

Novel relationships of markers of monocyte activation and endothelial dysfunction with pulmonary dysfunction in HIV-infected persons

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Objective: Chronic obstructive pulmonary disease is a common comorbidity in HIV, with prevalence and severity of disease incompletely explained by risk factors such as smoking and age. Unique HIV-associated factors, including microbial translocation, monocyte activation, and endothelial dysfunction, have been described in other comorbidities, but have not been investigated in relation to pulmonary abnormalities in HIV. This study assessed the relationship of these pathologic processes to pulmonary function in HIV-infected and uninfected individuals and determined if relationships were unique to HIV.

Design: Longitudinal observational study.

Methods: Total 274 participants completed pulmonary function testing. Markers of inflammation (IL-6, IL-8, and TNF α), microbial translocation (lipopolysaccharide, sCD14), monocyte activation (sCD163, sCD14, and IL-2 receptor), and endothelial dysfunction (endothelin-1) were measured at baseline. Cross-sectional and longitudinal analyses were performed, adjusting for pertinent covariates.

Results: In HIV-infected individuals, higher IL-6 and endothelin-1 associated with worse forced expiratory volume in one second (FEV₁) percentage-predicted, and higher sCD163 associated with worse FEV₁/forced vital capacity. IL-6, TNF α , lipopolysaccharide, sCD163, IL-2 receptor, and endothelin-1 associated with diffusing impairment. sCD163 and endothelin-1 interacted with HIV status in relationship to pulmonary function. In HIV-infected individuals only, baseline endothelin-1 was associated with lower FEV₁, and sCD163 and endothelin-1 were associated with lower diffusing capacity during follow-up.

Conclusion: Circulating markers of HIV-associated humoral abnormalities are associated with airflow obstruction and diffusing impairment and baseline measures of monocyte activation and endothelial dysfunction associate with lower pulmonary function over time in HIV-infected persons. These findings suggest mechanisms of the disproportionate burden of chronic obstructive pulmonary disease in HIV-infected persons.

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Introduction

Abnormal pulmonary function is well documented in persons with chronic HIV, and specific pulmonary diseases such as chronic obstructive pulmonary disease (COPD), asthma, and pulmonary vascular disease are also disproportionately frequent [1–15]. HIV appears to be an independent risk factor for these diseases, as these associations have been demonstrated despite consideration of known risk factors for chronic pulmonary disease such as smoking, prior pneumonia history, or injection drug use [2–4,7,14]. The biologic mechanisms that underlie pulmonary function abnormalities in this vulnerable population remain incompletely described.

Features unique to chronic HIV infection are likely to contribute to the unexplained differences in pulmonary disease incidence, prevalence, and progression between HIV-infected persons and the general population. One potential contributor is enhanced inflammation, generally in the context of persistent immune activation [16–18]. We have recently demonstrated cross-sectional relationships between peripheral T-lymphocyte activation and senescence and classic inflammatory markers IL-6 and C-reactive protein with pulmonary function testing (PFT) abnormalities in HIV [19], but longitudinal analyses are lacking. Additionally, these markers explain only a small percentage of the variance in pulmonary function among HIV-infected persons. Related and alternative processes characterizing chronic HIV that would plausibly associate with COPD include monocyte activation, microbial translocation, and endothelial dysfunction. These conditions have been related to other HIV-associated non-AIDS comorbidities, but have not been explored in HIV-associated pulmonary disease.

Abnormally elevated measures of monocyte activation, which persist despite antiretroviral therapy (ART), have been linked to HIV progression, mortality, and HIV-associated cardiovascular and neurocognitive conditions [20–29]. Given the centrality of the alveolar macrophage to pulmonary immunity, monocyte activation may be expected to relate to HIV-associated pulmonary disease. The potential causes of immune activation (T-lymphocyte or monocyte) in HIV are myriad, but one potential-specific driver is microbial translocation of bacteria across the gut epithelial barrier, with bacterial cell wall elements serving as immunogenic triggers [30–33]. Finally, endothelial damage and dysfunction are consequences of HIV infection that have been associated with cardiovascular and other HIV-associated comorbidities [34–40]. As translational and epidemiologic data suggest a link between endothelial damage and apoptosis with COPD in the general population [41–46], endothelial factors may be expected to play a role in PFT abnormalities in persons with HIV.

We investigated these three constructs (microbial translocation, macrophage activation, and endothelial dysfunction) in conjunction with classic inflammatory markers to describe their relationships to one another and to determine their potential role in HIV-related pulmonary disease. We measured markers of Th1 inflammation (IL-6, IL-8, and TNF α), microbial translocation [sCD14, lipopolysaccharide (LPS)], monocyte activation [sCD163, sCD14, and IL-2 soluble receptor α (IL-2sR α)], and endothelial dysfunction [endothelin-1] in a prospective cohort of HIV-infected and HIV-uninfected men. Using cross-sectional and longitudinal analyses, we determined which biomarkers independently associated with PFTs in HIV-infected participants, to what degree biologic markers from related pathways correlate in this population, and whether specific associations were unique to HIV-infected versus HIV-uninfected persons.

Methods

Participants

Participants included 293 men in the Lung HIV study enrolled from 2009–2011 at two participating sites [47]. Recruitment of Lung HIV study participants occurred from the Pittsburgh and Los Angeles sites of the Multicenter AIDS Cohort study [48], which enrolls and prospectively follows men with or at risk for HIV. Results of baseline PFTs from a subset of these men have previously been reported [4]. All participants provided informed consent, and study protocols were approved by the Institutional Review Boards of participating sites (University of Pittsburgh; University of California, Los Angeles).

