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Contributors to diffusion impairment in HIV-infected persons

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Summary: Diffusing capacity impairment in HIV infection is multifactorial, and causes may vary between smokers and never smokers.

Word Count: 2878

Abstract

Abnormal diffusing capacity is common in HIV-infected individuals including never smokers. Etiologies for diffusing capacity impairment in HIV are not understood, particularly in those without a history of cigarette smoking.

A cross-sectional analysis of 158 HIV-infected individuals without acute respiratory symptoms or infection to determine associations between a DL_{CO} % predicted and participant demographics, pulmonary spirometric measures (FEV_1 and FEV_1/FVC), radiographic emphysema (fraction of lung voxels <-950 Hounsfield units), pulmonary vascular/cardiovascular disease (echocardiographic tricuspid regurgitant jet velocity [TRV], N-terminal pro-brain natriuretic peptide), and airway inflammation (induced sputum cell counts), stratified by history of smoking.

Mean DL_{CO} was 65.9% predicted, and 55 (34.8%) participants had a significantly reduced DL_{CO} (<60 % predicted). Lower DL_{CO} % predicted in ever smokers was associated with lower post-bronchodilator FEV_1 % predicted ($p<0.001$) and greater radiographic emphysema ($p=0.001$). In never smokers, mean (standard deviation) DL_{CO} was 72.7% (13.4%) predicted, and DL_{CO} correlated with post-bronchodilator FEV_1 ($p=0.02$), sputum neutrophils ($p=0.03$), and sputum lymphocytes ($p=0.009$), but not radiographic emphysema.

Airway obstruction, emphysema, and inflammation influence DL_{CO} in HIV. Never smokers may have a unique phenotype of diffusing capacity impairment. The interaction of multiple factors may account for the pervasive nature of diffusing capacity impairment in HIV infection.

Word Count: 199

Introduction

Lung disease is an important cause of morbidity and mortality in the HIV-infected population even in the current era of combination antiretroviral therapy (ART). A greater incidence of several non-infectious lung diseases has been found in HIV-infected persons compared to HIV-uninfected persons,[1] and death from obstructive lung disease has increased in the HIV-infected population since the introduction of ART.[2] Abnormal diffusing capacity and an accelerated form of emphysema were associated with HIV infection prior to ART.[3-6] A recent study showed that abnormal diffusing capacity remains extremely common with over 64% of HIV-infected persons having an impaired diffusing capacity for carbon monoxide (DL_{CO}) (<80% predicted). Diffusing capacity impairment is not limited to smokers with HIV as over 47% of never-smokers have been reported to have a DL_{CO} below 80% percent predicted.[7] Additionally, diffusing capacity impairment in the HIV-uninfected population has been associated with increased mortality,[8] highlighting the importance of this abnormality in lung function.

DL_{CO} can be decreased by multiple mechanisms including parenchymal destruction, interstitial lung disease, loss of alveolar surface, or primary pulmonary vascular processes. The contributors to decreased diffusing capacity in HIV are not well-known, but studies prior to ART found the majority of HIV-infected individuals had impairments in DL_{CO} related to advanced HIV, infections, and emphysema.[3-6] One study from a pre-ART cohort of participants without AIDS-defining lung disease demonstrated impairment in diffusing capacity was related to a decrease in capillary blood volume and not an increase in the

membrane component of gas diffusion, suggesting emphysema or pulmonary vascular disease to be the significant contributors.[4] Although recent studies have found that abnormal DL_{CO} remains common in HIV infection in the post-ART era, specific contributors to this abnormality has not been examined, and associations in non-smokers who may represent a distinct phenotype have not been specifically investigated. Understanding causes of diffusion impairment in HIV might lead to development of novel therapies.

We investigated contributors to impaired diffusing capacity in an HIV-infected cohort. Factors examined included mechanical lung function, computed tomography (CT) assessed emphysema, echocardiographic pulmonary hypertension, markers of cardiac strain, and lung inflammation. We also investigated the same relationships to impaired diffusing capacity in the subset of participants who had never smoked.

Methods

Participants: Details of the methods can be found in the online data repository. In brief, participants were 158 HIV-infected outpatients and were a subset of an established cohort recruited from the University of Pittsburgh HIV/AIDS clinic who had a study visit between February 2009 and August 2011.[7] All participants signed written informed consent, and the University of Pittsburgh IRB approved the protocol. Standardized questionnaires were used to obtain demographic and clinical data including smoke exposure and smoking history, any occupational exposures to vapors, gases, dusts, or fumes, and prior pneumonia. Medical record review was used to obtain CD4+ T-lymphocyte count and plasma HIV RNA levels within the past six months.

