regression Models for Mild Anemia (Hemoglobin < 14 g/dL for men; Hemoglobin < 13 g/dL for women)

Variable[1]	Univariable Models			Multivariable Model[2]		
	Parameter estimate (SE)	OR (95% CI)	P	Parameter estimate (SE)[2]	aOR (95% CI)	P
Age, year	$3.51x10^{-2}$ ($1.87x10^{-2}$)	1.036 (0.998–1.074)	0.06	$8.21x10^{-2}$ ($2.95x10^{-2}$)	1.086 (1.025–1.15)	<0.01
Sex, 1=male	−1.83 (0.43)	0.161 (0.069–0.375)	<0.0001	−2.17 (0.62)	0.115 (0.034–0.386)	<0.001
Race, 1=Caucasian	−1.01 (0.44)	0.366 (0.154–0.867)	0.02			
Body Mass Index, kg/m²	$4.02x10^{-2}$ ($2.67x10^{-2}$)	1.041 (0.988–1.097)	0.13			
HIV-1 RNA <50 copies/mL, 1=yes	−0.55 (0.41)	0.577 (0.260–1.282)	0.18			
ART Duration, month	$-8.89x10^{-3}$ ($3.68x10^{-3}$)	0.991 (0.984–0.998)	0.02	$-1.84x10^{-2}$ ($5.7x10^{-3}$)	0.982 (0.971–0.993)	<0.01
White Blood Cell Count, x10⁹/L	−0.12 (0.08)	0.890 (0.757–1.046)	0.16	−0.25 (0.13)	0.780 (0.607–1.003)	0.05
Platelet Count, x10⁹/L	$8.52x10^{-3}$ ($2.84x10^{-3}$)	1.01 (1.004–1.015)	<0.001	$1.31x10^{-2}$ ($4.46x10^{-3}$)	1.013 (1.004–1.022)	<0.01
Total Monocyte Cell Count, cells	$1.422x10^{-6}$ ($1.4x10^{-5}$)	1 (1-1)	0.92	$-2x10^{-5}$ ($2.8x10^{-5}$)	1 (1-1)	0.38
$CD14^{dim}CD16^{+}$ Cell Count, cells[3]	$2.77x10^{-4}$ ($1.14x10^{-4}$)	1 (1–1.001)	0.02	$4.60x10^{-4}$ ($1.67x10^{-4}$)	1 (1–1.001)	<0.01
sCD14, ng/mL	$4.39x10^{-4}$ ($2.76x10^{-4}$)	1 (1–1.001)	0.11	$9.13x10^{-4}$ ($4.37x10^{-4}$)	1.001 (1–1.002)	0.04
IP-10, pg/mL	$8.58x10^{-4}$ ($5.56x10^{-4}$)	1.001 (1–1.002)	0.12			

[1] All variables with p<0.25 in univariable analysis are shown in the table. Variables tested but not shown include: Hepatitis C status, smoking status, lean body mass, $_{e}GFR_{cr}$, creatinine, protease inhibitor status, thymidine analogue status, HIV-1 RNA level (as continuous variable), current and nadir $CD4^{+}$ T cell count, HIV duration, $\%CD14^{+}CD16^{+}$, $CD14^{+}CD16^{+}$ cell count, $\%CD14^{dim}CD16^{+}$, sCD163, $\%CD4^{+}DR^{+}38^{+}$, $\%CD8^{+}DR^{+}38^{+}$, hsCRP, IL-6, sTNF-RI, sTNF-RII

[2] All variables with p<0.25 in univariable analysis were considered in the final model selection. Backward elimination was used for final model selection.

[3] Total monocyte count included in all models including $CD4^{dim}CD16^{+}$ cell count.

SE, standard error; CI, confidence interval; ART, antiretroviral therapy, sCD14, soluble CD14; IP-10, interferon γ inducible protein-1