Participants were seen at a baseline visit and at 18 and 36 months. Clinical characteristics including age, HIV status, race, BMI, cigarette smoking status, cumulative pack-years of cigarettes smoked (pack-years), history of injection drug use, use of ART, and history of *Pneumocystis* or bacterial pneumonia were recorded at each study visit. The most proximal CD4⁺ T-cell counts and HIV viral loads were included from the 12 months preceding PFTs.

Pulmonary function testing

Spirometry was performed before and after bronchodilator administration, and diffusing capacity for carbon monoxide (DL_{CO}) was measured following American Thoracic Society (ATS)/European Respiratory Society standards [49,50]. Spirometry reference values were determined from the third National Health and Nutrition Examination Survey equations [51], and reference values for DL_{CO} used Neas *et al.* [52] equations that were adjusted for hemoglobin and carboxyhemoglobin [50]. Bronchodilator response was defined as improvement in

either forced expiratory volume in one second (FEV₁) or forced vital capacity (FVC) of at least 200 ml and 12% between administration of bronchodilator [53]. DL_{CO} was not adjusted for alveolar volume. Trained research technicians performed PFTs, and site pulmonologists confirmed quality compatible with ATS guidelines. If PFTs did not meet ATS guidelines, they were reviewed by site pulmonologists who determined if they were acceptable for inclusion in the study.

Biomarker measurements

Protein levels of biomarkers of IL-8, sCD14, sCD163, IL-2sR α , and endothelin-1 were measured in serum via ELISA (R&D, Minneapolis, Minnesota, USA), and IL-6 and TNF α were measured via Luminex (Luminex corporation, Austin, Texas, USA). Serum aliquots obtained at baseline visits were stored at -80°C and were assayed after a single thaw. Technical replicates were performed in triplicate, and replicates with coefficients of variation greater than 20% were rejected and the assay repeated. LPS was assayed via *Limulus* Amebocyte Lysate assay (Lonza; Walkersville, Maryland, USA).

Statistical analysis

Variables were transformed as necessary using natural logarithm or square root to approximate normality. Baseline clinical, demographic, and laboratory markers as well as PFT measures were compared between HIV-infected and HIV-uninfected groups using Student's *t* test or χ^2 test. Pearson correlation coefficients between different biomarkers were calculated in all participants and separately by HIV status. Associations between serum biomarkers and PFT measures were calculated in HIV-infected and HIV-uninfected subgroups using standardized β in linear regression analysis (for continuous measures) or odds ratio in logistic regression (for binomial variables). We then introduced an interaction term between each serum marker and HIV status in the model to test the difference of these associations between HIV-infected and HIV-uninfected individuals.

In each model, clinical covariates entered the model if bivariate analysis demonstrated *P* value of less than 0.2, and were maintained in the model if *P* values remained at less than 0.05 after adjustment. Given the known strong association between smoking exposure and pulmonary function, pack-years smoked were forced into the models regardless of significance of association.

Longitudinal analyses assessing the association of baseline biomarkers with pulmonary function decline over time (18–36 months of follow-up) were performed for the HIV-infected subgroup using mixed-effect models (with random intercept for each participant). Participants were included in the analysis if they returned for one or both longitudinal visits. Time–biomarker interaction terms were tested in each mixed-effects model to measure the effect of biomarker on change of PFT during the time of

follow-up. Statistical analyses were performed using Stata version 14.0 (StataCorp; College Station, Texas, USA).

Results

Characteristics of the cohort

Overall, 274 of 293 men performed acceptable PFTs; 124 (45.3%) were HIV infected, and the majority of those infected were prescribed ART (87%) with clinically undetectable HIV viral load (81%) (Table 1). The median age of the cohort was 54 and was similar between HIV-infected and HIV-uninfected individuals. More than half of the cohort had history of smoking, with higher cumulative pack-years smoked among those with HIV infection. HIV-infected participants also had lower BMI, greater frequency of ever using injection drugs, and greater frequency of *Pneumocystis* pneumonia history.

Pulmonary function testing

Cross-sectional PFT analyses, including men from this cohort, have previously been reported [4], and data are summarized here. In general, spirometry was normal, with median FEV₁ percentage-predicted values of 98 and 103% before and after the administration of bronchodilator. FEV₁/FVC ratios were similarly normal, on average, but 15% of the cohort demonstrated an abnormal postbronchodilator FEV₁/FVC ratio (<0.7), consistent with COPD. A substantially higher number of participants [123 (45%)] had diffusing impairment (DL_{CO} $<80\%$ -predicted), and HIV-infected persons had higher odds of diffusing impairment and significantly lower diffusing capacity on average (Table 1).

Biomarker data

Data were available for the majority of participants, with some data incomplete because of absence of available serum or plasma samples (Supplemental Table 1, <http://links.lww.com/QAD/A902>). In unadjusted comparisons, biomarkers sCD163, sCD14, and IL-2sR α were significantly higher among participants with HIV (Supplemental Table 1a, <http://links.lww.com/QAD/A902>). One outlying influential point for IL-6 (>500 pg/ml, in an HIV-uninfected participant) was excluded from the PFT analyses, with the rationale that it was highly irregular in the cohort. Comparisons of biomarkers based on ART status in HIV-infected persons found that endothelin-1 was significantly lower among persons not receiving ART, but otherwise there were no differences in biomarkers (Supplemental Table 1b, <http://links.lww.com/QAD/A902>). The sample size (16 persons not receiving ART) was not adequate to draw conclusions from these data.

Correlations between biomarkers

In the overall cohort, moderate associations were found between IL-6 and TNF α ($r=0.67$, $P<0.0001$) and

Table 1. Baseline characteristics of study participants.