Testing procedures: Participants performed spirometry and single breath CO diffusing capacity (DL_{CO}) measurement per American Thoracic Society/European Respiratory Society guidelines.[9, 10] Race-adjusted predicted values for spirometry were determined using Hankinson formulas and for DL_{CO} using Neas formulas adjusted for carboxyhemoglobin and hemoglobin concentrations [9, 11, 12]. Standardized non-contrast CT scans of the entire thorax at end-inspiration were obtained in individuals who had less than approximately 10 rad exposure to radiation in the prior year. Percentage of lung voxels associated with emphysema defined as voxels below -950 Hounsfield units was calculated[13]. Scans were reviewed independently without knowledge of the participant's lung function or clinical characteristic by two pulmonologist (MG and CW) and a radiologist (CF) to determine the presence of interstitial lung

disease or fibrosis defined by the presence of diffuse, peripheral, or subpleural patchy ground glass opacities, reticular opacities, honeycomb changes, or signs of volume loss (traction bronchiectasis, displaced fissure)[14]. Echocardiography was performed to determine peak tricuspid regurgitant jet velocity (TRV),[15] left ventricular (LV) ejection fraction, LV hypertrophy, and diastolic dysfunction. A TRV ≥3.0 m/sec was defined as elevated.[16] Echocardiograms were added to the study protocol as of July 1, 2009 and were available in 126 individuals. Percentages of neutrophils, lymphocytes, and eosinophils were determined from sputum induced via inhalation of nebulized 3% saline.[17] Sputum samples were considered suitable for analysis if they contained fewer than 30% squamous cells (n=128). Plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured in participants who had a Modification of Diet in Renal Disease[18] estimated glomerular filtration rate ≥60 ml/min per 1.73 m^2 (n=143).[19] CT scans and echocardiograms were administered by trained clinical technicians. Pulmonary function tests were administered by either of 2 trained research nurses. Sputum analysis and NT-proBNP measurement were done in a research lab.

Statistical approach: Because it is likely that causes of diffusion impairment are different in those who had ever smoked and never smokers, analysis was stratified by smoking status. The dependent variable DL_{CO} % predicted was analyzed as a dichotomous variable, identifying those with more significantly impaired diffusing capacity (≤60% predicted) vs. >60%, and also as a continuous variable. This cut-off was chosen because it is used clinically to

define moderate impairment of DL_{CO} and we were interested in a more severe phenotype. Participant characteristics were summarized and compared between participants with a DL_{CO} >60% predicted and ≤60% predicted using *t*-tests, Wilcoxon rank-sum, chi-squared, or Fisher's exact tests where appropriate with a p-value less than 0.05 considered significant. Variables of interest associated with DL_{CO} were assessed from four categories: measures of obstructive lung disease (forced expiratory volume in 1 second [FEV_1] % predicted, forced vital capacity [FVC] % predicted, FEV_1/FVC, and percent of radiographic emphysema), interstitial lung disease (presence of interstitial changes on CT scan), pulmonary vascular/cardiovascular disease (TRV, elevated TRV, NT-proBNP, left ventricular function), and airway inflammation (induced sputum cell counts). To approximate normality, NT-proBNP was transformed using the natural logarithm and sputum lymphocyte counts using the square root. Sputum eosinophils were dichotomized for analysis to detectable versus absent. Associations between variables of interest and DL_{CO} % predicted were determined using simple linear regression.

Two linear regression models, one for ever smokers and another for never smokers, were created to determine independent associations between DL_{CO} % predicted and participant demographics, obstructive lung disease, pulmonary vascular disease, and inflammation. Variables were selected for the model that had univariate associations of $p<0.1$. Models were selected using stepwise regression.[20] The multivariable models were assessed for excess colinearity by checking variance inflation factors.[21] The effect of secondhand smoke

exposure was assessed in the multivariate models and no significant confounding was noted.

