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ARTICLE

Soluble CD14, CD163, and CD27 biomarkers distinguish ART-suppressed youth living with HIV from healthy controls

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Abstract

Objective: To define inflammatory pathways in youth living with HIV infection (YLWH), assessments of biomarkers associated with lymphocyte and macrophage activation, vascular injury, or bone metabolism were performed in YLWH in comparison with healthy controls (HC).

Design: Longitudinal multicenter study comparing biomarkers in YLWH suppressed on antiretroviral therapy (ART), those with ongoing viral replication, and HC were compared using single blood samples obtained at end of study.

Methods: Twenty-three plasma proteins were measured by ELISA or multiplex assays. Principal component analysis (PCA) was used to define contributions of individual biomarkers to define outcome groups.

Results: The study cohort included 129 predominantly African American, male participants, 21–25 years old at entry. Nine biomarkers of lymphocyte and macrophage activation and cardiovascular injury differed between HC and YLWH. Significant positive correlations were identified between lymphocyte and macrophage activation biomarkers among HC and YLWH. Correlations distinct to YLWH were predominantly between biomarkers of macrophage and vascular inflammation. PCA of outcome groups showed HC and suppressed YLWH clustering together for lymphocyte activation biomarkers, whereas macrophage activation markers showed all YLWH clustering distinct from HC. Cardiovascular biomarkers were indistinguishable across groups. Averaged variable importance projection to assess single biomarkers that maximally contribute to discriminate among outcome groups identified soluble CD27, CD14, and CD163 as the 3 most important with TNFα and LPS also highly relevant in providing separation.

Conclusions: Soluble inflammatory and lymphocyte biomarkers sufficiently distinguish YLWH from HC. Persistent macrophage activation biomarkers may provide a means to monitor consequences of HIV infection in fully suppressed YLWH.

KEYWORDS
adolescents, biomarker, chemokines, cytokines, HIV, inflammation, lymphocytes, macrophage

1 | INTRODUCTION

HIV infection perturbs inflammatory networks before the loss of CD4 T cells and development of immune deficiency.[1] Chronic lymphocyte and macrophage activation are implicated in the increase in non-AIDS related morbidity and mortality among chronically HIV-infected

Abbreviations: ART, antiretroviral therapy; CRP, C-reactive protein; HC, healthy controls; MPO, myeloperoxidase; MANOVA, multivariate analysis of variance; MMP-2, matrix metalloprotease-2; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; VIP, variable importance in projection; YLWH, youth living with HIV infection

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adults.[2–5] In contrast, few studies have focused on the inflammatory pathways and biomarkers associated with early infection in youth living with HIV infection (YLWH). YLWH, ages 13–24, represent over 25% of newly acquired HIV infections in the United States and are the most rapidly expanding population of infected individuals worldwide.[6] Recent changes in treatment recommendations for the initiation of combination antiretroviral therapy (ART) will lead to treatment of all YLWH independent of CD4 T cell counts or extent of HIV-associated disease.[7,8]

In general, at diagnosis YLWH have higher CD4 T cell counts, fewer comorbidities, and less advanced disease progression than their adult counterparts.[9,10] YLWH who initiate ART prior to CD4 T cell decline have a high capacity to reconstitute functional T cell immunity,[11] although macrophage and T cell activation, osteopenia, and biomarkers of vascular injury persist, even when viral replication is optimally controlled and CD4 T cell counts are normal.[11–13] For example, effective ART lowers plasma levels of biomarkers for lymphocyte activation, soluble CD27 (sCD27), and macrophage activation, soluble CD163 (sCD163), compared with untreated individuals,[14,15] even though biomarker levels among virally suppressed individuals remain elevated compared with HIV-uninfected youth.[11] A comprehensive, integrated assessment of biomarkers associated with lymphocyte, macrophage, vascular, and bone metabolism in YLWH compared with uninfected youth is lacking. The goal of the current study was to examine multiple biomarkers associated with distinct inflammatory pathways altered by HIV infection in YLWH using novel biostatistical modeling to identify inflammatory profiles among YLWH. Understanding the relationship between inflammatory pathways and disease progression in YLWH is critical to designing unique treatment interventions for this population.

