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Clinical and Immunological Markers of Pulmonary Impairment Among People With HIV in India

Anurima Baidya,[1] Shashikala Sangle,[2] Ivan Marbaniang,[3,4] Vandana Kulkarni,[4] Prasad Deshpande,[4] Smita Nimkar,[4] Amol Chavan,[4] Sonali Salvi,[2] Rahul Lokhande,[2] Dileep Kadam,[2] Amita Gupta,[5] Vidya Mave,[4,5] and Akshay N. Gupte[5]

[1]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA, [2]Byramjee-Jeejeebhoy Government Medical College, Pune, India, [3]Department of Epidemiology, McGill University, Montreal, Québec, Canada, [4]Johns Hopkins India, Pune, India, and [5]Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Background. Despite antiretroviral therapy, chronic lung diseases remain an important source of morbidity and mortality in people with HIV (PWH). We sought to identify clinical and immunological markers of pulmonary impairment among PWH in India.

Methods. Two hundred ten adult PWH receiving antiretroviral therapy (ART) were prospectively evaluated for 3 years. Plasma concentrations of interleukin (IL)-6, IL-10, tumor necrosis factor alpha, D-dimer, C-reactive protein, soluble (s)CD14, and sCD163 were measured at enrollment. We used multivariable linear and logistic regression to measure the association of baseline and time-varying clinical and immunological variables with spirometry-defined chronic obstructive pulmonary disease (COPD), restrictive spirometry pattern (RSP), preserved ratio impaired spirometry (PRISm), forced expiratory volume in 1 second (FEV1), and forced vital capacity (FVC) during the third year of follow-up.

Results. After adjusting confounders, females were 7 times more likely to have RSP (95% CI, 2.81 to 17.62; $P < .001$) and 22 times more likely to have PRISm (95% CI, 7.42 to 69.92; $P < .001$) compared with men. Higher IL-6 concentrations were associated with lower FEV1 z-scores (β, −0.14 per log-higher; 95% CI, −0.29 to 0.008; $P = .06$) and higher odds of COPD (adjusted odds ratio [aOR], 2.66 per log-higher; 95% CI, 1.16 to 6.09; $P = .02$). Higher D-dimer concentrations were associated with lower FVC z-scores (β, −0.40 per log-higher; 95% CI, −0.78 to −0.01; $P = .04$). Conversely, higher IL-10 concentrations were associated with lower odds of PRISm (aOR, 0.76 per log-higher; 95% CI, 0.59 to 0.99; $P = .04$).

Conclusions. Female sex, higher concentrations of IL-6 and D-dimer, and lower concentrations of IL-10 were associated with pulmonary impairment in adult PWH receiving ART in India.

Keywords. HIV; chronic lung disease; IL-6; IL-10; D-dimer; lung function; sex differences.

With the introduction of antiretroviral therapy (ART), the survival of people with HIV (PWH) and their overall life span have seen dramatic improvement. However, this has been accompanied by an increased risk of noncommunicable diseases. Obstructive and restrictive lung diseases, hereafter referred to as chronic lung diseases (CLDs), are now emerging as important comorbidities in PWH [1]. For instance, a recent meta-analysis reported that PWH were more likely to have COPD relative to HIV-negative controls, independent of smoking exposure [2]. The prevalence of CLD in PWH can range from 5%

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to 30% and is an important contributor to poor quality of life and excess mortality [3, 4].

A majority of the evidence generated on CLD in PWH is from high-income countries with at-risk populations, such as those with high levels of smoking exposure and intravenous drug use, or from individuals with poorly controlled HIV [3, 5]. Prior studies conducted in these settings have established the association of poorly controlled HIV with a rapid decline in lung function, reduced diffusion capacity, and worsening spirometry parameters [3, 5, 6]. However, these data may not be generalizable to populations with well-controlled HIV receiving ART under programmatic settings, especially in low- and middle-income countries (LMICs). For instance, our prior work in South Africa did not find a significant association of low CD4 cell counts and high viral load with lung impairment in a cohort of PWH receiving ART [7]. The epidemiology of CLD in PWH in LMICs may be different from that observed in high-income settings, likely due to an interplay of various social and environmental factors, such as higher exposure to biomass fuels, air pollution, recurrent respiratory infections, and high prevalence of tuberculosis and malnutrition [8].

In addition, HIV care itself may be delivered differently under programmatic settings in LMICs.

Furthermore, persistent immune activation in the setting of ART-mediated viral suppression has been associated with several chronic morbidities and excess mortality [9–17]. Prior studies have implicated inflammatory markers such as interleukin (IL)-1, IL-6, IL-8, IL-15, tumor necrosis factor (TNF) α, and C-reactive protein (CRP) and markers of monocyte activation such as soluble (s)CD14 and sCD163 as plausible determinants of persistent immune activation and cardio-pulmonary morbidity in PWH [9, 10, 12, 15, 17]. Although immunologic abnormalities are most apparent in patients who do not use ART, recent evidence suggests that these immunological abnormalities may continue to persist despite ART initiation [10]. Biomarkers of innate immune activation and chronic inflammation pathways have the potential to predict a broad spectrum of comorbid conditions that are likely increased in the context of treated HIV infection, suggesting that these pathways—and their key drivers—may be important targets for interventions to improve health outcomes of PWH in the ART era [10]. However, few studies have identified immunological markers associated with pulmonary impairment in PWH with well-controlled HIV disease who are receiving routine outpatient care.

Therefore, we prospectively evaluated adult PWH receiving ART under programmatic settings in Pune, India, to identify clinical and immunological markers associated with impaired lung function and CLD.

METHODS

Study Population

We established a prospective cohort of HIV-positive adults (≥18 years) receiving medical care through the National AIDS Control Organisation (NACO) outpatient ART Centre at Byramjee-Jeejeebhoy Government Medical College and Sassoon General Hospitals (BJGMC-SGH) in Pune, India, from 2014 to 2016. We excluded pregnant and breastfeeding women and participants with a life-threatening illness at enrollment.

Patient Consent

All participants provided written informed consent in their native language before enrollment. The study protocol was approved by the Institutional Review Boards of Johns Hopkins Medicine and Byramjee-Jeejeebhoy Government Medical College and Sassoon General Hospitals, Pune.

