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Markers of inflammation and immune activation are associated with lung function in a multicenter cohort of persons with HIV

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Abstract

Objectives: Studies have shown that persons with HIV (PWH) may be at increased risk for chronic lung diseases and lung function abnormalities, which may be associated with immune activation. We tested the association of a panel of twelve immune activation and inflammation biomarkers with spirometry and single-breath diffusing capacity for carbon monoxide (DLco).

Design: Cross-sectional, observational study.

Methods: Participants were enrolled from the I AM OLD cohort of PWH at two US sites. Biomarkers were examined and standardized spirometry and DLco testing were performed. We tested associations between each biomarker and lung function, examined individually and in combination, using multivariable linear and logistic regression.

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Author contributions: Conceived the study: LH, PWH, KC, and MML; designed the study: LH, MM, MHF, and PWH; acquired, analyzed, or interpreted the data: AKJ, JVM, LH, RJW, MM, CF, DM, MHF, RK, DJ, and EL; performed the primary statistical analyses: JVM; drafted the article: AKJ; critically revised the article for intellectual content, approved the final article, and take responsibility for the accuracy and integrity of the data: all authors.

Conflicts of interest

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All other authors have nothing to disclose.

Results: Among 199 participants, median FEV1 was normal (90% predicted) and median DLco was abnormal (69% predicted). The most common lung function abnormality (57%) was a normal FEV1 to forced vital capacity ratio with an abnormal DLco≤80% predicted (iso↓DLco). Two markers (IL-6, hsCRP) were associated with FEV1% predicted, whereas eight markers (sCD14, sCD163, IP10, sCD27, IL-6, sTNFR-I, sTNFR-II, D-dimer) were associated with DLco% predicted. Compared to those participants with normal spirometry and DLco, five markers (sCD14, sCD163, IP10, sTNFR-I, sTNFR-II) were associated with iso↓DLco.

Conclusions: Among PWH, different markers of immune activation and inflammation are associated with FEV1% predicted than with DLco% predicted and with an iso↓DLco, representing possible unique pathways of chronic lung disease. Identifying plausible drivers of these inflammatory pathways may clarify mechanisms underlying impaired lung function in HIV infection and may identify therapeutic avenues.

Keywords

HIV; pulmonary disease; pulmonary gas exchange; respiratory function tests; biomarkers; inflammation; chronic obstructive pulmonary disease

Introduction

In the modern era of antiretroviral therapy (ART) and early treatment of HIV, people with HIV (PWH) are living longer and developing chronic medical conditions as they age.[1] These illnesses contribute significantly to morbidity and mortality. Compared to those without HIV, PWH may develop these illnesses more commonly, earlier, or at a more severe disease stage.[1] Many of these conditions may result from persistent immune activation, even in the setting of viral suppression with ART.[2] Indeed, immune activation remains abnormally high in many individuals despite ART and higher levels strongly predict increased morbidity and mortality. Several markers of immune activation are also tied to cardiovascular events and end-organ disease in PWH.[3-19]

In addition to other morbidities, PWH also have a higher frequency of chronic obstructive pulmonary disease (COPD) and lung function abnormalities than the general population, even among never-smokers.[20,21] Cohorts of PWH have COPD frequencies as high as 16%-27%[22,23] compared to as low as 9%[24] in similar HIV-negative cohorts. Moreover, HIV infection is an independent risk factor for COPD.[22,24] Studies that performed spirometry (forced expiratory volume in 1 second/forced vital capacity, FEV1/FVC) and diffusion capacity for carbon monoxide (DLco) measurements, the main tests used to diagnose COPD, have found that abnormalities in both are frequent among PWH. Further, PWH more frequently have DLco defects compared to spirometry abnormalities.[25] One recent multicenter study found that PWH had worse DLco and a higher risk of moderate or greater DLco impairment (DLco<60% predicted) than those without HIV.[26]

A large proportion of PWH have impaired DLco. Cohorts of PWH have a moderate or greater reduction in DLco as high as 30%.[27] While diffusion abnormalities are non-specific and seen in many lung diseases, including COPD, many PWH have impaired DLco without spirometry abnormalities (referred to as iso↓DLco hereafter). Notably, an iso↓DLco

is the most frequent lung function abnormality seen in PWH[27,28] whereas, in individuals without HIV, it is an infrequent finding (<1.0%).[29] These studies demonstrating that an iso↓DLco is more common among PWH raise the question of an HIV-specific role in pathogenesis. Whether HIV infection causes impaired diffusion and, if so, by what mechanisms, is unknown.