2 | METHODS

2.1 | Study subjects

YLWH were enrolled in the Adolescent Trials Network (ATN) for HIV/AIDS Interventions protocol 071/101, *Assessment of Inflammatory Markers Associated with Neurocognitive Impairment in HIV-infected Adolescents*, a sub-study of ATN 071, *Neurocognitive Assessment in Youth Initiating ART*, a 3 year longitudinal study, initiated in April 2008 with the last participant completing the study in 2013 (ClinicalTrials.gov Identifier NCT00683579).[16] Enrollment was at 22 sites throughout the United States and Puerto Rico; procedures included routine monitoring of clinical HIV disease, neurocognitive assessments, and self-reported substance use.[16] A single blood sample to assess biomarkers was obtained at week 144. Inclusion criteria included age ≥18 and <25 years at enrollment, HIV-infection acquired via behavioral means, and no previous ART. Exclusion criteria included current pregnancy, substance use that would interfere with the ability to complete the study, and significant non-HIV-related cognitive or motor impairment. Participants were stratified at entry based on CD4 T cell counts into 3 study arms; (1) CD4 T cell counts > 350 cells/μL who at entry initiated ART consisting of a protease inhibitor plus 2 nucleotide reverse transcriptase inhibitors; (2) CD4 T cell counts > 350 cells/μL who remained untreated until CD4 counts declined to < 350 cells/μL; and (3) CD4 T cell counts < 350 cells/μL who initiated ART at entry.[16] Plasma viral levels, CD4 counts, diagnoses, and medications were abstracted from clinic records at study visits. At the end of study, participants were classified based on HIV RNA viral levels as viral load negative (VL^-) (<50 copies/mL) or viral load positive (VL^+) (>50 copies/mL).

Age, gender, and race-balanced self-declared healthy control (HC) participants, with no acute illnesses at the time of enrollment or immunizations during the past 3 months, were enrolled at the University of South Florida. Blood samples were obtained at a single time point. Clinical, demographic, and laboratory data, similar to ATN 071/101 data, were collected.[17] IRB approval was obtained at each study site and from the University of Florida and University of South Florida. Informed consent was obtained from each participant.

2.2 | ELISA and multiplex assays for plasma cytokine/chemokine

Biomarkers included cytokines, chemokines, growth factors, enzymes, proteins, or soluble receptors associated with lymphocyte activation, macrophage activation, or markers of vascular or bone injury impacted by HIV infection in different cohorts/studies.[13–15,18–25] Six biomarkers associated with lymphocyte activation (sCD27, IFNγ, sCD25, IL-10, IL-2, and IL-5),[14,19,24] 12 associated with macrophage activation (sCD163, sCD14, TNFα, TNF-β, GM-CSF, MCP-1, IL-1β, IL-1α, IL-6, IL-8, LPS, and matrix metalloprotease-2 [MMP-2]),[4,14,15,18,20,23] 4 with vascular injury (myeloperoxidase [MPO], sICAM-1, sVCAM-1, and C-reactive protein [CRP]), and 1 with bone metabolism (osteopontin) were selected.[13,21,22,25] ELISA assays were performed per manufacturer's instructions: sCD14, sCD163, MMP-2, osteopontin (R&D Systems, Minneapolis, MN) and sCD27 (eBioscience, San Diego, CA). Three distinct multiplex analyses were performed using the Luminex platform (Millipore, Darmstadt, Germany): a 13-plex assay for IFNγ, IL-2, IL-5, IL-10, sCD25 (sIL-2Rα), CCL2 (MCP-1), TNFα, TNFβ, IL-1α, IL-1β, IL-6, IL-8, and GMCSF; a 3-plex assay for sICAM-1, sVCAM-1, and MPO; and a 1-plex assay for CRP. Plasma was assayed undiluted for the 13-plex panel, and diluted 1:200 or 1:2000 per manufacturer's instructions for 3- or 1-plex panels. To normalize for lower limits of detection, standard curves from each assay were combined to provide one least detectable value. Imputation process and sensitivity analysis were performed to provide a robust data set. Samples below the level of detection were recoded to one significant figure below the lowest detectable value for each biomarker. Plasma LPS was measured after 1:2 dilution in 0.15 M NaCl and heat inactivated at 65°C, using the limulus amebocyte assay Chromogenic Endpoint Assay.[14,18] Because over 40% of HC had levels of IL-2, IL-5, TNF-β, or IL-1α below detection in their respective assays, these biomarkers were excluded from further analysis, leaving 19 biomarkers.

2.3 | Data processing

Raw values for each biomarker were used for correlation analysis. Natural log-transformed values were used for group comparison on

TABLE 1 Demographics of the study population

	Healthy control	HIV+	*P* value	HIV VL$^-$ <50 copies/mL	HIV VL$^+$ >50 copies/mL	*P* value
N	56	129		80	48	
ART[a]	NA	110 (85.3%)		79 (98.75%)	31 (64.58%)	
Age in years[b,d]	22 (18, 25)	24 (21, 28)	<0.001[d*]	24 (21, 28)	24 (21, 28)	0.870[d]
Gender[a,c]						
Male	41 (73.2%)	110 (85.3%)	0.082	68 (86%)	41 (85.4%)	>0.999
Female	15 (26.8%)	19 (14.7%)		11 (14%)	7 (14.6%)	
Race[a,c]						
African American	38 (67.9%)	87 (67.4%)	>0.999	52 (65.8%)	33 (68.75%)	0.884
Other	18 (32.1%)	42 (32.6%)		27 (34.2%)	15 (31.25%)	
CD4%[b,d]	46.1 (27.4, 64.3)	34 (7, 53)	<0.001[e]	35.5 (11, 52)	30.5 (7, 50)	<0.005[e]
CD4 (cells/μL)[b,d]	723 (192, 1416)	651 (80, 1320)	0.149	668 (231, 1280)	564.5 (80, 1320)	0.038
Viral load (copies/mL)[b]	NA	<50 (<50 to 500,000)		<50	7856 (65, 500,000)	

[a]Number of subjects (%).
[b]Median (range).
[c]Chi-square test.
[d]Wilcoxon rank-sum test.
[e]Indicate statistical significance.

single biomarkers and multiple biomarkers as a bioprofile. Natural log-transformed values were standardized to a mean of zero and standard deviation of one to use for principal component analysis (PCA) or partial least squares discriminant analysis (PLS-DA).