	Overall cohort (n = 274)	HIV-infected (n = 124)	HIV-uninfected (n = 150)	P value ^a
Age (years), median (IQR)	54 (48–61)	53 (48–59)	55 (49–62)	0.1
Race/ethnicity				0.7
White, n (%)	213 (78%)	98 (79%)	115 (77%)	
Black, n (%)	47 (17%)	22 (18%)	25 (17%)	
Hispanic, n (%)	8 (3%)	2 (2%)	6 (4%)	
Other, n (%)	6 (2%)	2 (2%)	4 (3%)	
BMI, median (IQR)	26.1 (23.7–29.7)	25.0 (23.1–27.9)	26.7 (24.1–30.6)	0.001
Smoking history (pack years), median (IQR)	1.9 (0–22.1)	5.7 (0–30.8)	0 (0–16.5)	0.01
Smoking history, categorized				
Ever smoker, n (%)	148 (54%)	74 (57%)	74 (49%)	0.1
Injection drug use				
Last 6 months, n (%)	1 (3%)	1 (6%)	0	0.9
Ever, n (%)	28 (10%)	18 (15%)	10 (7%)	0.03
Prior pneumonia history				
Bacterial, n (%)	22 (8%)	14 (11%)	8 (5%)	0.1
Pneumocystis, n (%)	4 (1%)	4 (3%)	0	0.04
Using ART, n (%)	n/a	108 (87%)	n/a	n/a
HIV viral load, median (IQR)	na	49 (49–49)	n/a	n/a
Viral load undetectable, n (%)	n/a	97 (81%)	n/a	n/a
CD4 ⁺ cell count, median (IQR)	n/a	560 (431–804)	n/a	n/a
CD4 ⁺ cell count <200 cells/ μ l, n (%)	n/a	2 (2%)	n/a	n/a
FEV ₁ %predicted, pre-BD, median (IQR)	98 (87–110)	98 (86–109)	99 (89–110)	0.3
FEV ₁ %predicted, post-BD, median (IQR)	103 (91–112)	101 (90–111)	104 (92–113)	0.3
FEV ₁ /FVC ratio, pre-BD, median (IQR)	77 (71–80)	77 (70–80)	77 (72–80)	0.9
FEV ₁ /FVC ratio, post-BD, median (IQR)	79 (74–84)	80 (74–84)	79 (74–83)	0.4
FEV ₁ /FVC ratio <70%				
Pre-BD, n (%)	62 (23%)	33 (27%)	29 (19%)	0.1
Post-BD, n (%)	41 (15%)	19 (15%)	22 (15%)	0.9
Positive bronchodilator response ^b , n (%)	22 (8%)	13 (11%)	9 (6%)	0.2
DL _{CO} %predicted, median (IQR)	82 (71–92)	77 (68–92)	84 (73–92)	0.03
DL _{CO} <80 %predicted, n (%)	123 (45%)	69 (56%)	54 (36%)	0.001

ART, antiretroviral therapy; BD, bronchodilator; DL_{CO}, diffusing capacity for carbon monoxide; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; IQR, interquartile range.

^aFrom comparison of HIV– and HIV+ group.

^b'Bronchodilator response' defined as change in FEV₁ or FVC by 12% and 200 ml followed administration of 400 μ g albuterol, as per American Thoracic Society/European Respiratory Society criteria.

between sCD163 and IL-2sR α ($r=0.39$, $P<0.0001$); significant but weaker associations between other markers are shown in Supplemental Table 2a, <http://links.lww.com/QAD/A902>. Among HIV-infected individuals, associations were more robust, with a strong association between IL-6 and TNF α ($r=0.87$, $P<0.0001$), moderate associations between IL-8 and sCD14 ($r=0.32$, $P<0.001$), IL-8 and sCD163 ($r=0.30$, $P<0.001$), TNF α and LPS ($r=0.31$, $P<0.001$), and sCD163 and IL-2sR α ($r=0.38$, $P<0.0001$). Weaker associations reaching significance are shown in Supplemental Table 2b, <http://links.lww.com/QAD/A902>. LPS and sCD14, both included as potential markers of microbial translocation, did not correlate, nor were there strong correlations among all of the Th1 biomarkers or between the monocyte activation markers sCD163 and sCD14.

Biomarker associations with pulmonary function Spirometry, cross-sectional analyses

Results of analyses for prebronchodilator and post-bronchodilator spirometry were not meaningfully different; therefore, we report only postbronchodilator analyses. Pack-years smoked retained significance in nearly all multivariable models. In only one instance did an additional variable enter a model: age retained

significance in the model examining sCD163 and FEV₁/FVC ratio in HIV-infected persons, but did not significantly change the association.

In the HIV-infected group, several significant associations were found. Higher IL-6 was associated with lower postbronchodilator FEV₁ percentage-predicted ($\beta=-0.24$, $P=0.01$). Higher sCD163 was associated with lower postbronchodilator FEV₁/FVC ratios ($\beta=-0.23$, $P=0.01$) and greater odds of bronchodilator response (OR 4.9, $P=0.04$). Higher endothelin-1 was also associated with lower postbronchodilator FEV₁ percentage-predicted values ($\beta=-0.36$, $P<0.001$), greater odds of postbronchodilator airflow obstruction (FEV₁/FVC < 0.7; OR 4.8, $P=0.04$), and greater odds of bronchodilator response (OR 7.6, $P=0.03$). Among HIV-uninfected participants, higher IL-6 ($\beta=-0.20$, $P=0.01$) and IL-2sR α ($\beta=-0.20$, $P=0.02$) were associated with lower postbronchodilator FEV₁ percentage-predicted (Table 2).

Diffusing capacity, cross-sectional analyses

Among HIV-infected participants, several variables were found to associate independently with lower DL_{CO} percentage-predicted values, including IL-6 ($\beta=-0.35$,

$P < 0.001$), TNF α ($\beta = -0.38$, $P < 0.001$), LPS ($\beta = -0.19$, $P = 0.03$), sCD163 ($\beta = -0.18$, $P = 0.04$), IL-2sR α ($\beta = -0.25$, $P = 0.005$), and endothelin-1 ($\beta = -0.32$, $P < 0.001$). In the HIV-uninfected group, IL-6, TNF α , LPS, and IL-2sR α demonstrated similar associations with DL_{CO} percentage-predicted (Table 2).