Immune activation is one potential mechanism, as markers of inflammation and immune activation have been associated with chronic lung disease and lung function abnormalities in PWH. Elevated levels of monocyte activation markers, sCD14 and sCD163, are associated with an increased risk of radiographic emphysema[30] and chronic lung disease,[31] respectively. Increased IL-6 and CRP, non-specific markers of inflammation, are associated with worse FEV1 and DLco in PWH.[32] Similarly, IL-6, sCD163, and TNF-α are associated with diffusing impairment.[33] In a cross-sectional analysis of PWH in 20 countries, IL-6 and D-dimer were found to be associated with worse airflow obstruction (FEV1/FVC).[34] Nevertheless, more work is needed to understand the relationship between immune activation and spirometry and DLco in concert. Thus, we enrolled a multicenter cohort of PWH and evaluated the relationship between plasma immune activation markers and spirometry and DLco, including an iso↓DLco.

Methods

Study Cohort

Inflammation, Aging, Microbes and Obstructive Lung Disease (I AM OLD): I AM OLD is a longitudinal cohort of PWH in San Francisco, CA and Seattle, WA assessed for change in lung function over time. It is comprised of adult PWH (aged ≥18 years) at San Francisco General Hospital or Seattle's Harborview Medical Center. We recruit participants who seek routine outpatient HIV care or are diagnosed with pneumonia at either hospital. One of the study aims relates to pneumonia, a known risk for COPD in PWH,[35] and this method of recruitment enriches the cohort for patients with or at risk for COPD and/or lung function abnormalities. Participants provide written informed consent and the study protocol is approved by the Institutional Review Boards of University of California, San Francisco and University of Washington.

The current study is a cross-sectional analysis of participants at their baseline visit when paired blood samples and pulmonary function tests (PFTs) were obtained. Participants underwent PFTs only if they denied symptoms of acute respiratory illness. For participants recruited after pneumonia diagnosis, the baseline visit occurred at least 3 months after completion of pneumonia treatment and only if participants were at their baseline respiratory status with no new or worsening respiratory symptoms.

Data Collection

Clinical and Laboratory Data: Study personnel obtained information on demographic characteristics, smoking and illicit drug history, ART use, and prior pulmonary illnesses using a standardized questionnaire conducted on the same day as blood draw and PFTs. Blood was sent for CD4 and CD8 cell counts and HIV viral load.

Biomarkers of Immune Activation and Inflammation: Participants provided plasma for enzyme-linked immunosorbent assays (ELISAs) of 12 biomarkers of immune activation and inflammation performed at Case Western Reserve University. We selected these markers based on previously-shown associations with other HIV-associated morbidities and mortality and to represent distinct mechanistic pathways.[3-19] Specifically, we chose: (1) intestinal fatty acid binding protein (*I-FABP*), a marker of gut barrier dysfunction; (2) soluble CD14 (*sCD14*) and soluble CD163 (*sCD163*), markers of monocyte activation; (3) interferon gamma-inducible protein-10 *(IP10*), a marker of Type I and II interferon response; (4) *sCD27* (ThermoFisher), a marker of lymphocyte activation; (5) interleukin-6 (*IL-6*), high-sensitivity C-reactive protein (*hsCRP*), *fibrinogen* (AbCam), and soluble tumor necrosis factor receptors 1 and 2 (*sTNFR-I* and *sTNFR-II*), all markers of inflammation; (6) *D-dimer* (Diagnostic Stago), a marker of fibrin breakdown; and (7) *hyaluronic acid*, a marker of fibrosis. ELISAs were from R&D Systems unless otherwise noted.

Pulmonary Function Tests: PFTs consisted of pre- and post-bronchodilator (BD) spirometry and DLco measurement. Spirometry was performed before and after administration of Albuterol 360μg from a metered-dose inhaler. Diffusing capacity was corrected for hemoglobin and carboxyhemoglobin obtained on the day of PFTs. PFTs were performed in accordance with American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines.[36-38] A single trained reader over-read the PFTs, and spirometry and DLco results were included only if they met ATS/ERS criteria for acceptability and reproducibility. Percentage predicted values were calculated using standard NHANES III values and account for age, gender, race, and height.[38,39]

PFT Outcomes: We defined an abnormal FEV1/FVC using a post-BD FEV1/FVC<LLN, the lower limit of normal,[38] and a post-BD FEV1/FVC<0.70.[40] We defined an abnormal DLco as a DLco<LLN and a DLco≤80% predicted. As prior studies in PWH have done, we also examined a moderately-to-severely abnormal DLco, defined as ≤60% predicted.[27,28] We also examined these lung function measurements in combination. We defined an emphysema-like lung function phenotype as an abnormal FEV1/FVC and an abnormal DLco% predicted. We defined an iso↓DLco phenotype as an abnormal DLco% predicted, but with a normal FEV1/FVC. Although we identified participants with spirometric evidence of restriction, we did not measure total lung capacity (TLC), the gold standard lung function test for restriction, and thus we did not examine the potential association of our markers with this abnormality.