2.4 | Statistical analysis

Chi-square test compared gender and race distribution, whereas Wilcoxon rank-sum test compared distribution of age, total CD4 count, and CD4 percent between HC and HIV-infected VL$^+$ and VL$^-$ participants. Linear mixed models, with adjustment for clustering resulting from a shared assay plate, were fit to test the effect of HIV-1 infection, therapy, or virus on each individual biomarker. Multivariate analysis of variance (MANOVA) was conducted to examine the effect of age, gender, ethnicity, HIV infection, ART, and viral levels on bioprofiles consisting of full or refined biomarker panels. Semiparametric Spearman's rank correlation coefficient was used to quantify statistical dependence between pairs of biomarkers in HIV-infected subjects and HC, separately. Heatmaps were produced to visualize the strength of the correlations. PCA biplots were generated to visualize 2-dimensional representation of bioprofiles among groups, whereas the supervised PLS-DA was applied to identify biomarkers that provide the most information in separating of designated groups. Q^2 criterion of leave-one-out cross-validation was used to select the optimal number of PLS-DA components. Identified important factors were ordered using averaged variable importance in projection (VIP) scores for the selected number of components. VIP scores above 1 were considered relevant for group separation. Bonferroni correction was considered to adjust for multiple comparisons based on biomarker categories and control the overall Type I error rate at 0.05. All statistical analysis results were generated using R 3.1.2 and SAS® 9.4 software.

3 | RESULTS

3.1 | Demographics of the study cohort

YLWH were 85% male and 67% African American. At entry, average length of infection was 10.75 months based on medical history (Table 1). At week 144, median age of the 129 YLWH was 24 years median, whereas CD4 T cell count was 651 cells/μL (34%) (Table 1) Most YLWH, 110 /129 (85%), initiated ART while on study. One subject had missing CD4 and viral load data and was not included in the analysis for Tables 1 and 2. Of those receiving ART, 79/110 (72%) with viral suppression were classified as VL$^-$, whereas those with detectable plasma virus (28%) were classified as VL$^+$. Among the HC, median CD4 T cell percentage was 46.1%, significantly higher than YLWH, but absolute CD4 T cell counts were similar (723 cells/μL).

3.2 | HIV infection disrupts normal biomarker levels

There were no significant pairwise differences between HC and YLWH for 10 biomarkers, including osteopontin, lymphocyte markers (sCD25 and IL-10), macrophage (CCL2, IL-1β, IL-6, IL-8, and MMP-2), and cardiovascular (sVCAM-1 and CRP). (Table 2) Plasma levels of 9 biomarkers, including lymphocyte (sCD27 and IFNγ), monocyte/macrophage (sCD163, sCD14, LPS, TNFα, and GM-CSF) and cardiovascular markers (MPO and sICAM-1), were significantly different between HC and YLWH.

To identify the impact of persistent viral replication, biomarkers were evaluated between HC and VL$^+$ YLWH (Table 2). Five monocyte/macrophage biomarkers, as well as sCD27 (lymphocyte) and MPO (cardiovascular), differed between HC and VL+, similar to the comparison between HC and all YLWH. In addition, VL$^+$ youth differed from HC in levels of sCD25 (lymphocyte biomarker)