Interaction with HIV infection

Given the observed differences in relationships of several biomarker levels and PFTs between HIV-infected and HIV-uninfected participants, interaction testing was performed. Endothelin-1 demonstrated interaction with HIV in relationship to FEV₁ percentage-predicted and DL_{CO} percentage-predicted values, and sCD163 demonstrated interaction with HIV related to bronchodilator response (Table 2, Fig. 1).

Longitudinal analyses

Total 70 of 124 HIV-infected participants and 72 of 150 HIV-uninfected persons with biomarker data returned for at least one planned follow-up visit and were included in the longitudinal analyses. Compared to those lost to follow-up, those who returned for scheduled visits were younger, more frequently of black race, had worse baseline pulmonary function on all measures, and had higher levels of several serum inflammatory markers. There were no differences in follow-up by HIV status, and no difference in HIV-related variables between those who did and did not return (Supplemental Table 3, <http://links.lww.com/QAD/A902>).

In those with longitudinal data, postbronchodilator FEV₁ percentage-predicted values decreased over time, and trends for decrease were similar among HIV-infected and HIV-uninfected individuals (Fig. 2). Percentage-predicted diffusing capacity appeared to increase over time (similarly in both groups, Fig. 2), though this was likely because of surveillance bias or regression to the mean, as including all participants at each time point (i.e., not excluding participants' values from the baseline visit if they had no subsequent visit) revealed that DL_{CO} may in fact remain stable, as persons who did not follow-up tended to have better DL_{CO} percentage-predicted at baseline than those who did not (Supplemental Figure 1, <http://links.lww.com/QAD/A902>).

In HIV-infected participants with repeated measures of PFTs, in models adjusting for time constants and pack-years smoked, two circulating biomarkers contributed significantly to lower PFT. Baseline levels of endothelin-1 significantly independently associated with lower post-bronchodilator FEV₁ percentage-predicted over time, and baseline levels of sCD163 and endothelin-1 independently associated with lower diffusing capacity percentage-predicted over time (Table 3). Time-biomarker interaction terms were all nonsignificant (endothelin-1 with FEV₁ percentage-predicted: $P = 0.16$; endothelin-1 with diffusing capacity percentage-predicted: $P = 0.9$; sCD163

Table 2. Adjusted associations between circulating biomarkers and pulmonary function testing in HIV-infected and HIV-uninfected participants.

	HIV-infected		HIV-uninfected		<i>P</i> int HIV
	β	<i>P</i> value	β	<i>P</i> value	
IL-6					
FEV ₁ %predicted, post-BD	-0.24	0.01	-0.20	0.01	0.6
FEV ₁ /FVC ratio, post-BD	-0.13	0.2	0.02	0.8	0.3
DL _{CO} %predicted	-0.35	<0.001	-0.37	<0.001	0.7
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	1.04	0.09	1.0	0.9	0.2
Positive BD response	1.06	0.051	1.0	0.9	0.1
IL-8					
FEV ₁ %predicted, post-BD	-0.17	0.06	0.05	0.6	0.08
FEV ₁ /FVC ratio, post-BD	-0.16	0.08	0.04	0.7	0.1
DL _{CO} %predicted	-0.12	0.2	0.0	n/a	0.3
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	2.4	0.1	1.0	0.9	0.3
Positive BD response	1.8	0.4	1.3	0.8	0.8
TNF α					
FEV ₁ %predicted, post-BD	-0.18	0.07	-0.05	0.5	0.3
FEV ₁ /FVC ratio, post-BD	-0.04	0.7	0.10	0.2	0.3
DL _{CO} %predicted	-0.38	<0.001	-0.32	<0.001	0.6
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	1.01	0.2	1.0	0.9	0.3
Positive BD response	1.02	0.03	1.0	0.6	0.06
LPS					
FEV ₁ %predicted, post-BD	0.07	0.5	-0.06	0.5	0.5
FEV ₁ /FVC ratio, post-BD	0.07	0.5	-0.01	0.9	0.7
DL _{CO} %predicted	-0.19	0.03	-0.24	0.004	0.9
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	0.7	0.4	1.0	0.9	0.7
Positive BD response	1.2	0.7	1.0	0.9	0.8
sCD14					
FEV ₁ %predicted, post-BD	-0.08	0.5	-0.11	0.2	0.7
FEV ₁ /FVC ratio, post-BD	-0.12	0.2	0.04	0.6	0.2
DL _{CO} %predicted	-0.06	0.5	-0.05	0.5	0.9
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	2.4	0.4	0.5	0.5	0.2
Positive BD response	0.2	0.09	0.6	0.7	0.5
sCD163					
FEV ₁ %predicted, post-BD	-0.13	0.2	-0.12	0.2	0.8
FEV ₁ /FVC ratio, post-BD	-0.23	0.01	-0.07	0.4	0.2
DL _{CO} %predicted	-0.18	0.04	-0.15	0.07	0.6
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	3.8	0.06	1.2	0.7	0.2
Positive BD response	4.9	0.04	0.6	0.5	0.05
IL-2sR α					
FEV ₁ %predicted, post-BD	-0.12	0.2	-0.20	0.02	0.4
FEV ₁ /FVC ratio, post-BD	-0.04	0.6	-0.07	0.4	0.8
DL _{CO} %predicted	-0.25	0.005	-0.24	0.004	0.9
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	1.4	0.6	1.6	0.5	0.9
Positive BD response	1.4	0.6	1.4	0.7	0.9
ET-1					
FEV ₁ %predicted, post-BD	-0.36	<0.001	-0.04	0.6	0.003
FEV ₁ /FVC ratio, post-BD	-0.14	0.1	-0.11	0.2	0.8
DL _{CO} %predicted	-0.32	<0.001	-0.07	0.4	0.02
	OR	<i>P</i> value	OR	<i>P</i> value	
FEV ₁ /FVC <70%, post-BD	4.8	0.04	1.9	0.3	0.3
Positive BD response	7.6	0.03	4.9	0.053	0.8