Data Collection

Enrolled participants were prospectively evaluated for 3 years. History of ever smoking (defined as ≥100 tobacco products smoked), tuberculosis, alcohol consumption, and exposure to biomass fuels (defined as the burning of wood, coal, animal dung, or crop residues for cooking or heating purposes) were obtained at baseline by self-report. Diabetes was classified at enrollment as receipt of any antidiabetic medication, a glycated hemoglobin (HbA1c) level ≥6.5%, or a fasting blood glucose (FBG) ≥126 mg/dL [18]. Anthropometric measurements were noted for all participants at enrollment, and body mass index (BMI) was calculated as weight (kg)/height (m)^2. CD4 cell count at HIV diagnosis, duration of HIV disease, duration of ART, and receipt of protease-based ART regimen were ascertained by reviewing medical records. We assessed CD4 cell counts and viral load at enrollment and annually thereafter until the end of the study follow-up. We measured the nadir and average CD4 count during the 3-year study period. Viral load <40 copies/mL was defined as the limit of detection.

We assessed lung function of study participants in the third year of follow-up by pre- and postbronchodilator spirometry. Spirometry was performed by trained study staff using the EasyOne ultrasonic flow spirometer (ndd Medical, Zurich) in the sitting position according to American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines for acceptability and reproducibility. Postbronchodilator spirometry was performed 15 minutes after administering 200 μg of salbutamol sulphate via capsule-based dry powder inhaler (Cipla Ltd, Mumbai, India). We calculated prebronchodilator percent-predicted values and z-scores of forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), and the FEV1-to-FVC ratio (FEV1/FVC) for each participant using reference equations derived from an adult population of healthy nonsmokers from India [19]. Airflow obstruction (AO) was classified as having prebronchodilator FEV1/FVC less than the lower limit of normal (LLN), defined as z-scores <–1.64, which corresponds to the fifth percentile of the normal FEV1/FVC distribution obtained from the healthy reference population. Restrictive spirometry pattern (RSP) was defined as prebronchodilator FVC less than the LLN and an FEV1/FVC ratio greater than the LLN [20]. Preserved ratio impaired spirometry (PRISm) was defined as having an FEV1/FVC ratio ≥0.70 and FEV1 <80% of the predicted value [21]. Additionally, we classified participants with postbronchodilator FEV1/FVC ratio <0.70 as having chronic obstructive pulmonary disease (COPD), in accordance with Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [22]. A positive bronchodilator response was defined as a 12% or 200-mL increase in FEV1 and/or FVC relative to prebronchodilator values [23].

Plasma samples collected at enrollment underwent cytokine testing using multiplex Luminex assay (Bio-Rad, Hercules, CA, USA) at BJGMC. We a priori selected 7 markers of inflammation based on their associations with comorbidities and mortality in PWH. Specifically, we chose D-dimer as a marker of fibrin breakdown; IL-6, CRP, IL-10, and TNF-α as nonspecific markers of inflammation; and sCD14 and sCD163 as markers of monocyte activation [9, 10, 15, 24, 25].

Statistical Analysis

Baseline characteristics between participants with AO, RSP, and normal spirometry were compared using the Kruskal-Wallis test and Fisher exact test for continuous and categorical data, respectively. We used uni- and multivariable logistic regression to determine the association of AO, RSP, PRISm, and COPD with inflammatory markers and clinical variables including markers of HIV disease as time-updated exposures, assessed during the preceding 3 years. Similarly, we used uni- and multivariable linear regression to measure the association between FEV1 and FVC z-scores and percent-predicted values, inflammatory markers, and clinical variables during the preceding 3 years. Predictor variables were modeled either as continuous exposures (age, BMI, CD4 cell counts, $\log_2$-transformed viral load, and CRP) or categorically at relevant thresholds (BMI at 18.5 kg/m^2). Smoking, diabetes, ART receipt, and prior tuberculosis were modeled as dichotomous exposures. Inflammatory markers were modeled as $\log_2$-transformed continuous exposures. In addition to inflammatory markers, the final multivariable model included variables known to be associated with pulmonary impairment (age, sex, BMI, smoking, prior tuberculosis), markers of HIV disease ($\log_2$-transformed average viral load during follow-up, CD4 cell counts at ART initiation, and duration of ART), and variables that were statistically significantly associated with the outcome in univariable analysis (diabetes, average CD4 cell count during follow-up). Variance inflation factor analysis was used to test for multicollinearity. Additionally, we performed a sex-stratified analysis of the association between inflammatory markers and lung function outcomes (Supplementary Tables 7 and 8) and a separate secondary analysis of the association between inflammatory and clinical markers with pulmonary impairment using Global Lung Function Initiative (GLI) reference equations for mixed ethnicity (Supplementary Tables 3–6) [26]. We performed a separate analysis of the association between inflammatory markers and a positive bronchodilator response restricted to participants with CLD (Supplementary Table 9). Adjusted and unadjusted odds ratios, regression coefficients, and 95% CIs are presented. Analyses were done in Stata, version 16.0 (StataCorp, USA).

RESULTS

Overall, 212 patients received a spirometry assessment in the third year of follow-up, and 210 (99%) participants who satisfied ATS/ERS quality criteria for spirometry were included in the analysis. The median (interquartile range [IQR]) age of participants was 40 (36–45) years, and 89 (42%) were male. The median (IQR) body mass index was 21 (18–24) kg/m^2, and 57 (27%) had BMI ≤18.5 kg/m^2. Ever smoking, alcohol consumption, exposure to biomass fuels, and diabetes were reported by 34 (16%), 19 (9%), 33 (16%), and 44 (21%) participants, respectively. At enrollment, the median (IQR) duration of HIV disease was 8.2 (4.1–12.1) years, and the median viral load (IQR) was 40 (40–134) copies/mL. The median (IQR) CD4 counts at ART initiation and enrollment were 178 (105–300) and 452 (314–651) cells/mm^3, respectively. At enrollment, 184 (88%) participants were receiving ART, with a median (IQR) treatment duration of 5.6 (2.0–8.7) years, and 37 (18%) were receiving protease inhibitor–based ART. All study participants were receiving ART by 6 months of follow-up. The median (IQR) percent-predicted FEV1 and FVC were 87% (70%–102%) and 79% (69%–96%), with AO and RSP being detected in 6 (3%) and 94 (45%) participants, respectively (Table 1). Additionally, 96 (46%) and 5 (3%) participants were classified as having PRISm and COPD, respectively.