Statistical Analysis

Demographic, clinical, and laboratory (e.g., CD4 cell count) characteristics, as well as biomarker levels and PFT outcomes, were summarized using medians with interquartile ranges (IQRs) for continuous variables and percentages with 95% confidence intervals (CIs) for categorical variables. Continuous variables (e.g., HIV viral load) were also categorized using clinically-relevant cutoffs, and sensitivity analyses restricted to those with a viral load<40 copies/mL were done post hoc.

Regression diagnostics suggested that for several biomarkers, model residuals were not normally distributed and the variance for each biomarker was large. To satisfy modelling assumptions, biomarker levels were $\log_{10}$-transformed and then divided by the IQR of the $\log_{10}$-transformed biomarker. Dividing by the IQR standardized each biomarker to its own range of test values and allowed for comparison of the relative strength of association of each biomarker to the others. Strength of associations between clinical characteristics and $\log_{10}$-transformed biomarkers and our continuous outcomes of interest (post-BD FEV1% predicted, post-BD FVC% predicted, and DLco% predicted) were assessed using simple linear regression with 95% CIs. Similarly, logistic regression was performed to evaluate associations between clinical factors and $\log_{10}$-transformed biomarkers on our categorical outcomes of interest (post-BD FEV1/FVC defined as <0.70 or ≥0.70 and <LLN or ≥LLN, and DLco% predicted at ≤0.80 or >0.80, <LLN or ≥LLN, and ≤0.60 or >0.60, as done in prior studies). For analysis of iso↓DLco and the emphysema-like phenotype, comparison was made to those with normal spirometry and DLco.

Factors known to be related to lung disease, such as cigarette smoking, and those that were also significant at p-value<0.2 in bivariate analysis were included in multivariate analysis. To evaluate factors associated with low post-BD FEV1% predicted, post-BD FVC% predicted, and DLco% predicted, multiple linear regression models were fit for each outcome of interest. Each outcome of interest was a continuous outcome in which each $\log_{10}$-transformed biomarker was the main predictor of interest; co-variates included smoking history (pack years), recreational drug use, T-cell count, and history of lung infection. Additional multivariable linear regression analyses were carried out to evaluate all factors associated with DLco% predicted after stratification of all participants with a post-BD FEV1/FVC <0.70 or ≥0.70. Co-variates included smoking history, recreational drug use, T-cell count, and history of lung infection. The same covariates were used in all multivariate analyses for consistency, but best-fit covariates were also used. Any differences in analyses with the consistent covariates vs. the best-fit covariates are explicitly listed in the Results. Since we selected our biomarkers based on *a priori* mechanistic hypotheses, we did not adjust for multiple comparisons.[41] Data were analyzed with R Studio, 1.1.453.

Results

Cohort

The study enrolled 224 participants between July 2013 and July 2018 (Fig. 1). Of these, seven patients enrolled at the time of acute pneumonia died and 14 were lost to follow-up. Three participants were unable to provide blood, four were unable to complete spirometry, 19 had post-BD spirometry that failed ATS/ERS criteria, and one had DLco that failed criteria. Thus, 177 participants were included in the spirometry analyses and 199 were included in the diffusing capacity analyses.

Participants

Participants' median age was 53 years, 84% were male, and 39% identified as white (Supplemental Table 1). Race, age, and gender were representative of PWH in San Francisco and Seattle and the US.[42, 43] Overall, 37% of participants were current tobacco smokers

and 66% were ever-smokers. In addition, 87% had smoked marijuana, 47% had smoked crack cocaine, and 36% had a history of injecting drugs in their lifetime. Participants who were enrolled at the time of acute pneumonia were comparable in age, race, and smoker status to participants not enrolled at acute pneumonia.

The majority (84%) had taken ART in the past week by self-report. The median CD4 and CD8 cell counts were 474 cells/μL and 782 cells/μL, respectively; the median CD4/CD8 ratio was 0.65. Overall, 81% of participants had an HIV viral load<40 copies/mL. Given the study's focus on pneumonia, 63% of participants had bacterial pneumonia, 27% had *Pneumocystis* pneumonia (PCP), and 7% had tuberculosis (TB) in their lifetime.

Spirometry and Association of Inflammatory Biomarkers with Spirometry

The spirometry outcomes are shown in Supplemental Table 1. A substantial proportion of participants met spirometric criteria for COPD: 22% of participants had a post-BD FEV1/FVC<0.70 and 25% had a post-BD FEV1/FVC<LLN.