Results

One hundred and fifty-eight participants completed pulmonary function testing, 125 who had ever smoked and 33 who had never smoked. The majority of participants (71.3%) had undetectable plasma HIV RNA levels, and CD4 counts at the most recent assessment within 6 months prior to study visit were generally high (median CD4 cells/μL, 561; range, 24-2094). The mean (standard deviation [SD]) DL_{CO} was 64.1% (15.0%) predicted in ever smokers and 72.7% (13.4%) in never smokers (Table 1). Eighty-four percent of participants had a DL_{CO} <80% predicted. Forty-seven (37.6%) smokers had a more significantly reduced DL_{CO} (≤60% predicted), and even 8 (24.2%) never smokers had a DL_{CO} ≤60% predicted (Figure 1). Among smokers, those with a reduced DL_{CO} had lower BMI, smoked more pack-years, and were more likely to have used cocaine (Table 1). In 125 participants with data collected on secondhand smoke exposure, secondhand smoke exposure during childhood was more common in ever smokers with significantly reduced DL_{CO}.

Measures of obstructive lung disease. Diffusing capacity was associated with several measures of obstructive lung disease. In ever smokers, a lower DL_{CO} % predicted was associated with lower post-bronchodilator FEV_1, FVC, and FEV_1/FVC (Table 2), but in never smokers, lower DL_{CO} % predicted was only associated with FEV_1 and FVC. In 117 participants who had CT scans, a lower DL_{CO} % predicted was seen with a higher fraction of lung voxels <-950 HU in ever smokers but not never smokers (Figure 2).

Measures of interstitial lung disease. None of the CT exams reviewed were rated as positive for the presence of interstitial lung disease or fibrosis.

Measures of pulmonary vascular/cardiovascular disease. Lower DL_{CO} % predicted was seen with a higher TRV and higher NT-proBNP levels in ever smokers. Ten participants (7.9%), all ever smokers, had echocardiograms with an abnormal TRV (≥3.0m/sec), and an abnormal TRV was associated with a lower DL_{CO} % predicted. Measures of left ventricular function were not associated with DL_{CO} in ever smokers, but lower LVEF was associated with lower DL_{CO} % predicted in never smokers (Table 2).

Measures of airway inflammation. The mean (SD) percent neutrophils in 128 participants with adequate induced sputum was 50.5% (19.8%). None of the sputum cell counts were associated with diffusing in ever smokers, but higher sputum neutrophil percent was associated with a lower DL_{CO} % predicted in never smokers (Figure 2). The median (range) lymphocyte percentage was 0.7% (0-9.0%), and lower sputum lymphocyte percents were associated with lower DL_{CO} in never smokers.

While the associations between DL_{CO} and post-BD FEV_1 % predicted, TRV, and sputum neutrophils (Figures 2a, 2b, 2c) were similar in smokers and never smokers, the associations appeared different between smokers and never smokers for post-BD FEV_1/FVC and radiographic emphysema (Figures 2d and 2e).

Multivariable regression. Regression models were evaluated to determine specific factors independently associated with impaired diffusion in

ever smokers and never smokers separately (Table 3). In ever smokers, the final model showed that lower post-bronchodilator FEV_1 % predicted and greater percent emphysema measured by the fraction of lung <950 HU were independently associated with worse DL_{CO} % predicted. Opposed to this, in never smokers, lower DL_{CO} % predicted was associated with lower post-bronchodilator FVC % predicted, a greater percent of neutrophils in sputum, and a lower percent of lymphocytes in sputum.

Discussion

In an HIV-infected cohort, we found that impaired diffusing capacity is common, even in never smokers, and may result from different mechanisms in smokers and non-smokers. Overall, in smokers, impaired diffusing capacity was associated with measures related to obstruction and emphysema (lower FEV_1 % predicted and greater radiographic emphysema), and in never smokers, diffusing capacity impairment was associated with FVC and airway inflammation, but not lower FEV_1/FVC or radiographic emphysema.

Degree of diffusing capacity impairment in this cohort is comparable to findings in prior studies of diffusing capacity in HIV-infected persons.[3-6] We found a mean DL_{CO} of 65.9% predicted, and most previous studies find low DL_{CO} in HIV-infected individuals. In our cohort, 34.3% had a DL_{CO} less than 60% predicted and 84.8% had a DL_{CO} less than 80% predicted, while prior studies found 55% had a DL_{CO} below 80% predicted[3, 6] or 25% had a DL_{CO} below 72% predicted.[4]

The pathogenesis for diffusing capacity impairment in the HIV-infected population is not completely understood. Early in the HIV epidemic, it was thought primarily due to HIV-related inflammation or infection[3, 5], and diffusing capacity seemed to worsen with HIV progression.[3] Prior work was performed before widespread use of effective ART, and many participants had acute pulmonary processes.[3-6] In contrast, 87% of participants in the current study were using ART and the majority had CD4+ T-cell counts greater than 500 cells/μl and undetectable viral loads (< 50 copies/ml by ultrasensitive assays).