Female sex was independently associated with a 7-fold (95% CI, 2.81 to 17.62; $P < .001$) and 22-fold (95% CI, 7.42 to 69.92; $P < .001$) higher odds of having RSP and PRISm compared with males after adjusting for age, low BMI, history of tuberculosis, smoking, diabetes, CD4 count at ART initiation, duration of ART, average viral load, and average CD4 count (Table 2). Similarly, female sex was associated with lower FEV1 (2.36 lower z-score; 95% CI, −2.92 to −1.80; $P < .001$) and lower FVC (1.89 lower z-score; 95% CI, −2.39 to −1.39; $P < .001$) relative to males after adjusting for covariates. We also observed lower FEV1 (0.67 lower z-scores; 95% CI, −1.21 to −0.12; $P = .01$) scores in participants with BMI ≤18.5 kg/m^2 in the multivariable analysis (Figure 1A and B; Supplementary Table 1). We did not find a significant association between markers of HIV disease (CD4 count at ART initiation, average CD4 count, average viral load, and duration of ART) and pulmonary impairment in the multivariable analysis (Table 2, Figure 2A and B).

Higher levels of circulating IL-6 were associated with lower FEV1 (0.14 lower z-score per log-higher IL-6; 95% CI, −0.29 to 0.00; $P = .06$), while higher levels of circulating D-dimer were associated with lower FVC (0.40 lower z-score per log-higher D-dimer; 95% CI, −0.78 to −0.01; $P = .04$) (Figure 3A and B; Supplementary Table 2). We observed an association of circulating IL-6 with AO (adjusted odds ratio [aOR], 1.68 per log-higher IL-6; 95% CI, 0.97 to 2.90; $P = .06$) and COPD (aOR, 2.66 per log-higher IL-6; 95% CI, 1.16 to 6.09; $P = .02$). Conversely, participants with higher levels of circulating IL-10 were less likely to have PRISm (aOR, 0.76 per log-higher IL-10; 95% CI, 0.59 to 0.99; $P = .04$) and RSP (aOR, 0.80 per log-higher IL-10; 95% CI, 0.63 to 1.02; $P = .07$) (Table 3).

In the sex-stratified analysis, we found that higher levels of circulating D-dimer were associated with lower FEV1 (1.07 lower z-score per log higher D-dimer; 95% CI, −2.17 to 0.02; $P = .05$) and lower FVC (0.79 lower z-score per log higher D-dimer; 95% CI, −1.40 to −0.17; $P = .01$) among male participants; we did not find similar associations between inflammatory markers and pulmonary impairment in females

Table 1. Airflow Obstruction and Restrictive Spirometry Pattern by Participant Characteristics at Enrollment

	Full Cohort (n = 210)	AO (n = 6)	RSP (n = 94)	No CLD (n = 110)	P Value
Socio-demographic indicators					
Age, median (IQR), y	40 (36–45)	45 (38–49)	40 (36–44)	41 (25–59)	.21
Male sex, No. (%)	89 (42)	3 (50)	14 (15)	72 (65)	<.001
Education					
Illiterate	24 (11)	1 (17)	12 (13)	11 (10)	.43
Primary school	24 (11)	1 (17)	8 (9)	15 (14)	
High school	77 (37)	1 (17)	37 (39)	39 (35)	
College	85 (41)	3 (49)	37 (39)	45 (41)	
BMI, median (IQR), kg/m^2	21 (18–24)	19 (17–20)	21 (18–24)	22 (19–24)	.10
Ever-smoking, No. (%)	34 (16)	1 (17)	3 (9)	30 (27)	<.001
Alcohol use, No. (%)	19 (9)	3 (16)	1 (1)	15 (14)	<.001
Biomass use, No. (%)	33 (16)	0	12 (13)	21 (19)	.31
Prior TB, No. (%)	85 (40)	3 (50)	37 (39)	45 (41)	.80
Diabetes, No. (%)	44 (21)	2 (33)	21 (22)	15 (14)	.50
Markers of HIV disease, median (IQR)					
HIV duration, y	8.2 (4.1–12.1)	6.6 (2.7–9.5)	8.8 (5.0–13.9)	8.1 (3.0–11.9)	.25
ART duration, y	5.6 (2.0–8.7)	4.7 (0–8.8)	5.1 (2.1–8.5)	6.1 (1.9–8.8)	.87
CD4 at ART initiation, cells/mm^3	178 (105–300)	140 (127–225)	204 (141–341)	156 (80–282)	.02
CD4 at enrollment, cells/mm^3	452 (314–651)	343 (206–550)	513.5 (356–719)	430 (296–605)	.03
CD4 nadir, cells/mm^3	360 (277–546)	310 (206–332)	401 (312–615)	346 (248–492)	.01
Undetectable HIV RNA at enrollment, No. (%)	92 (43.8)	3 (50.00)	48 (52.2)	41 (44.6)	.13
Inflammatory markers at enrollment, median (IQR)					
Il-6, pg/mL	1.9 (0.8–4.8)	6.1 (3.0–12.2)	2.4 (0.8–5.4)	1.54 (0.8–3.8)	.03
D-dimer, mcg/mL	0.22 (0.15–0.13)	0.24 (0.15–0.42)	0.25 (0.19–0.35)	0.18 (0.15–0.3)	<.001
IL-10, pg/mL	2.9 (1.63–5.41)	2.63 (2.3–4.43)	2.61 (1.44–4.56)	3.0 (1.78–5.82)	.42
CRP, µg/mL	0.205 (0.08–0.55)	0.207 (0.066–0.42)	0.205 (0.08–0.41)	0.208 (0.08–0.71)	.73
TNF-α, pg/mL	3.1 (0.1–5.08)	1.0 (1.0–3.1)	2.3 (0.9–5.6)	3.1 (1.0–4.3)	.81
sCD14, ng/mL	9396.5 (6495.3–13 605)	6403.8 (3581.9–11 541)	10 297 (6659.2–14 173)	8858.4 (6441.7–13 303)	.22
sCD163, ng/mL	1995.4 (1261.8–2941.1)	4222.7 (3730.06–4258.4)	2019 (1177.4–2863.5)	1939.2 (1308.2–2871.8)	.03
Lung function parameters in year 3, median (IQR)					
FEV1					
% predicted	86.6 (69.7–102.1)	54.4 (36.4–61.9)	68.4 (61.2–75.5)	103.8 (85.9–117.9)	<.001
z-score	−1.3 (−2.5 to 0.18)	−3.1 (−3.9 to −2.6)	−2.4 (−3.0 to −1.9)	0.14 (−1.0 to 1.3)	<.001
FVC					
% predicted	78.7 (66.8–95.8)	68 (53.3–83.4)	63.6 (58.7–70.3)	94.9 (83.4–107.0)	<.001
z-score	−2.3 (−2.9 to −1.5)	−2.4 (−3.5 to −0.9)	−2.6 (−3.2 to −2.2)	−0.36 (−1.2 to 0.5)	<.001
FEV1/FVC					
% predicted	69.7 (68.5–71.8)	68.6 (67.4–72.2)	69.3 (68.6–71.1)	70.0 (68.4–72.2)	.41
z-score	1.07 (−0.1 to 2)	−2.8 (−4.7 to −2.4)	1.3 (0.17 to 2.3)	1.04 (−0.04 to 1.9)	.0002