TABLE 2 Comparison of biomarker levels across outcome groups

Group comparison	HC	HIV	VL$^+$	VL$^-$
N	56	129	48	80
Lymphocyte biomarkers				
sCD27 (U/mL)	23.18 (11.54)[a]	57.10 (36.90)[b]	75.69 (46.63)[b]	45.45 (23.33)[b]
IFN-γ (pg/mL)	23.27 (33.50)	12.43 (36.05)[b]	20.67 (49.20)	7.62 (24.47)
sCD25 (pg/mL)	50.06 (34.30)	67.40 (80.26)	106.72 (116.17)[b]	43.90 (30.15)
IL-10 (pg/mL)	12.61 (18.65)	5.99 (13.08)	9.59 (20.03)	3.73 (4.98)
Monocyte/macrophage biomarkers				
sCD163 (ng/mL)	351.14 (168.21)	546.48 (304.55)[b]	711.58 (401.55)[b]	446.99 (166.58)[b]
sCD14 (ng/mL)	991.05 (501.97)	1463.09 (422.77)[b]	1428.84 (450.53)[b]	1482.36 (409.40)v
LPS (EU/mL)	0.16 (0.07)	0.21 (0.08)[b]	0.23 (0.06)[b]	0.20 (0.09)
TNFα (pg/mL)	4.68 (3.26)	11.31 (25.62)[b]	13.71 (24.80)[b]	9.95 (26.29)
GM-CSF (pg/mL)	2.60 (4.60)	0.90 (1.99)[b]	0.62 (1.33)[b]	1.08 (2.30)
CCL2 (pg/mL)	142.88 (98.34)	208.88 (303.25)	273.27 (468.82)	167.80 (116.30)
IL-1β (pg/mL)	3.82 (7.58)	28.10 (101.36)	57.17 (151.15)	10.99 (47.26)
IL-6 (pg/mL)	6.68 (10.83)	13.80 (66.69)	30.44 (106.95)	3.98 (11.56)
IL-8 (pg/mL)	7.93 (7.92)	64.22 (290.41)	128.28 (464.51)	21.61 (50.32)
MMP-2 (ng/mL)	206.03 (35.14)	195.61 (39.13)	196.38 (39.28)	194.29 (38.75)
Cardiovascular biomarkers				
MPO (ng/mL)	181.13 (211.48)	280.54 (269.37)[b]	314.82 (256.49)[b]	260.98 (277.92)
sICAM-1 (ng/mL)	1780.94 (9454.89)	214.33 (302.62)[b]	201.15 (243.60)	219.32 (335.03)[b]
sVCAM-1 (ng/mL)	633.28 (202.84)	597.08 (238.86)	718.78 (286.44)	522.98 (170.22)[b]
CRP (ng/mL)	1987.63 (6700.38)	1417.70 (5496.65)	1016.86 (3651.08)	1675.71 (6390.02)
Bone related				
Osteopontin (ng/mL)	60.50 (34.58)	51.53 (21.21)	48.88 (20.93)[b]	53.68 (20.85)

Bonferroni-corrected significance cut-off to adjust for multiple comparisons: $P < 0.002$ for lymphocyte and cadiovascular biomarkers, $P < 0.00083$ for monocyte/macrophage biomarkers, $P < 0.0083$ for bone-related biomarker.
[a]All values shown are mean (standard deviation).
[b]Indicate statistical significance compared with HC. *P* values are calculated using mixed model after log transformation.

and osteopontin (bone). When the VL$^-$ group was compared with HC, 5 biomarkers (sCD27, sCD163, sCD14, sVCAM, and sICAM) remained different, while sCD25, LPS, TNFα, GM-CSF, and MPO were no longer significantly different (Table 2). The potential effect of ART, independent of viral replication, was assessed, but no significant difference for any biomarkers between the groups was detected (Supplemental Table S1). No clear confounding variables such as gender, age, ethnicity, or substance use fully provided an explanation for pairwise differences between groups and biomarkers (data not shown).

3.3 | Impact of HIV infection on biomarker networks

To evaluate the relationships among biomarkers within either the HC or YLWH groups, pairwise Spearman correlations between all 19 biomarkers were performed (Fig. 1 and Supplemental Table S2). Among HC, significant positive correlations with *rho* values ranging from 0.485 to 0.822 ($P < 0.0002$) were identified for 8 lymphocyte and monocyte/macrophage biomarkers (Fig. 1A). Biomarkers of lymphocyte and monocyte/macrophage activation markers TNFα, IL-6, and IL-8 were positively correlated with IFNγ, IL-8 positively correlated with TNFα and IL-6, IL-10 with IL-1β, and CD25 with CCL2. With the exception of TNFα with IFNγ and sCD25 with CCL2 all of these biomarkers also positively correlated with YLWH (Figs. 1B and 1C).

Among the 129 YLWH 16 significant relationships with *rho* values ranging from 0.334 to 0.570 were observed (Fig. 1B and Supplemental Table S2). Most correlations were distinct to YLWH, including activation biomarkers for lymphocytes (TNFα and IL-10 with sCD25), macrophages (IL-1β with IL-6 or IL-8 and TNFα), and vascular inflammation biomarkers (CRP significantly correlated with sVCAM-1 and sICAM-1). Macrophage biomarkers sCD163 correlated with sCD25, while sCD14 correlated with IL-10. Macrophage and vascular inflammation biomarkers also significantly correlated within YLWH as shown by correlations between IL-1β with MPO, sVCAM-1 with TNFα, sCD163, sCD25, IL-10, and CRP. A summary of the biomarker correlation networks across lymphocyte, macrophage, and cardiovascular markers combined data from both HC and YLWH (Fig. 1C). An unexpected finding was lack of significant correlations between many of the lymphocyte and macrophage activation markers,