BD, bronchodilator; DL_{CO}, diffusing capacity for carbon monoxide; ET-1, endothelin-1; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; IL, interleukin; IL-2sR α , IL-2 soluble receptor- α ; LPS, lipopolysaccharide; *P* int HIV, *P* value for interaction of biomarker and HIV status with pulmonary function outcome. Italics indicates significant values.

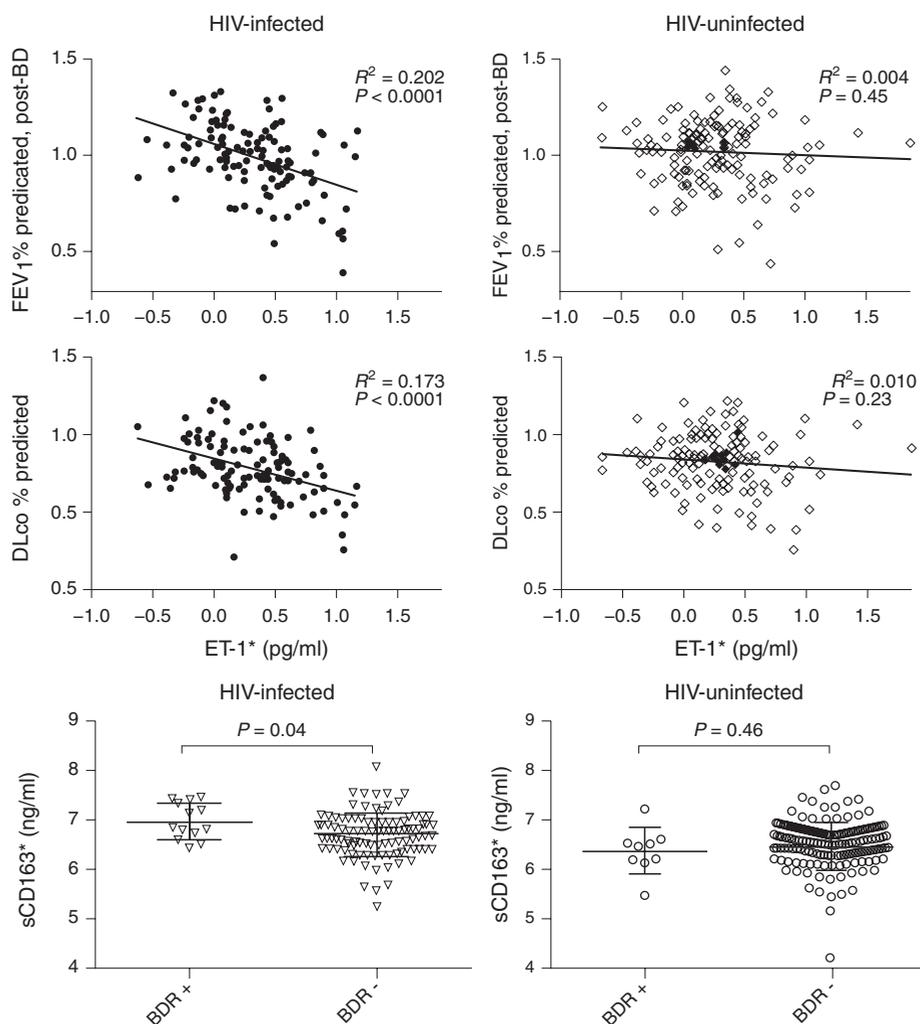


Fig. 1. Unadjusted relationships between biomarkers and pulmonary function in instances where HIV status modifies the association. BDR, bronchodilator response; DLco, diffusing capacity for carbon monoxide; ET-1, endothelin-1* log transformation; FEV₁, forced expiratory volume in one second. Line and whiskers represent mean and standard deviation.

with diffusing capacity percentage-predicted: $P=0.3$), suggesting that while higher baseline biomarker levels are associated with lower pulmonary function over time, they do not independently predict a more rapid rate of decline of FEV₁ or DL_{CO} percentage-predicted. No associations were found between baseline biomarkers and PFT changes in HIV-uninfected persons.

To further evaluate the impact of smoking status on the relationship between biomarkers and pulmonary function in HIV-infected participants, we compared the longitudinal relationships between biomarkers and PFTs between never smokers and smokers (current or former). Although the relationships between endothelin-1 and FEV₁ percentage-predicted did not vary by smoking status, the relationships between both endothelin-1 and sCD163 with DL_{CO} percentage-predicted appeared to be limited to smokers; though inferences should be made with caution given the small number ($n=28$) of

HIV-infected nonsmokers with longitudinal follow-up (Supplemental Table 4, <http://links.lww.com/QAD/A902>).