P values reported are comparing participants with AO, RSP, and no CLD by t test, Kruskal-Wallis test, or Fisher exact test, as appropriate.

Abbreviations: AO, airflow obstruction; ART, antiretroviral therapy; BMI, body mass index; CLD, chronic lung disease; IQR, interquartile range; RSP, restrictive spirometry pattern; TB, tuberculosis.

(Supplementary Tables 7 and 8). In the analysis restricted to participants with CLD, we did not find a statistically significant association between any of the inflammatory markers and a positive bronchodilator response (Supplementary Table 9). The findings from our secondary analyses using GLI equations are reported in Supplementary Tables 3–6.

DISCUSSION

Our study sought to identify clinical and immunological markers of pulmonary impairment among PWH receiving ART under programmatic settings in India. We found a high burden of RSP and PRISm, phenotypes of CLD that are associated with excess morbidity and mortality yet remain largely underrecognized in PWH. Importantly, we found a disproportionately high likelihood of RSP and PRISm among female PWH after adjusting for sex-related physiological differences in lung volumes and gender-related confounders such as smoking and biomass fuel use. Finally, we observed that higher plasma concentrations of IL-6 and D-dimer and lower concentrations of IL-10 were associated with pulmonary impairment independent of severity of HIV disease. These data suggest a potential role of circulating IL-6, D-dimer, and IL-10 as biomarkers for CLD and

Table 2. Participant Characteristics Associated With Airflow Obstruction, Restrictive Spirometry Pattern, Preserved Ratio Impaired Spirometry, and Chronic Obstructive Pulmonary Disease

|	Airflow Obstruction				Restrictive Spirometry Pattern				Preserved Ratio Impaired Spirometry				Chronic Obstructive Pulmonary Disease			
	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
Age	1.23 (0.48 to 3.15)	.65	1.06 (0.38 to 2.93)	.90	0.70 (0.50 to 0.99)	.04	1.04 (0.67 to 1.61)	.84	0.62 (0.44 to 0.88)	.008	1.04 (0.64 to 1.68)	.87	1.47 (0.54 to 3.99)	.44	0.93 (0.25 to 3.45)	.92
Sex	0.74 (0.14 to 3.69)	.70	1.24 (0.15 to 10.26)	.83	10.45 (5.27 to 2.70)	<.001	7.04 (2.81 to 17.62)	<.001	22.74 (10.27 to 50.36)	<.001	22.78 (7.42 to 69.92)	<.001	0.17 (0.01 to 1.61)	.12	0.20 (0.01 to 3.04)	.24
Low BMI	2.77 (0.54 to 14.18)	.21	1.75 (0.25 to 12.04)	.56	1.54 (0.82 to 2.84)	.16	1.73 (0.71 to 4.22)	.22	1.94 (1.06 to 3.63)	.03	3.02 (1.06 to 8.57)	.03	4.19 (0.68 to 25.78)	.12	2.39 (0.28 to 20.20)	.42
Smoking	1.03 (0.11 to 9.16)	.97	0.99 (0.07 to 13.41)	.99	0.09 (0.02 to 0.30)	<.001	0.24 (0.04 to 1.31)	.10	0.08 (0.02 to 0.29)	<.001	0.56 (0.09 to 3.18)	.51	1.30 (0.14 to 12.03)	.81	0.69 (0.05 to 9.69)	.78
Past TB	1.48 (0.29 to 7.55)	.63	1.13 (0.16 to 7.04)	.89	0.84 (0.48 to 1.47)	.56	1.55 (0.67 to 3.62)	.30	0.62 (0.35 to 1.09)	.09	1.19 (0.47 to 3.00)	.70	2.25 (0.36 to 13.76)	.38	0.84 (0.08 to 8.77)	.88
Diabetes	0.82 (0.40 to 1.04)	.07	0.96 (0.23 to 3.97)	.96	0.88 (0.58 to 1.32)	.54	1.20 (0.66 to 2.17)	.54	0.65 (0.43 to 0.99)	.04	0.89 (0.47 to 1.69)	.73	0.52 (0.08 to 3.25)	.49	0.47 (0.05 to 4.02)	.49
CD4 level at HIV diagnosis	0.77 (0.41 to 1.44)	.42	0.85 (0.45 to 1.61)	.63	1.09 (0.96 to 1.24)	.16	1.11 (0.91 to 1.10)	.28	1.13 (0.98 to 1.24)	.07	1.17 (0.92 to 1.48)	.19	0.61 (0.25 to 1.44)	.26	0.81 (0.35 to 1.85)	.62
Average CD4 count	0.65 (0.40 to 1.04)	.07	0.69 (0.38 to 1.22)	.20	1.16 (1.04 to 1.34)	.008	1.12 (0.95 to 1.32)	.15	1.12 (1.00 to 1.26)	.03	0.99 (0.83 to 1.17)	.94	0.57 (0.31 to 1.01)	.05	0.63 (0.30 to 1.34)	.23
ART duration at month 36	0.94 (0.76 to 1.16)	.59	1.00 (0.79 to 1.28)	.95	1.00 (0.94 to 1.07)	.83	0.99 (0.90 to 1.10)	.99	0.98 (0.92 to 1.05)	.70	1.05 (0.94 to 1.18)	.33	1.03 (0.83 to 1.27)	.78	1.13 (0.87 to 1.47)	.33
Average viral load	1.16 (0.88 to 1.53)	.27	1.12 (0.81 to 1.55)	.47	1.02 (0.90 to 1.22)	.65	1.05 (0.88 to 1.25)	.57	1.02 (0.89 to 1.17)	.73	0.99 (0.82 to 1.19)	.92	1.18 (0.88 to 1.59)	.24	1.27 (0.85 to 1.91)	.23