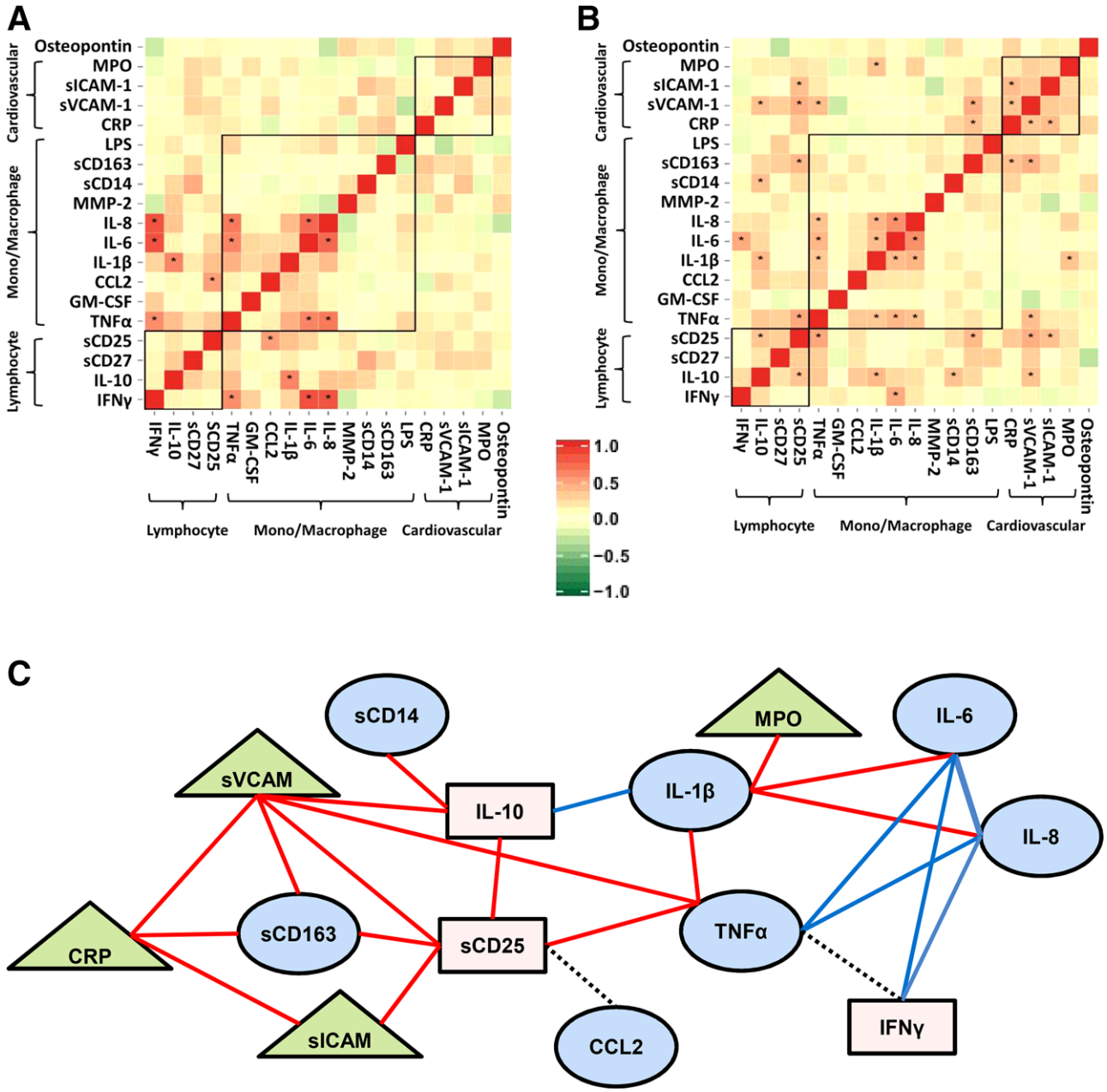

FIGURE 1 **Biomarker correlation networks.** Heatmap of correlation matrix for HC (A) and YLWH (B). Biomarkers by classification are clustered top to bottom and right to left. Asterisks within black box indicate significant correlations ($P < 0.0002$ based on Bonferroni correction). Heat map colors and respective *P* are shown in the legend; positive red, negative green. C shows significant pairwise correlations indicated by lines connecting biomarkers. Red lines indicate correlations found only in YLWH, blue lines indicate correlations found only in both YLWH and HC, and black dashed lines indicate correlations found in HC only. Monocyte/macrophage related biomarkers are depicted in blue ovals, lymphocyte biomarkers are pink rectangles, and cardiovascular biomarkers are green triangles.

including sCD163 with sCD14 or IL-6; LPS with sCD14; or IFNγ with sCD27.