Two biomarkers of inflammation, IL-6 and hsCRP, were significantly associated with spirometry results in multivariate analyses. For each IQR increase in log-transformed IL-6 and hsCRP levels, the post-BD FEV1% predicted declined by approximately 9% and 10% predicted (p=0.007 and p=0.004), respectively (Table 1). Similarly, for each interquartile increase in log-transformed hsCRP, the post-BD FVC% predicted decreased by approximately 10% predicted (Supplemental Table 2, p=0.002). These associations remained significant in sensitivity analyses restricted to those 158 (81%) participants with an HIV viral load<40 copies/mL to exclude confounding caused by active viral replication (data not shown). IL-6 was the only biomarker significantly associated with post-BD FEV1/FVC<0.70 and FEV1/FVC<LLN (both p=0.03) in multivariate analyses (Supplemental Table 3).

DLco and Association of Inflammatory and Immune Activation Biomarkers with DLco

The DLco outcomes are shown in Supplemental Table 1. A majority of participants had abnormal diffusion.

A greater number of biomarkers were associated with DLco results compared to spirometry results. Higher levels of eight different markers, sCD14 (p=0.03), sCD163 (p=0.0003), IP10 (p<0.0001), sCD27 (p=0.03), IL-6 (p=0.04), sTNFR-I (p=0.01), sTNFR-II (p<0.0001), and D-dimer (p=0.0009), were significantly associated with lower DLco% predicted in multivariate analyses (Table 1). The three strongest effects were seen with IP10, sTNFR-II, and D-dimer; for each IQR increase in these log-transformed biomarkers, DLco% predicted decreased by approximately 12%, 10%, and 9% predicted, respectively. These results remained largely the same when sensitivity analyses restricted to those participants with an HIV viral load<40 copies/mL were performed (not shown) and also when DLco% predicted was dichotomized to <LLN, ≤80% predicted (Supplemental Tables 4a and 4B), and ≤60% predicted (not shown).

Biomarkers associated with DLco% predicted differed when the cohort was stratified into two groups according to whether spirometry was abnormal or normal. Among participants

with post-BD FEV1/FVC≥0.70, higher levels of sCD14 (p=0.002), sCD163 (p=0.0006), IP10 (p=0.0006), sCD27 (p=0.04), sTNFR-I (p=0.048), and sTNFR-II (p<0.0001) were associated with lower DLco% predicted (Table 2). The strongest effects were seen with IP10, and sTNF-RII; for each IQR increase in these log-transformed biomarkers, DLco% predicted decreased by approximately 13% predicted. Among participants with an FEV1/FVC<0.70, fewer and slightly different biomarkers were associated with DLco% predicted. Here, only higher levels of IFABP (p=0.02), IP10 (p=0.005), and sTNFR-II (p=0.04) levels were associated with lower DLco% predicted (Table 2).

iso↓DLco and Association of Inflammatory and Immune Activation Biomarkers with iso↓DLco

In our cohort, DLco abnormalities without spirometric abnormalities (an iso↓DLco lung function phenotype) were more frequent than DLco abnormalities in the presence of spirometric abnormalities (an emphysema-like phenotype) (Table 3). Using both post-BD FEV1/FVC and DLco% predicted to characterize lung function phenotypes, a high proportion, 18-20%, had an emphysema-like phenotype. However, an even higher proportion, 34-56%, had an iso↓DLco phenotype. Using a more restrictive DLco≤60% predicted cut-off, nearly 16% of our cohort had an iso↓DLco lung function phenotype of at least moderate severity (not shown). Thus, an iso↓DLco phenotype was the most frequent lung function abnormality seen in our cohort.

Compared to PWH who had normal spirometry and diffusing capacity, higher levels of sCD14 (p=0.02), sCD163 (p=0.008), IP10 (p=0.01), sTNFR-1 (p=0.02), and sTNFR-II (p=0.0001) are associated with an iso↓DLco phenotype in multivariate analyses (Table 4). The strongest effects were seen with sTNFR-II and IP10; for each IQR increase in these log-transformed biomarkers, the adjusted odds of having an iso↓DLco phenotype was greater than eight-fold and greater than five-fold higher, respectively. On the other hand, compared to PWH with normal PFTs, IP10 (p=0.02), IL-6 (p=0.007), fibrinogen (p=0.02), and sTNFR-II (p=0.02) are associated with an emphysema-like phenotype in multivariate analyses (Supplemental Table 5).

Discussion

Our study analyzed 12 plasma biomarkers in association with both spirometry and DLco outcomes, examined singly and in combination with each other, in a cohort of PWH. Our study has four main findings: (1) non-specific inflammatory markers (IL-6 and hsCRP) were associated with spirometry; (2) sCD14, sCD163, IP10, sCD27, IL-6, sTNF-RI, sTNF-RII, and D-dimer were associated with diffusion; (3) more and different biomarkers were associated with diffusion when spirometry was normal than when spirometry was abnormal; and (4) markers of monocyte/macrophage activation (sCD14, sCD163) and Type I and II interferon response (IP10), in addition to non-specific inflammatory markers (sTNF-RI and sTNF-RII) were associated with an iso↓DLco lung function phenotype.