Logistic regression adjusted for age, sex, BMI, smoking, prior TB, diabetes, CD4 count at HIV diagnosis, duration of ART at month 36, average viral load, and average CD4 count.

Abbreviations: AO, airflow obstruction; ART, antiretroviral therapy; COPD, chronic obstructive pulmonary disease; OR, odds ratio; PRISm, preserved ratio impaired spirometry; RSP, restrictive spirometry pattern.
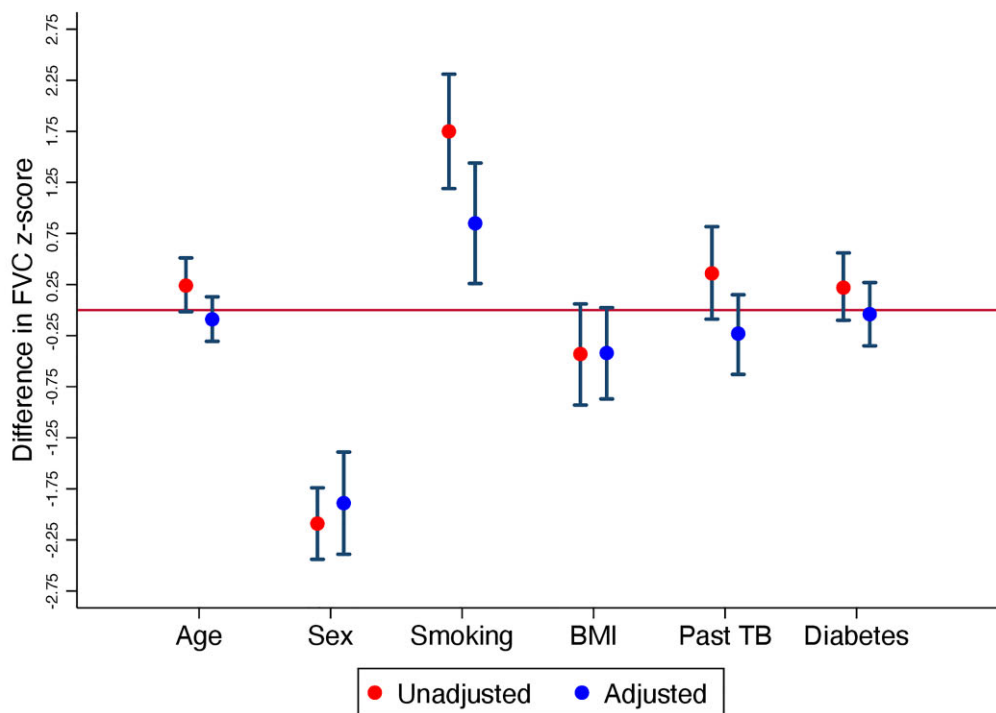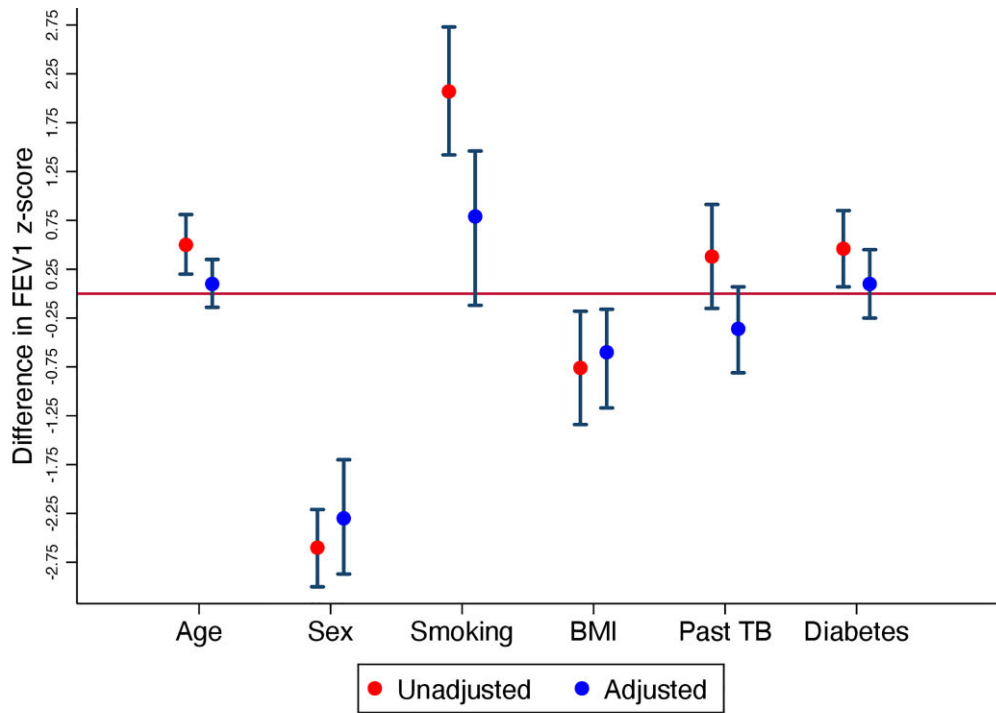
Figure 1. Differences in FEV1 (Figure 1A) and FVC (Figure 1B) z-scores by participant characteristics at enrollment. Adjusted models include age, sex (male/female), BMI, smoking (yes/no), history of TB (yes/no), diabetes (yes/no), average viral load and CD4 counts, CD4 count at ART initiation, and duration of ART in months. Abbreviations: ART, antiretroviral therapy; BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TB, tuberculosis.