3.4 | Macrophage and lymphocyte related cytokines contribute to bioprofiles unique to YLWH

PCA was performed to reduce 19 biomarkers into a smaller set of components while retaining most of the variation. Biomarkers were evaluated within 4 outcome groups; HC, HIV$^+$ ART$^+$ VL$^-$, HIV$^+$ ART$^+$ VL$^+$, and HIV$^+$ ART$^-$ VL$^+$. Principal component (PC)1 vs. PC2 for all 19 biomarkers explained over 30% of the total variance (Fig. 2A). Although the HC group was generally distinct from YLWH, considerable overlap between HC and virally suppressed (ART$^+$ VL$^-$) groups was apparent, while VL$^+$ groups clustered together independent of treatment. Similar results occurred when the analysis was limited to the subset of biomarkers of lymphocyte activation (Fig. 2B). In contrast, the PCA biplot of monocyte/macrophage activation markers showed overlap among all YLWH, independent of treatment or viral load, all distinct from HC. (Fig. 2C) Based on cardiovascular biomarker-specific PCA biplot, all outcome groups clustered and were indistinguishable

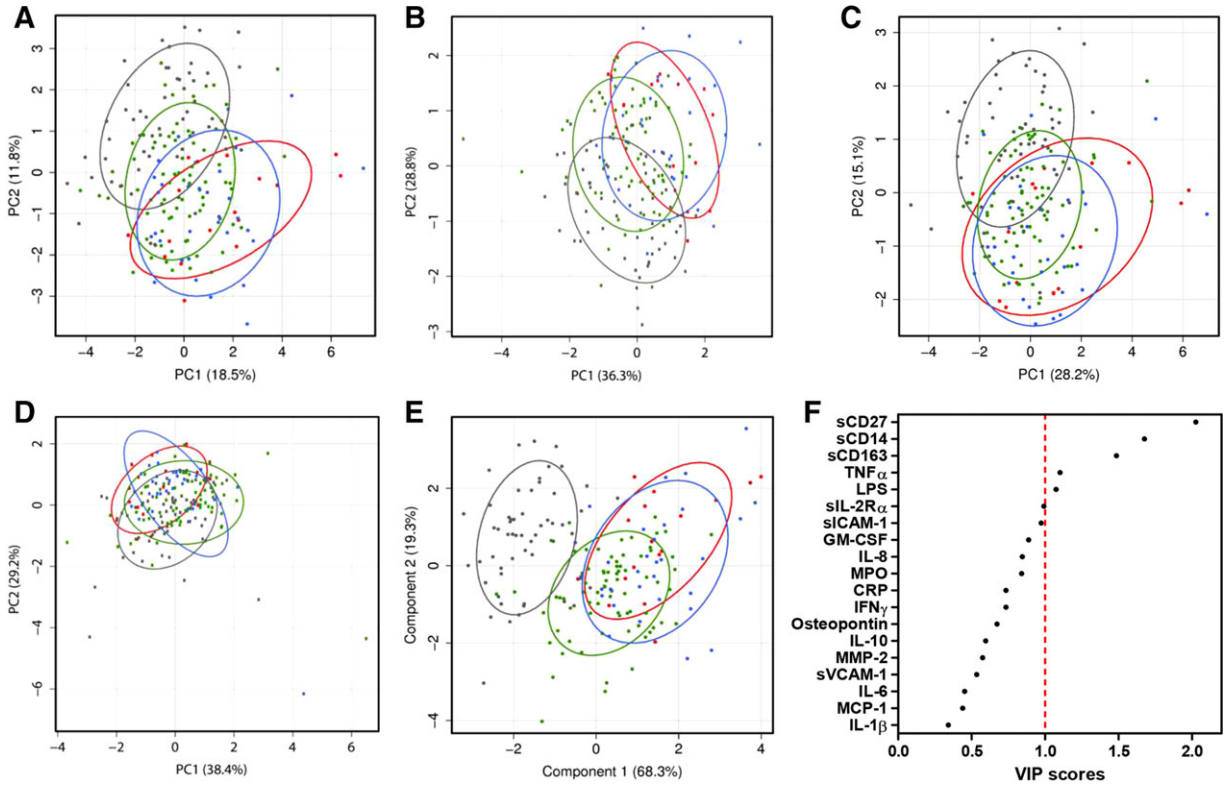

FIGURE 2 **Effect of HIV on bioprofile visualization**. PCA biplots from first and second principal components utilizing on all 19 biomarkers (A), lymphocyte biomarkers (B), monocyte/macrophage biomarkers (C), and cardiovascular biomarkers (D) are shown. Percentage shown on the *X* and *Y*-axes indicates percentage of total variance explained by the components. In E, all 19 biomarkers were utilized to create PLS-DA plot maximizing separation among 4 groups using first and second components. Percentage shown on the *X* and *Y*-axes indicates percentage of intergroup variance explained by the components. F shows a VIP plot depicting relative contribution of all 19 soluble factors to 4 group classification. Healthy controls are represented by gray dots and ellipses, ART^+VL^- are green dots and ellipses, ART^+VL^+ are blue dots and ellipses, and ART^-VL^+ are red dots and ellipses. Ellipses indicate normal contour line within one standard deviation away from mean.

from each other (Fig. 2D). PCA analysis of the 19 biomarkers based on CD4% did not distinguish among groups of YLWH (data not shown). Overall, clustering the biomarkers into specific inflammatory pathways revealed trends toward differences among the outcome groups.