Exploratory multivariable regression path analyses were performed to assess whether the effects of either sCD163 or endothelin-1 on pulmonary function in HIV-infected participants were mediated by the other. In these models, we assumed that each biomarker had two distinct effects on pulmonary function, one direct and one indirect. Models were adjusted for pack-years smoked. We also performed cross-sectional cyclic path analyses to explore the converse hypothesis that impaired pulmonary function may be driving the biomarker abnormalities – these models had fewer significant associations for direct and indirect effects and were not as well fitted as the models describing a path proceeding from biomarker to pulmonary function. The best-fitted models suggested that part of the effects of sCD163 on diffusing capacity

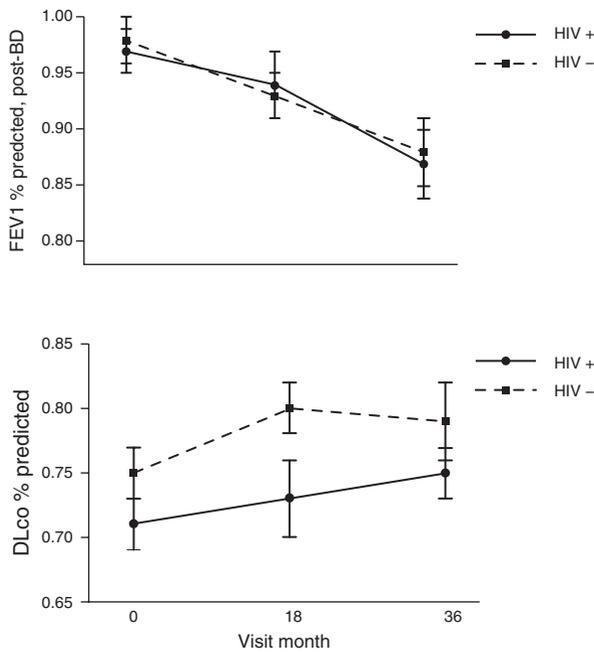


Fig. 2. Unadjusted trends of pulmonary function over longitudinal follow-up in HIV-infected and HIV-uninfected persons. Data are included only for persons who returned for at least one follow-up visit. HIV-positive, *n* = 70; HIV-negative, *n* = 98. Error bars represent standard error of the mean.

and postbronchodilator FEV₁ percentage-predicted may be mediated through endothelin-1 (Supplemental Figure 2, <http://links.lww.com/QAD/A902>).

Table 3. Multivariable models for baseline biomarkers significantly associated with lower pulmonary function in HIV-infected participants during follow-up.

Postbronchodilator FEV ₁ percentage predicted	β (95% CI)	<i>P</i> value
ET-1 ^a	-0.20 (-0.30 to -0.11)	<0.001
Pack-years smoked ^b	-0.01 (-0.03 to -0.002)	0.02
Time (per year)	-0.03 (-0.04 to -0.02)	<0.001
Interaction term between ET-1 and time	-	0.16
DL _{CO} percentage-predicted		
sCD163 ^a	-0.08 (-0.16 to -0.001)	0.047
Pack-years smoked ^b	-0.02 (-0.03 to -0.005)	0.005
Time (per year)	0.02 (0.006 to 0.04)	0.005
Interaction term between sCD163 and time	-	0.90
DL _{CO} percentage-predicted		
ET-1 ^a	-0.10 (-0.20 to -0.006)	0.04
Pack-years smoked ^b	-0.01 (-0.03 to -0.001)	0.04
Time (per year)	0.02 (0.007 to 0.04)	0.005
Interaction term between ET-1 and time	-	0.30

CI, confidence interval; DL_{CO}, diffusing capacity for carbon monoxide; ET-1, endothelin-1; FEV₁, forced expiratory volume in one second.
^aLog transformation.
^bSquare root transformation.

Discussion

In a well characterized cohort of both HIV-infected and HIV-uninfected men, we found cross-sectional associations of several inflammatory biomarkers (IL-6, TNFα, LPS, sCD163, and IL-2sRα) and the endothelial mediator endothelin-1 with worse pulmonary function in the cohort. Additionally, we identified interactions between HIV seropositivity and two mediators (sCD163 and endothelin-1) with measures of obstruction or diffusing impairment. Finally, we demonstrated that among HIV-infected participants with serial PFT, baseline sCD163 and endothelin-1 continued to be associated with lower pulmonary function in longitudinal analysis.

Prior studies have demonstrated associations between chronic HIV infection and pulmonary dysfunction; with reports of more frequent respiratory symptoms [14], worse diffusing capacity [4,7], and more frequent COPD diagnoses and radiographic emphysema [1–3,14,54] among persons with HIV. These observations are in part related to known exposures, most notably high frequency and intensity of cigarette smoking [55,56], but analyses in well conducted studies have established that a gap persists between HIV-infected and HIV-uninfected groups independent of age, smoking, and prior pulmonary infection [1–3,14,54]. We have previously shown an association between systemic inflammation and T-lymphocyte activation with HIV-associated COPD [19], but data are lacking regarding integration with specific pathways. Features relatively unique to persons with HIV that may contribute to pulmonary pathophysiology include persistent immune activation, microbial translocation, and endothelial dysfunction.

A major finding of this study is the novel discovery that endothelin-1 is independently associated with worse airflow obstruction and diffusing capacity impairment in persons with HIV, and that it is associated with lower FEV₁ percentage-predicted and lower DL_{CO} percentage-predicted over time. Endothelial dysfunction is a complication of acute and chronic HIV [35,36], and markers of endothelial activation (including cellular adhesion molecules, methylarginines, fibrinogen, and D-dimer) have been associated with cardiovascular disease in HIV [34,37,39,40]. Endothelial damage markers have also been associated with COPD in the general population [42–46]. Interestingly, parallel associations between endothelin-1 and PFTs were not observed in the HIV-uninfected participants, despite similar median levels of endothelin-1 in HIV-infected and HIV-uninfected persons. The lack of association in the HIV-uninfected subset is unlikely to be explained by lower statistical power because of sample size or precision relative to HIV-infected, given that the HIV-uninfected group was larger, and the statistical variances of affected PFT values were similar between the two groups. Interaction testing confirmed significant interactions

between endothelin-1 levels and HIV serostatus with postbronchodilator FEV₁ percentage-predicted and DL_{CO} percentage-predicted in this cohort, supporting a larger effect size of endothelin-1 relative to PFT outcomes in HIV-infected persons.