The frequency of obstruction (post-BD FEV1/FVC<LLN) in our cohort was 25%. This is in the higher range reported by prior studies of PWH (16%-27%),[22,23] perhaps because our cohort includes participants with prior pneumonia, a known risk factor for COPD.[35]

Acute-phase inflammatory markers IL-6 and hsCRP were significantly associated with FEV1% predicted, a measure of airflow obstruction severity. This agrees with prior studies that found IL-6 was associated with obstruction in PWH.[32-34] Another study found inflammatory mediators Th1, Th2, and Th17 to be associated with airway obstruction,[44] supporting the association between inflammation and spirometric defects in our cohort. Our findings are further reinforced as IL-6 is a primary inducer of CRP production in the liver and thus these markers are mechanistically linked. However, since COPD may be a cause of systemic inflammation, our study does not elucidate whether IL-6 and hsCRP are a cause or consequence of lung function abnormalities, confounded by other immunologic pathways, or signs of the mechanisms underlying them; further investigation is needed.

The frequency of diffusion abnormalities, including moderate or greater reductions, was also high in our cohort and in the higher range reported by prior studies of PWH (13.7%-64.1%).[25-27] An iso↓DLco was the most frequent lung function abnormality in our cohort and prior studies of PWH (12%-43%).[25,27] We found sCD14, sCD163, IP10, sCD27, IL-6, sTNF-RI, sTNF-RII, and D-dimer were significantly associated with DLco% predicted. A prior study also found sCD163 to be associated with diffusing impairment, but also found hsCRP to be associated, which we did not find.[33] We also found that most of these markers were associated with DLco% predicted among participants with normal spirometry and fewer were associated with DLco% predicted among those with abnormal spirometry. Compared to participants with normal spirometry and diffusion, sCD14, sCD163, IP10, sTNFR-I, and sTNFR-II were significantly associated with an iso↓DLco phenotype. In prior studies, sCD163 was independently associated with chronic lung disease[31] and diffusion impairment.[33]

The disparate biomarkers associated with DLco imply that multiple, different mechanisms may underlie diffusion impairments in PHW and this may contribute to the high frequency of this abnormality. Intriguingly, there is substantial overlap between the biomarkers associated with DLco—especially iso↓DLco—in our study and those that declined with treatment of asymptomatic CMV replication in a recent trial of valganciclovir in PWH.[45] For example, sCD14, sTNFR-I, and sTNFR-II are associated with an iso↓DLco, and they are also the most strongly affected biomarkers in the valganciclovir trial.[45] CMV infects myeloid cells in the lung and vasculature, so CMV may be an important co-factor contributing to these relationships and merits further study. We do not have the CMV data to pursue this line of investigation at present, but we intend to collect CMV serostatus data in future projects to explore the possible relation of CMV to pulmonary dysfunction in PWH.

A main strength of this analysis is the measurement of spirometry and DLco on the same day that plasma markers were drawn and the use of both of these lung function tests to classify participants into four lung function phenotypes. Our observation of distinct plasma biomarker profiles related to DLco in the context of spirometry is thus unique. Our findings linking several biomarkers to an iso↓DLco phenotype indicate that specific and non-specific mechanisms may be involved in lung pathology that specifically affects the alveolar-capillary bed and therefore diffusing capacity. Future studies should explore the relationship between these biomarkers and lung disease in order to identify mechanisms underlying an iso↓DLco and other abnormalities in PWH.

Our study also has several limitations. First, we performed a cross-sectional analysis, which limits our ability to distinguish causality. However, I AM OLD continues to conduct longitudinal PFTs paired with same-day plasma biomarker levels. These longitudinal data will better indicate what mechanisms underlie long-term, progressive diseases, and can potentially serve as a platform for future interventional trials. Next, we selected 3 months after pneumonia as the earliest time-point for PFT measurement based on a study of serial PFTs that found that patients with PCP had an abnormal DLco at the time of PCP, and then DLco partially improved over the ensuing 3 months and stabilized thereafter.[46] Whether this is the best time-point for "baseline" PFTs after PCP or non-PCP pneumonia is unclear. Another limitation is that we did not control for some variables that may contribute to biomarker levels or for multiple comparisons. These variables include comorbidities like pulmonary arterial hypertension, which is increased in PWH, or a history of pulmonary emboli that might be the cause of a low DLco. Though the rate of such comorbidities in our cohort is relatively low, these potential confounding variables should be explored in the future. Finally, in the absence of comprehensive testing, including chest CT scans, to further characterize our lung function findings, it is likely that our iso↓DLco group contains some individuals with emphysema, pulmonary fibrosis, etc. in addition to a potential novel HIV-specific iso↓DLco pathology. However, this misclassification would tend to bias our findings against detecting significant associations and further characterization might actually lead to stronger associations than those observed.