3.5 | sCD27, sCD14, and sCD163 are important contributors to separating biomarker bioprofiles in YLWH

To identify biomarkers unique to YLWH a PLS-DA was applied. Components in PLS-DA, designed to separate groups of interest, were applied to visualize the bioprofile based on 19 biomarkers with maximum segregation between HC and the 3 HIV-infected groups. Cross-validation results suggested 2 components were optimal for classification; PLS scores for the first 2 components of the group separation explained 88% of the intergroup variance (Fig. 2E).

Averaged VIP scores for the first 2 components identified the single biomarkers sCD27, sCD14 and sCD163 as most critical for the discrimination between the outcome group classification, while contributions to the separation by TNFα and LPS (Fig. 2F). Taken together, these results indicate that a subset of markers from lymphocyte and macrophage activation domains contributed to discrimination between HIV^+ (with or without viral suppression) and HC bioprofiles.

3.6 | Key biomarker bioprofiles distinguishing YLWH

To determine if bioprofiles containing all 19 biomarkers were significantly altered by HIV infection, therapy, or viral load a MANOVA was conducted using all participants (Table 3). Information deduced from 19 biomarkers generated unique bioprofiles distinguishing HC from YLWH regardless of ART or VL outcome ($P < 0.0001$). This bioprofile also identified significant differences within HIV-infected outcome groups based on whether virus was fully suppressed or detectable. However, the 19-factor bioprofile was unable to distinguish a therapy effect alone, as untreated YLWH were not significantly different from those on ART. Similar results were obtained when the bioprofile was limited to only sCD27, sCD14, and sCD163, the biomarkers identified as top contributors in the PLS-DA. In this case the 3 biomarkers distinguished HC from all HIV^+, VL^+ or ART^+ VL^-. In addition, the 3-biomarker bioprofile was significantly different when VL^- and VL^+ YLWH with or without consideration of ART were compared. No effect of age, gender, or ethnicity was observed for any of the biomarkers in YLWH or HC. Overall, the application of sCD27, sCD14, and sCD163 provided the most discrimination between YLWH from HC.

TABLE 3 Group comparison of bioprofiles utilizing all 19 biomarkers or 3 biomarkers with highest VIP scores

Bioprofile comparison	*N*	19 Biomarker	3 Biomarker
HC vs. HIV	56 vs. 129	<0.0001[a]	<0.0001[a]
HC vs. VL^+	56 vs. 48	<0.0001[a]	<0.0001[a]
HC vs. ART VL^-	56 vs. 79	<0.0001[a]	<0.0001[a]
VL^- vs. VL^+	80 vs. 48	<0.0001[a]	<0.0001[a]
ART VL^- vs. ART VL^+	79 vs. 31	0.0026[a]	0.0002[a]
ART^- vs. ART^+	18 vs. 111	0.0533	0.0055[a]
ART^-VL^+ vs. ART^+VL^+	17 vs. 31	0.5314	0.5039

Bonferroni-corrected significance cutoff: $P < 0.007$.
[a]Indicate statistical significance.

4 | DISCUSSION

HIV perturbs multiple inflammatory pathways through effects on both macrophages and lymphocyte function. As a result biomarkers associated with HIV pathogenesis and complications vary greatly based on extent of CD4 T cell decline, viral load, age, length of infection, and infectious comorbidities.[1] Biomarkers specific for YLWH are needed for the early identification HIV-associated comorbidities when initiating ART before CD4 T cell decline. These biomarkers provide biologic distinctions among individuals with normal CD4 T cells with, or without, suppressed virus. Interpretation of the relevance of inflammatory biomarkers in HIV-infected individuals is often hampered by the lack of appropriate HIV-uninfected control groups balanced for age, gender, ethnicity and other confounding variables. A unique aspect of the current study is inclusion of a balanced population of HIV-uninfected predominantly African American young men allowing comparisons between HC and YLWH with optimally suppressed or ongoing viral replication.[26] Comparisons among YLWH with and without viral suppression were enhanced because both groups were similar with respect to their CD4 T cells counts, demographics, and disease stage. While the impact of gender, ethnicity, and race could not be thoroughly evaluated due to the relatively homogenous make up the study population, nonetheless biomarker profiles emerged specific to YLWH with normal CD4 T cells many of whom initiated ART prior to CD4 decline. The findings of this study contrast to biomarker assessments within populations of chronically HIV-infected adults who have advanced disease where biomarkers such as IL-6 are associated with disease progression and morbidity.[3,23,27,28] This is one of few studies of HIV-associated biomarkers among recently infected youth and are highly relevant because they identify the earliest inflammatory pathways perturbed by HIV. The distinct bioprofiles among YLWH are likely to enhance longitudinal assessments of interventions designed to attenuate long-term metabolic, inflammatory, cardiovascular, and neurologic complications of HIV.