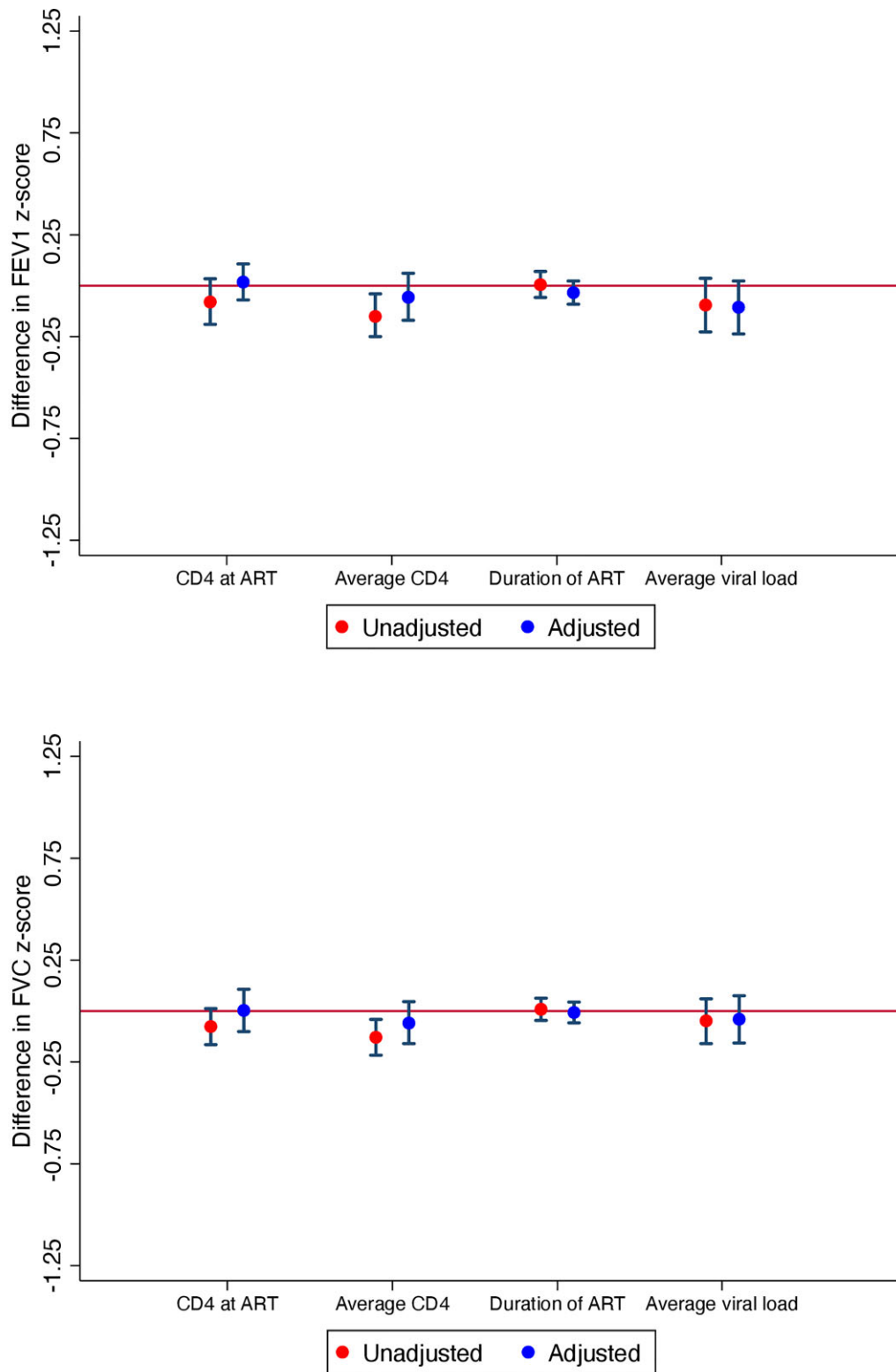
Figure 2. Differences in FEV1 (Figure 2A) and FVC (Figure 2B) z-scores by markers of HIV disease. Adjusted models include age, sex (male/female), BMI, smoking (yes/no), history of TB (yes/no), and diabetes (yes/no). Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TB, tuberculosis.
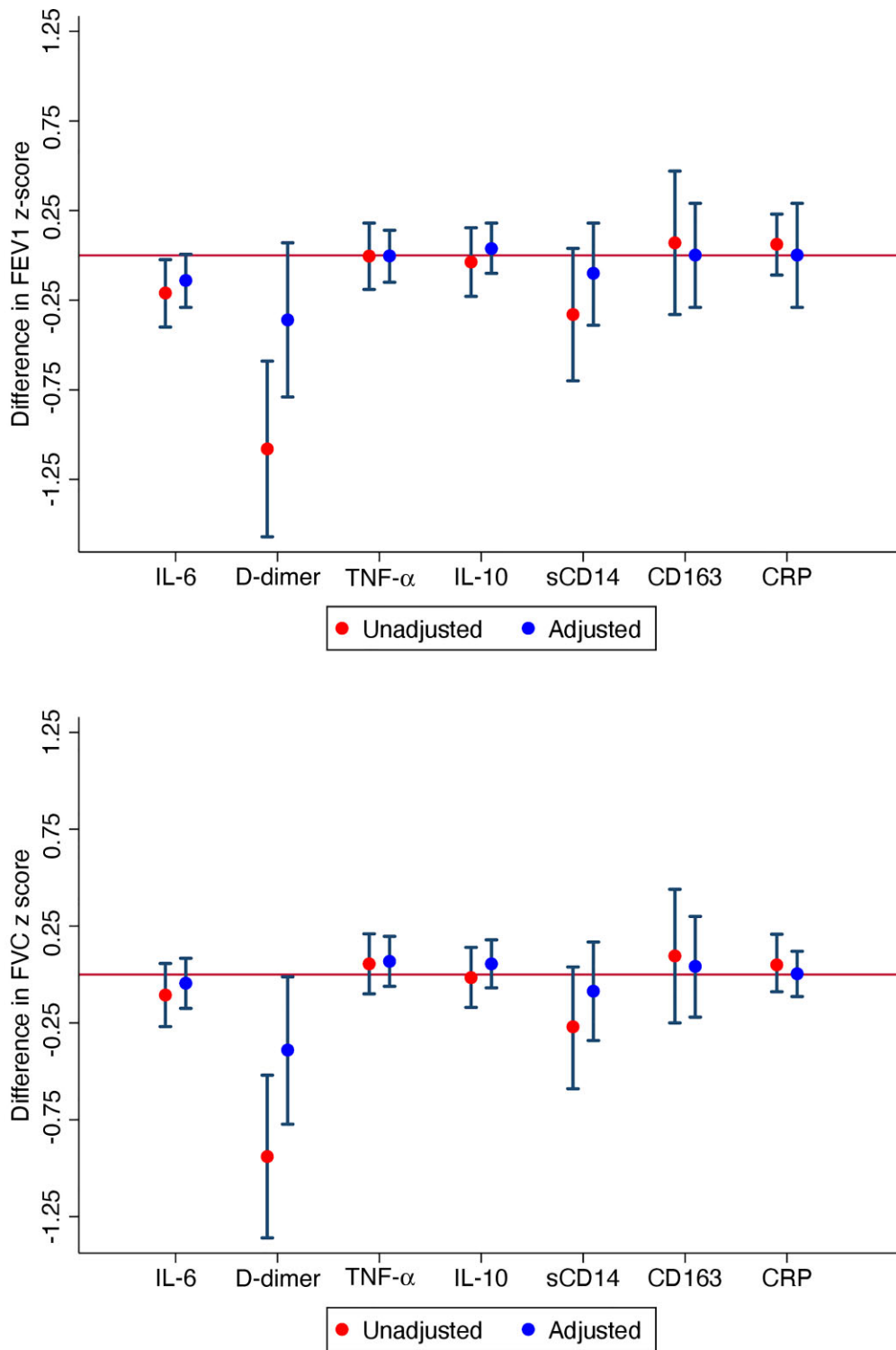
Figure 3. Differences in FEV1 (Figure 3A) and FVC (Figure 3B) z-scores by markers of inflammation. Adjusted models include age, sex (male/female), BMI, smoking (yes/no), history of TB (yes/no), diabetes (yes/no), viral load, CD4 count, and ART at enrollment. Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TB, tuberculosis.