The finding of circulating endothelin-1 in association with PFT abnormalities adds to the existing literature describing a relationship between endothelial dysfunction and pulmonary disease, though in our cohort the association was only seen in HIV-infected persons. In a small study of individuals with COPD, plasma endothelin-1 negatively correlated with FEV₁, and endothelin-1 was increased in sputum and plasma during exacerbations, compared to controls during clinical stability [45]. Additionally, endothelin-1 is increased in exhaled breath condensate in exercise-induced bronchospasm and asthma [57], and has been related to the development of bronchiolitis obliterans syndrome in pulmonary allografts [58]. Recently, a very large population study (not selecting for persons with respiratory disease) found endothelin-1 levels to negatively correlate with FEV₁ [44]. Other investigations have related pulmonary outcomes to vascular measures of endothelial function; one such study demonstrated cross-sectional associations between brachial artery flow-mediated dilation, FEV₁, and quantitative CT emphysema scores [41]. Another recent investigation found a relationship between quantitative CT measure of lung total tissue volume and DL_{CO}, further supporting that the pulmonary microvasculature plays a significant role in the gas exchange abnormalities of COPD [59].

The drivers of endothelin-1 expression are not completely elucidated, but include reactive oxygen species, inflammatory mediators, and hypoxia-related factors [60]. In addition to its most clearly defined function as a potent vasoregulator, endothelin-1 is a bronchoreactive mediator [61,62] and an inflammatory regulator, having been found *in vivo* to affect regional expression of IL-6 [63,64], monocyte chemoattractant protein-1 [63], CX3CL1 [65], and other inflammatory cytokines [66]. Animal and *in-vivo* studies suggest potential mechanisms of endothelial-pulmonary associations, including emphysema secondary to endothelial apoptosis and endothelial inflammation, regional inflammation and chemotaxis, and matrix metalloproteinase production [43,46,67–70].

The greater relationship between endothelin-1 and PFTs in HIV-infected persons, despite similar circulating endothelin-1 levels to HIV-uninfected persons in the cohort, is not explained. It is possible that persons with HIV demonstrate more local variation in endothelin-1 regulation or receptor expression – for instance, one study has found that in response to vasoreactive challenge, local endothelin-1 remained elevated in persons with hypertension, but not in healthy volunteers, suggesting regional dysregulation with lack of adaptation [71].

Whether a similar local adaptation failure may be present in HIV is unknown. Additionally, the source of elevated circulating endothelin-1 in our participants is not known. Although pulmonary vascular endothelium is the most common location of endothelin-1 synthesis, endothelin-1 can be produced by many cell types, notably epithelium, smooth muscle, and immune cells, including alveolar macrophages [72,73]. Endothelin-1 expression from alveolar macrophages has been shown to be increased in congestive heart failure patients, although the enhanced elaboration from macrophages did not correlate with serum levels [73]. Future studies measuring endothelin-1 levels from the pulmonary compartment or assessing regional endothelin-B receptor expression may help explain the mechanism of its differential effect in HIV-infected versus HIV-uninfected persons.

The second novel finding of this study, equally important, is the association between markers of monocyte activation with pulmonary function in persons with HIV. Monocyte activation is a feature of both acute and chronic HIV infection [20] and is an important correlate of HIV-associated morbidity in the ART era [21]. Commonly measured markers of monocyte activation include sCD163, a circulating molecule which is enzymatically cleaved from monocytes upon activation [74], and sCD14. Both markers are elevated in HIV infection, including among persons with viral control [24,25,27,28]. Additionally, both are increased in inflammatory HIV-associated non-AIDS conditions such as cardiovascular disease, atherosclerosis [20,29,75–78], and HIV-associated neurocognitive disorders [22,23,26,79]. Soluble IL-2 receptor α (IL-2sR α , or sCD25), the circulating cleavage product of the IL-2 receptor (CD25), is a marker of disease activity in chronic inflammatory diseases [80–82]. CD25 is present on many leukocytes, including activated T-lymphocytes, and IL-2sR α has thus been used as a circulating surrogate of T-lymphocyte activation [83,84]; as such, it has been found to associate with cardiovascular risk among women with HIV [84]. Of interest, however, monocytes also elaborate CD25, and IL-2sR α levels have been associated with percentage of nonclassical monocytes while failing to associate with T-lymphocyte subsets [81]. In this cohort, regardless of HIV serostatus, IL-2sR α significantly correlated with sCD163 and sCD14, suggesting that it may be linked to monocyte activation. Given the importance of macrophages to pulmonary immunity and their relationship to COPD [85], monocyte contributions to pulmonary dysfunction may also be significant among persons with HIV, but these markers had not previously been investigated with reference to PFTs.

In the current study, median sCD163, sCD14, and IL-2sR α values were significantly higher in the HIV-infected than HIV-uninfected group, in keeping with prior data [24,28,29,84]. sCD163 independently associated with worse pulmonary dysfunction, including lower

FEV₁/FVC postbronchodilator ratio, greater odds of bronchodilator responsiveness, and lower DL_{CO} percentage-predicted in HIV-infected participants. Similar associations were not demonstrated in the HIV-uninfected group, though there were fewer persons with a bronchodilator response (9/150 [6%] vs. 13/124 [11%]), which reduced power to detect an association. Nonetheless, a statistical interaction was detected between HIV seropositivity and positive bronchodilator response. IL-2sR α was also associated with DL_{CO} percentage-predicted among HIV-infected persons in this cohort, but unlike sCD163, relationships were nearly identical in the HIV-uninfected group. Importantly, sCD163 baseline levels also were associated with lower DL_{CO} over the period of follow-up, though no significant longitudinal association was found with FEV₁. Exploratory path analyses supported that the effect of sCD163 on FEV₁ and DL_{CO} percentage-predicted are at least partially mediated by endothelin-1, suggesting that monocyte activation and endothelial dysfunction may be linked with regard to pulmonary disease in HIV-infected persons. These findings require further exploration in mechanistic studies.