Our study investigated biomarkers associated with abnormal spirometry, diffusion, and an iso↓DLco. It will be important to investigate pathways related to these markers—especially those related to CMV—because of the frequency of an iso↓DLco defect in PWH. Moreover, spirometry and diffusing capacity impairments are progressive and associated with decreased health-associated quality of life, increased healthcare costs, and increased mortality.[47-49] Logically, an iso↓DLco may have similar effects on quality of life that should be explored further. Understanding these pathways and whether there are distinct and different underlying mechanisms is crucial in treating and potentially preventing lung disease in PWH, especially as they live longer. Future studies should attempt to elucidate the relationship between HIV, immune pathways, and lung disease, especially an iso↓DLco.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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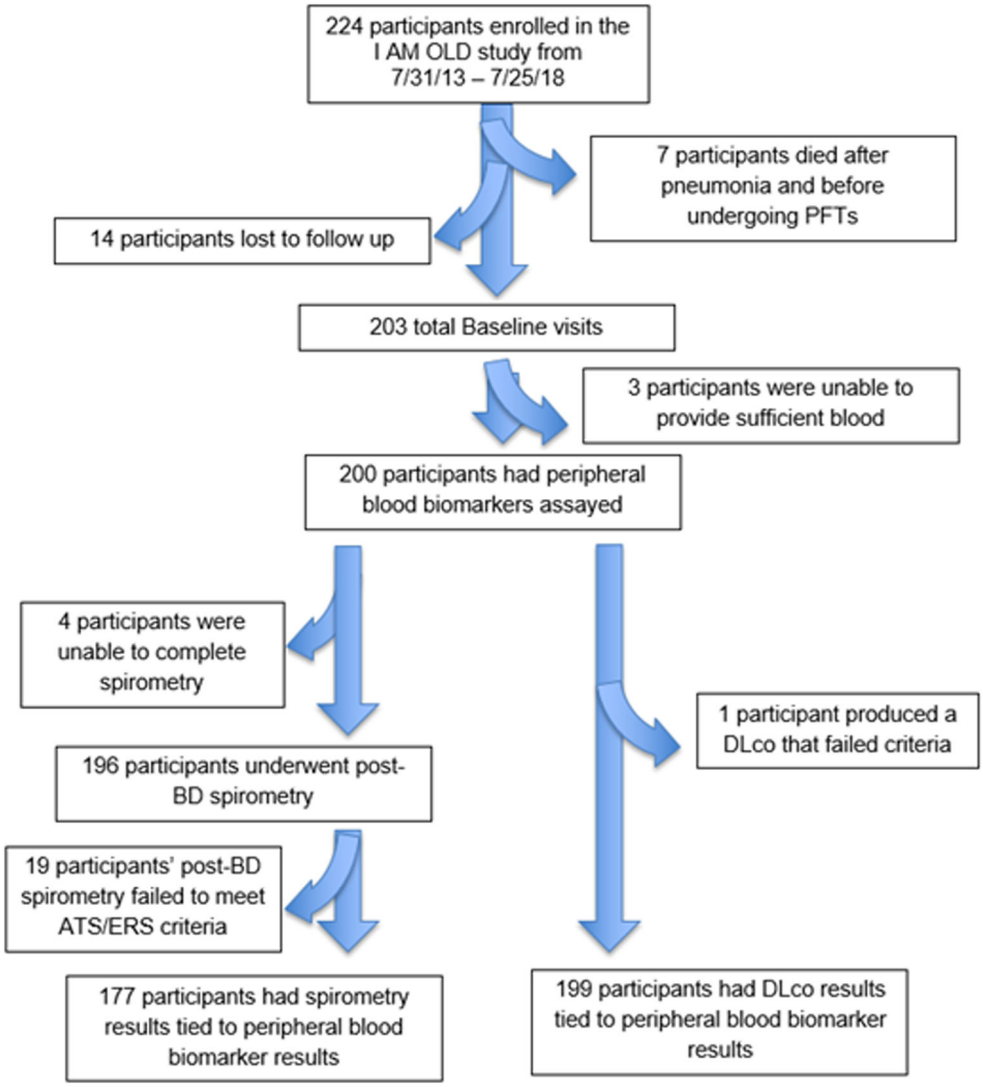

Figure 1.

Participants who were enrolled in the cohort but did not undergo a Baseline visit were unable to undergo PFTs, lost to follow up, or deceased.

Table 1.