highlight the importance of further studying the biological mechanisms underpinning sex differences in CLD risk and presentation among PWH.

An important finding of our study was the association of female sex with lower lung function and higher likelihood of RSP and PRISm, despite accounting for sex-related physiological

Table 3. Markers of Inflammation Associated With Airflow Obstruction, Restrictive Spirometry Pattern, Preserved Ratio Impaired Spirometry, Postbronchodilator Chronic Obstructive Pulmonary Disease

Inflammatory Markers (per Log Increments)	Airflow Obstruction				Restrictive Spirometry Pattern				Preserved Ratio Impaired Spirometry				Chronic Obstructive Pulmonary Disease			
	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value	Unadjusted OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
IL-6	1.46 (0.98 to 2.17)	.06	1.68 (0.97 to 2.90)	.06	1.01 (1.35 to 4.22)	.857	0.91 (0.69 to 1.19)	.49	1.23 (0.99 to 1.53)	.06	1.24 (0.92 to 1.69)	.15	1.62 (0.27 to 6.67)	.02	2.66 (1.16 to 6.09)	.02
D-dimer	1.33 (0.30 to 5.81)	.69	1.06 (0.17 to 6.31)	.94	2.39 (1.35 to 4.22)	.003	1.28 (0.62 to 2.64)	.49	2.10 (1.20 to 3.68)	.00	0.85 (0.39 to 1.85)	.69	1.34 (0.27 to 6.67)	.71	2.59 (0.35 to 19.55)	.35
IL-10	0.78 (0.36 to 1.67)	.53	0.71 (0.27 to 1.82)	.47	0.87 (0.71 to 1.07)	.21	0.80 (0.63 to 1.02)	.07	0.86 (0.69 to 1.05)	.15	0.76 (0.59 to 0.99)	.04	1.29 (0.87 to 1.90)	.19	1.48 (0.86 to 2.55)	.14
TNF-α	0.94 (0.51 to 1.70)	.84	0.94 (0.49 to 1.79)	.86	1.02 (0.84 to 1.24)	.78	0.94 (0.74 to 1.20)	.64	0.98 (0.81 to 1.19)	.91	0.93 (0.72 to 1.19)	.57	1.33 (0.91 to 1.03)	.13	1.63 (0.95 to 2.79)	.07
sCD163a	…	…	…	…	0.83 (0.54 to 1.27)	.39	0.80 (0.47 to 1.36)	.42	1.04 (0.68 to 1.58)	.84	1.18 (0.67 to 2.07)	.55	…	…	…	…
sCD14	0.59 (0.22 to 1.54)	.28	0.66 (0.24 to 1.81)	.42	1.19 (0.81 to 1.74)	.35	1.03 (0.64 to 1.64)	.89	1.21 (0.83 to 1.77)	.31	1.02 (0.62 to 1.69)	.92	0.57 (0.20 to 1.59)	.29	0.76 (0.24 to 2.37)	.64
CRP	0.42 (0.03 to 5.40)	.50	0.85 (0.48 to 1.48)	.57	0.89 (0.72 to 1.10)	.31	0.96 (0.75 to 1.22)	.75	0.87 (0.69 to 1.09)	.24	0.87 (0.65 to 1.16)	.35	0.94 (0.44 to 1.99)	.87	0.67 (0.27 to 1.67)	.39

Logistic regression adjusted for age, sex, BMI, smoking, prior TB, diabetes, CD4 count at enrollment, ART receipt at enrollment, and viral load at enrollment.