Of the humoral inflammatory markers investigated, IL-6 and TNF α were higher in HIV-infected individuals with worse pulmonary function. Higher plasma IL-6 levels were associated with both lower postbronchodilator FEV₁ percentage-predicted and diffusing impairment and, and TNF α was associated with bronchodilator response and lower DL_{CO} percentage-predicted. Relationships were similar between HIV-infected and HIV-uninfected participants. IL-6 and TNF α levels were closely correlated in this cohort, suggesting they represent the same Th1 inflammatory pathway. Consistent with findings in some general population COPD analyses [86,87], these markers associated with spirometry in the HIV-uninfected group. We have found similar associations with IL-6 and with C-reactive protein (not measured in this study) with PFTs in a different HIV-infected cohort [19], and these inflammatory markers have been associated with other HIV-associated chronic diseases, including cardiovascular disease [17,88], and with COPD outcomes in case of IL-6 [87,89]. One prior HIV study comparing IL-6 to radiographic emphysema found no relationship between the two, though that study did not include PFTs to allow for direct comparison to our data [54]. Notably, despite previous findings of IL-8 associating with elevated pulmonary artery systolic pressure and dyspnea in HIV-infected persons [12], IL-8 did not significantly associate with PFTs in this cohort. This finding is in keeping with our previous data in a different HIV-infected cohort [19] and with available COPD literature [86].

Microbial translocation is a feature of HIV infection [33] and has been linked to chronic immune activation despite ART [30–32]; because of this association with systemic

inflammation, potential for contribution to pulmonary disease, particularly COPD, is compelling. A previous investigation of potential microbial translocation markers in persons with HIV found an independent association between sCD14 and risk of radiographic emphysema, though this study did not assess spirometry [54]. In our analysis, we measured circulating levels of microbial translocation markers LPS and the soluble form of its ligand (sCD14). LPS was associated with lower DL_{CO} in both HIV-infected and HIV-uninfected groups, but there was no association with obstruction. There were no associations between sCD14 levels and any pulmonary function outcome in either HIV-infected or HIV-uninfected participants. In the current study, with mostly well controlled HIV (81% of HIV-infected participants virally suppressed, 2% with CD4⁺ T-cell counts <200 cells/ μ l), LPS and sCD14 did not correlate. This lack of association suggests that in this cohort, sCD14 does not specifically represent the construct of ‘microbial translocation,’ but may represent nonspecific monocyte activation, as discussed above. Although prior microbial translocation data have demonstrated that high levels of sCD14 in the plasma reflect LPS exposure [30], the two markers have not been reliably correlated in persons without significant immune suppression [90]; furthermore, in-vivo work has shown IL-6 or IL-1 β stimulation can induce sCD14 production comparable to that induced by LPS [91], suggesting inflammatory signaling independent of microbial translocation. Microbial translocation cannot be discounted as a factor in HIV-associated pulmonary disease based on this relatively small sample, but the contributions compared to other pathways appear to be limited.

We did not find differences in PFT decline (FEV₁ or DL_{CO} percentage-predicted) between HIV-infected and HIV-uninfected participants in this cohort, possibly because of the relatively limited number of participants who returned for serial testing. Our results were similar to those from another longitudinal cohort, where no differences were found in longitudinal spirometry between HIV-infected and HIV-uninfected groups unless participants were analyzed in strata of poor viral control or CD4⁺ cell deficiency [92]. We did not have an adequate number of poorly controlled participants to independently assess this factor in this study. In addition, because the Multicenter AIDS Cohort study was started in the 1980s, the cohort may suffer from survivor bias, and current individuals may have better pulmonary function than those who have died.

The study has several limitations. First, only circulating biomarkers were assessed. Evaluation of biomarker and receptor concentrations from respiratory samples or human bronchial epithelial cells with similar correlations to circulating markers and to pulmonary function will be revealing regarding systemic and local relationships of these molecules. Additionally, complementary vascular

mediators (such as endothelial growth factors, cellular adhesion molecules, and methylarginines) and other markers of endothelial apoptosis, such as endothelial microparticles, were not examined in this cohort. Further studies of the associations between levels of endothelin-1 and other vascular mediators and pulmonary outcomes in HIV-infected populations are warranted. A final important limitation of this study is that the findings are limited to men. COPD phenotypes and pathogenesis demonstrate sex variation [93,94]; additionally, biomarker levels and activity may vary between men and women. Endothelin-1 demonstrates sex differences in the cardiovascular system [95] and kidney [96], and sCD163 has been found to be higher in women [97,98], with possible modulation by sex hormones [99]. These associations require confirmation in a cohort, including female participants.

A major strength of our current study is the longitudinal follow-up, which is more strongly suggestive of a relationship between immune cell activation and endothelial dysfunction with reduction in pulmonary function than are prior cross-sectional analyses. Additionally, including both HIV-infected and HIV-uninfected participants allowed direct comparison between groups and identification of pathophysiology that may be particularly enhanced in persons with HIV. Finally, assessing multiple biomarkers in one cohort permitted comparison of their relationships, allowing hypothesis-generating inferences for possible interactions between biologic pathways. In conclusion, this study discovered two new potential arenas contributing to the disproportionate burden of pulmonary dysfunction among persons with HIV. Further exploration of monocyte activation and endothelial dysfunction in the role of HIV-associated lung disease and symptoms may lead to new implications for treatment in this population.

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Conflicts of interest

There are no conflicts of interest.

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