Assessment of multiple biomarkers associated with different inflammatory pathways identified factors that normalized with ART when compared with HC, improved with ART but remain elevated relative to HC, or remained abnormal in spite of optimal ART. Differences among outcome groups identified inflammatory mechanisms independent or dependent on viral replication. For example, it was expected that effective ART would result in improved sCD27, sCD163, and TNFα levels,[14,15,24] but unexpected that even with prolonged viral suppression these biomarkers would remain significantly higher in YLWH compared with HC. In contrast, effective viral suppression by ART normalized sCD25, TNFα, GM-CSF, and MPO. The results indicate that early use of ART and prolonged viral suppression can reverse many but not all of the inflammatory pathways impacted by HIV in other cohorts. Previous studies show that control of viral replication by ART has no impact on sCD14 levels, a biomarker of macrophage activation primarily mediated by microbial translocation.[11,14] This inflammatory pathway remained unchanged even after years of viral suppression and confirms the persistence of macrophage activation due to gastrointestinal microbial translocation and LPS binding to TLR4/CD14.[18]

Biomarker measurements have a high degree of variability due to quantitative differences in assay methods, variation in plasma half-life, and overall production levels.[29] Several plasma biomarkers related to key immune pathways were below detectable levels of the assay in a significant proportion of the participants preventing statistical comparisons and therefore not included in the study. However, correlations between inflammatory biomarkers were identified. Associations between biomarkers of lymphocyte and macrophage activation revealed similarities and sharp contrasts between YLWH and HC. Highly associated biomarkers in HC generally involved factors associated with acute immune activation such as IFNγ, TNFα, IL-8, IL-6, IL-1β, and IL-10. These biomarkers are driven in general by viral and bacterial infections and inflammation and are not unique to HIV.[30–36] In contrast, associations among biomarker networks involved in chronic macrophage activation and vascular injury were seen among YLWH. sCD163 was associated with higher levels of sCD25, a known biomarker of macrophage, activation syndrome, and was also associated with sVCAM and CRP in conditions associated with vascular injury in both HIV and non-HIV associated conditions such as autoimmune disease.[13,23,26,37–40] While both sCD14 and sCD163 are associated with macrophage activation, the markers represent distinct pathways impacted by HIV; therefore, the lack of associations between these 2 biomarkers is not surprising but indicate complex effects by HIV infection on multiple innate immunity pathways.[41,42]

Complex relationships between HIV-related inflammatory pathways, some related to direct viral replication and others indirect, complicates the assessment of biomarkers distinct to YLWH. PCA enabled better identification of the biomarkers unique to YLWH compared with

HC. When aggregated, all biomarkers discriminate HC from YLWH. Comparisons of biomarkers associated with lymphocyte activation identified clear differences based on viral outcomes. HC and YLWH who suppressed viral replication clustered together and were distinct from YLWH who were VL^+ whether receiving ART or not. In contrast, PCA of biomarkers of macrophage activation showed all YLWH clustering together regardless of viral outcome and distinct from HC. There were no distinguishing factors that differentiated HIV outcome groups and HC in the assessment of vascular markers. Simultaneous assessments of multiple biomarkers identify individual bioprofiles that normalize relative to HC or remain perturbed independent of viral replication. Although PCA provides an aggregate view of differences among outcome groups, the analysis does not reveal the extent of the contribution of specific biomarkers in defining individual groups.

Supervised PLS-DA provided distinct separation between HC and YLWH and revealed 3 biomarkers; sCD27, sCD14, and sCD163 showed the greatest separation between infected and uninfected youth. Refined bioprofile analysis provides sufficient information to recognize immune normalization and will be helpful in the identification of YLWH whose biomarker profiles show optimal responses to interventions designed to decrease viral replication and HIV-associated inflammation. Conversely, biomarker profiles showing persistent immune activation in spite of optimal viral suppression may become apparent. However, they do provide accurate appraisals of the pathways most perturbed by HIV infection. This is the first study to simultaneously evaluate perturbations in inflammation and lymphocyte activation among YLWH who are in the early stages of infection. The pathways identified in YLWH can be compared with the case-controlled studies of HIV-infected adults who have developed HIV-associated comorbidities such as myocardial infarction and stoke.[27] Plasma biomarkers similar to both groups include TNF and sCD14, whereas IL-6 is more associated as biomarkers of HIV comorbidities in older adults.[4,27,39]

The current study identifies similarities and differences among viral outcome groups and is unique in its focus on YLWH who are still healthy and early in disease. YLWH ages 18–24 years are the predominant populations for newly infected youth.[43] The exclusion of younger adolescents under 18 years limits the applicability of the findings to all YLWH. However, examining changes in inflammatory biomarkers over time provides an opportunity to create novel models to assess the development of HIV-associated comorbidities across the life span. These biomarkers can serve as intermediate outcomes for interventions to attenuate HIV complications. Now that initiation of ART is recommended for all infected individuals most YLWH should start therapy prior to CD4 T cell decline.[44] This study reveals interventions targeting inflammation mediated by macrophages are needed for YLWH to prevent long-term complications of HIV.[45]

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DISCLOSURES

The authors declare no conflicts of interest.

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