Associations between Plasma Biomarkers and Post-Bronchodilator FEV1% Predicted (N=177) and DLco% predicted (N=199)

			Post-BD FEV1% Predicted		DLco% predicted	
			Adjusted for pack years, CD4/CD8[S], bacterial pneumonia ever, PCP ever		Adjusted for pack years, smoke crack ever, CD4/CD8[S], bacterial pneumonia ever	
		Median [P25-P75]	Adjusted Estimate [95% CI] (% predicted)	p-value	Adjusted Estimate [95% CI] (% predicted)	p-value
Gut barrier integrity	**IFABP**[T,1]	1.59 [0.91, 2.46]	0.06 [−3.95, 4.06]	0.98	−1.96 [−4.92, 1.00]	0.19
Monocyte/ Macrophage activation	**sCD14**[T,2]	1,924.17 [1609.15, 2500.60]	0.31 [−4.11, 4.73]	0.89	−3.53 [−6.86, −0.20]	0.03
	sCD163[T,3]	806.50 [526.51, 1339.02]	−1.66 [−5.92, 2.60]	0.44	−5.74 [−8.83, −2.65]	0.0003
Interferon response	**IP10**[T,4]	173.92 [108.64, 291.52]	−1.56 [−9.29, 6.18]	0.69	−12.48 [−17.61, −7.35]	<0.0001
Lymphocyte activation	**sCD27**[T,5]	69.12 [46.91, 110.54]	−0.54 [−4.82, 3.74]	0.80	−3.61 [−6.77, −0.46]	0.03
Non-specific inflammation	**IL-6**[T,6]	1.70 [0.91, 3.25]	−8.91 [−15.37, −2.44]	0.007	−5.20 [−10.17, −0.24]	0.04
	hsCRP[T,7]	2584.68 [961.20, 6420.60]	−9.65 [−16.19, −3.10]	0.004	−1.68 [−6.84, 3.48]	0.52
	Fibrinogen[T]	4.63 [3.52, 6.82]	1.43 [−4.53, 7.39]	0.64	−1.50 [−5.66, 2.66]	0.48
	sTNFR-I[T,8]	1314.91 [986.31, 1699.12]	−2.26 [−7.09, 2.58]	0.36	−4.68 [−8.26, −1.11]	0.01
	sTNFR-II[T,9]	3243.77 [2508.85, 4837.85]	−2.64 [−7.80, 2.53]	0.31	−9.94 [−13.43, −6.45]	<0.0001
Fibrin breakdown	**D-dimer**[T]	202.41 [132.55, 363.68]	−5.92 [−12.97, 1.14]	0.10	−8.89 [−14.09, −3.68]	0.0009
Marker of fibrosis	**Hyaluronic Acid**[T]	52.77 [34.47, 81.51]	2.47 [−2.39, 7.33]	0.32	−0.80 [−3.91, 2.31]	0.61

Post-BD FEV1 = Post-bronchodilator forced expiratory volume in 1 second

DLco = Diffusion capacity for carbon monoxide adjusted for hemoglobin and carboxyhemoglobin measured on day of PFTs

[S] Square Root Transformed for Regression Analysis

[T] Log Transformed expressed per interquartile range increase

[1] intestinal fatty acid binding protein

[2] soluble CD14

[3] soluble CD163

[4] interferon gamma-inducible protein-10

[5] soluble CD27

[6] interleukin-6

[7] high-sensitivity C-reactive protein

[8] soluble tumor necrosis factor receptor 1

[9] soluble tumor necrosis factor receptor 2

Table 2.

Associations between Plasma Biomarkers and DLco% predicted According to Post-Bronchodilator FEV1/FVC

		DLco% predicted			
		Post-BD FEV1/FVC<0.70		Post-BD FEV1/FVC>0.70	
Adjusted for pack years, smoke crack ever, CD4/CD8[S], bacterial pneumonia ever					
		Adjusted Estimate[1,2] [95% CI] (% predicted)	p-value	Adjusted Estimate[1,2] [95% CI] (% predicted)	p-value
Gut barrier integrity	**I-FABP**[T,1]	−8.48 [−15.38, −1.59]	0.02	−1.80 [−5.14, 1.54]	0.29
Monocyte/Macrophage activation	**sCD14**[T,2]	−0.97 [−7.19, 5.25]	0.75	−6.02 [−9.88, −2.17]	0.002
	sCD163[T,3]	−6.33 [−13.91, 1.25]	0.09	−6.11 [−9.54, −2.68]	0.0006
Interferon response	**IP10**[T,4]	−16.66 [−27.85, −5.48]	0.005	−13.47 [−19.56, −7.38]	0.0006
Lymphocyte activation	**sCD27**[T,5]	−4.57 [−9.62, 0.49]	0.07	−4.19 [−8.23, −0.16]	0.04
Non-specific inflammation	**IL-6**[T,6]	−3.33 [−14.90, 8.24]	0.56	−3.75 [−9.69, 2.20]	0.21
	hsCRP[T,7]	4.29 [−7.06, 15.64]	0.45	−3.43 [−9.26, 2.40]	0.25
	Fibrinogen[T]	0.80 [−10.37, 11.96]	0.89	−2.74 [−7.67, 2.19]	0.27
	sTNFR-1[T,8]	−2.70 [−8.84, 3.44]	0.38	−4.58 [−9.11, −0.05]	0.048
	sTNFR-II[T,9]	−6.24 [−12.28, −0.21]	0.04	−12.95 [−17.37, −8.52]	<0.0001
Fibrin breakdown	**D-dimer**[T]	−8.48 [−19.04, 2.09]	0.11	−6.12 [−12.36, 0.11]	0.05
Marker of fibrosis	**Hyaluronic Acid**[T]	−0.19 [−8.81, 8.43]	0.96	−3.81 [−7.68, 0.05]	0.05