Abbreviations: AO, airflow obstruction; ART, antiretroviral therapy; OR, odds ratio; RSP, restrictive spirometry pattern.

aORs for AO and COPD had limited sample size for estimation.

differences in lung volumes and gender-related confounders. There is growing evidence examining sex differences in CLD [27, 28]. However, the burden, phenotype, and risk factors of CLD in female PWH, an understudied population in CLD research, remain unclear. Furthermore, the majority of the prior studies have focused on airflow obstruction and COPD. There is growing recognition of restrictive lung diseases and PRISm, that is, low FEV1 without overt airflow obstruction, as important contributors to the burden of CLD in high-risk populations [29–31]. Given the adverse impact of RSP and PRISm on all-cause mortality and quality of life [32–35], understanding their predictors is key to understanding long-term health outcomes in vulnerable groups like PWH. Female sex, being underweight, and reduced physical activity have been implicated as plausible determinants of RSP in adults [36–38]. The findings from our study are consistent with those from prior studies from high- and low-income settings that have reported greater odds of RSP in females, compared with males [30, 34, 36]. Studies have posited that female susceptibility to restrictive lung diseases may be associated with low circulating levels of the sex steroid, dehydroepiandrosterone (DHEA), that inhibits secretion of pro-inflammatory cytokines like IL-6, TNF-α, and IL-2, which consequently may attenuate elastic recoil of the lungs via inflammation-driven pathways [39]. Additionally, a recent meta-analysis on early life risk factors for CLDs reported that being underweight in childhood, maternal nutritional status, and intrauterine growth restriction (IUGR) are independent predictors of RSP in adult life [38]. While we did not find an association between inflammatory markers and lung function outcomes in our analysis restricted to females, we did find a significantly higher burden of low BMI, a marker of chronic malnutrition, among female participants. Furthermore, none of the female participants had ever smoked, and exposure to biomass fuels, which was higher among women, was not associated with lower lung function in our cohort. These data suggest that chronic malnutrition and greater sex-specific biological susceptibility to lung injury may play an important role in explaining the association between female sex and pulmonary impairment among PWH. Further research is needed to determine the pathophysiological mechanisms underpinning sex differences in CLD risk and presentation.

Our study found an association between higher levels of circulating IL-6 and D-dimer and impaired lung function in PWH. IL-6, a pro-inflammatory marker appearing later in the inflammatory cascade, and D-dimer, a fibrin degradation product and a marker of inflammation and hypercoagulation, have been associated with HIV-related mortality and morbidity in prior studies [24, 25, 40–43]. IL-6 exerts pro-inflammatory effects through the activation of JAK-STAT signaling and results in the downstream production of acute phase reactants like CRP [44]. Prior studies have found an association of higher concentrations of IL-6 in serum and broncho-alveolar lavage

with lower lung function in COPD, suggesting a role of IL-6 in the chronic inflammatory processes linked to disease progression [45, 46]. The etiology of chronic inflammation and altered coagulation in treated HIV is likely multifactorial and may include low-level viral replication in tissues [42]. Inflammatory responses subsequent to HIV establishment within the lungs also promote local fibrin formation and endogenous fibrinolytic activity, which can result in elevated D-dimer levels [25]. D-dimer is associated with acute exacerbations in patients with interstitial lung disease, endothelial dysfunction, and microbial translocation, suggesting an important role of the coagulation cascade in lung pathology [41, 42]. Interestingly, IL-6 has been shown to activate the coagulation cascade by increasing tissue factor production and factor VIII transcription in hepatocytes. [46, 47]. Furthermore, D-dimer and other products of fibrin degradation modulate the production of IL-6 and other inflammatory mediators like IL-1β through their interaction with monocytes [46, 47]. Our findings are consistent with prior studies in PWH from high-income settings that reported similar associations of IL-6 and D-dimer with abnormal spirometry and impaired diffusing capacity for carbon monoxide [48]. These data suggest an immunological link between pro-inflammatory cytokines like IL-6 and markers of the coagulation cascade like D-dimer in the pathophysiology of CLD in PWH.

Our study also found that high circulating IL-10 levels were associated with lower odds of pulmonary impairment, specifically PRISm, in PWH. IL-10 has been widely recognized as an anti-inflammatory cytokine that modulates the host immune response by regulating antigen presentation by dendritic cells and inhibiting macrophage activation [49]. In the context of lung pathology, IL-10 has pleomorphic and, occasionally, antagonistic effects. For instance, short-term IL-10 overexpression has been associated with decreased pulmonary inflammation following a lipopolysaccharide insult, whereas long-term IL-10 overexpression has been shown to induce lung fibrosis by increasing the recruitment and infiltration of T cells, B cells, macrophages, and collagen-producing fibrocytes [49–53]. While our study results are consistent with the hypothesis that excessive inflammation is associated with pulmonary impairment in PWH independent of immunosuppression, longitudinal studies with serial cytokine assessments are needed to better describe the role of IL-6, D-dimer, and IL-10 as biomarkers of CLD in PWH.

A conflicting finding of our study was the association of smoking with higher FVC z-scores in the full cohort. While the precise reasons for this are unclear, none of the female participants in our study had ever smoked, and smoking was not associated with poor lung function or CLD in a sensitivity analysis restricted to male participants. However, we did not have data on pack-year exposure to tobacco smoke, and inadequate adjustment of this cumulative exposure and a "healthy cohort effect" may have contributed to this finding. We did not perform objective assessments of indoor air pollution, and the self-reported biomass fuel exposure in our study may be biased. An important limitation of our study was the lack of spirometry at enrollment, preventing the assessment of changes in lung function during follow-up and its temporal association with baseline clinical and immunological markers. Similarly, we did not measure cytokine levels and lung function during all follow-up visits and are therefore unable to assess the prognostic role of IL-6, D-dimer, and IL-10. Some of the associations of inflammatory markers with pulmonary impairment reported in our study retain a small probability of type 1 error and should be validated in independent cohorts. Our study did not enroll an HIV-uninfected control group, and we are unable to compare the burden of pulmonary impairment between HIV-positive and HIV-uninfected women. Finally, our study was conducted in a single study site and may not be generalizable to PWH elsewhere.

Nevertheless, our study reports an important association between circulating IL-6, D-dimer, and IL-10 and pulmonary impairment in a relatively large cohort of PWH with HIV receiving routine clinical care under programmatic settings in India. These data suggest a potential role of IL-6, D-dimer, and IL-10 as biomarkers for CLD in PWH. Furthermore, we also report significantly lower lung function and a higher likelihood of RSP and PRISm among HIV-positive females after adjusting for sex-related physiological differences in lung volumes and gender-related confounders. Our study results add to the growing recognition of biological sex differences as a key determinant of CLD risk and presentation.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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