Post-BD FEV1/FVC = Post-bronchodilator forced expiratory volume in one second/forced vital capacity

DLco = Diffusion capacity for carbon monoxide adjusted for hemoglobin and carboxyhemoglobin measured on day of PFTs

[S] Square Root Transformed for Regression Analysis

[T] Log Transformed expressed per interquartile range increase

[1] intestinal fatty acid binding protein

[2] soluble CD14

[3] soluble CD163

[4] interferon gamma-inducible protein-10

[5] soluble CD27

[6] interleukin-6

[7] high-sensitivity C-reactive protein

[8] soluble tumor necrosis factor receptor 1

[9] soluble tumor necrosis factor receptor 2

Table 3.

Lung Function Phenotypes According to Post-Bronchodilator FEV1/FVC and DLco% Predicted

% participants (n)	Post-BD FEV1/FVC<0.70 (n=39)	Post-BD FEV1/FVC≥0.70 (n=137)
DLco≤80% predicted (n=133)	19.9% (35)	55.7% (98)
DLco>80% predicted (n=43)	2.3% (4)	22.2% (39)

% participants (n)	Post-BD FEV1/FVC<LLN (n=44)	Post-BD FEV1/FVC≥LLN (n=132)
DLco<LLN (n=91)	17.6% (31)	34.1% (60)
DLco≥LLN (n=85)	7.4% (13)	40.9% (72)

Post-BD FEV1/FVC = Post-bronchodilator forced expiratory volume in one second/forced vital capacity

DLco = Diffusion capacity for carbon monoxide adjusted for hemoglobin and carboxyhemoglobin measured on day of PFTs

LLN = Lower Limit of Normal

Table 4.

Associations between Plasma Biomarkers and iso↓DLco (DLco% predicted≤80% with Post-Bronchodilator FEV1/FVC≥0.7)[R]

Adjusted for pack years, CD4 count [S], PCP ever			
		Adjusted OR [95% CI] (% predicted)	**p-value**
Gut barrier integrity	**I-FABP**[T,1]	1.15 [0.68, 1.97]	0.59
Monocyte/Macrophage activation	**sCD14**[T,2]	2.36 [1.17, 5.22]	0.02
	sCD163[T,3]	2.26 [1.26, 4.27]	0.008
Interferon response	**IP10**[T,4]	5.43 [1.62, 21.60]	0.01
Lymphocyte activation	**sCD27**[T,5]	1.74 [0.92, 3.50]	0.10
Non-specific inflammation	**IL-6**[T,6]	1.67 [0.66, 4.35]	0.29
	hsCRP[T,7]	1.74 [0.69, 4.51]	0.24
	Fibrinogen[T]	1.05 [0.48, 2.30]	0.90
	sTNFR-1[T,8]	2.76 [1.19, 7.05]	0.02
	sTNFR-II[T,9]	8.37 [3.04, 26.94]	0.0001
Fibrin breakdown	**D-dimer**[T]	1.84 [0.69, 5.20]	0.23
Marker of fibrosis	**Hyaluronic Acid** [T]	1.48 [0.76, 3.00]	0.26

DLco = Diffusion capacity for carbon monoxide adjusted for hemoglobin and carboxyhemoglobin measured on day of PFTs

FEV1/FVC = Forced expiratory volume in one second/forced vital capacity

[R] Reference = Participants with normal spirometry and normal DLco

[T] Log Transformed and divided by IQR for Regression Analysis

[S] Square Root Transformed for Regression Analysis

[1] intestinal fatty acid binding protein

[2] soluble CD14

[3] soluble CD163

[4] interferon gamma-inducible protein-10

[5] soluble CD27

[6] interleukin-6

[7] high-sensitivity C-reactive protein

[8] soluble tumor necrosis factor receptor 1

[9] soluble tumor necrosis factor receptor 2