None of the participants had acute pulmonary infections, and these factors were not associated with DL_{CO} impairment in the cohort.

The impairment in diffusing capacity in HIV in the pre-ART era was also related to a decrease in the capillary blood volume in the lung and an accelerated form of emphysema.[4] The current study supports emphysema contributing to diffusing capacity impairment in smokers; however, the prevalence of diffusing capacity impairment in never smokers suggests diffusing capacity impairment is not entirely smoking-related.

Prior work has also demonstrated reductions in diffusing capacity in HIV related to lung infection or inflammation.[3, 5] In the HIV-uninfected population, increased sputum neutrophils are a hallmark of COPD and correlate with disease severity.[22, 23] In our cohort, airway inflammatory markers were associated with reduced diffusing capacity, only in never smokers. However, because we do not see an association between measures of emphysema and diffusing impairment in never smokers, other mechanisms linking increased neutrophils and diffusing impairment may also be important.

Pulmonary vascular disease (or pulmonary arterial hypertension) is more prevalent in HIV-infected persons[16, 24] and could also contribute to impairment in diffusing capacity. We found that elevated pulmonary artery pressures (as measured by increased TRV on echocardiography) correlated with decreases in diffusing capacity. We have previously shown that lower diffusing capacity is associated with elevated TRV, but our analysis demonstrates that there is an independent contribution of pulmonary artery pressures to diffusing capacity.[25]

It is also possible that COPD contributed to the increased pulmonary artery pressures seen, and longitudinal or animal studies will be helpful in examining cause and effect of these abnormalities.

Finally, it is possible that there are extra-pulmonary causes for reduced diffusing capacity. We corrected for anemia, but hyperglycemia is under-appreciated in HIV-infected persons, and chronic hyperglycemia can reduce diffusing capacity.[26, 27] We did not have measures of hemoglobin A1C to assess chronic glucose levels. Cardiac function might also impact diffusing capacity, but there was no correlation between LV function (both systolic or diastolic) or hypertrophy and DL_{CO} except in the never smokers, although this association was quite modest. Cardiac strain, as indicated by NT-proBNP levels, was associated with DL_{CO} abnormalities and may reflect right heart strain in smokers given the association of elevated pulmonary artery pressures with DL_{CO} or left heart strain in non-smokers given the association with LV function.

The degree of diffusing capacity impairment in never smokers is striking (over ¼ with a DL_{CO} less than 60% predicted) and may indicate causes for diffusing capacity impairment that are independent of smoking. Although a small sample, we found that impaired diffusing capacity in never smokers was associated with reduced FVC, but not a reduced FEV_1/FVC or radiographic emphysema. Because decreased FEV_1/FVC ratio and radiographic emphysema are suggestive of an emphysema phenotype,[28] emphysema may play a large role in diffusing capacity impairment in smokers, but in never smokers, diffusing capacity impairment may be driven by a process other than emphysema or a

process that might later develop into emphysema. We speculate that a component of restrictive lung physiology and inflammation or immune reconstitution may be in part responsible for impaired diffusing capacity in HIV-infected individuals. These mechanisms may be overshadowed by smoking, but still could be important contributors to diffusing capacity impairment.

Taken together, these data suggest diffusing capacity impairment in HIV infection is multifactorial and not solely the result of accelerated COPD that has been reported. The associations of diffusing capacity impairment with measures of airflow obstruction, pulmonary vascular disease, and airway inflammation indicate that several pathologic processes contribute to the reduction in diffusing capacity commonly seen in HIV-infected individuals. Various pathways may be contributing to these pathologies such as smoking leading to airway obstruction and emphysema, HIV-viral proteins contributing to pulmonary vascular disease,[29] or immune reconstitution leading to lung inflammation.[30] Vascular dysfunction may also link smoking and emphysema.[31] However, it is possible these are tied together by a single underlying mechanism such as immune activation related to HIV infection.[32] Chronic immune stimulation is believed to cause premature aging and immune senescence in HIV-infected persons[33] and has been implicated in both emphysema[34] and pulmonary vascular disease in the HIV-uninfected population.[35] Understanding individual factors important in diffusing capacity abnormalities in this population could have direct implications for guiding appropriate diagnostic evaluation in HIV-infected patients with

respiratory symptoms and an abnormal diffusing capacity and influencing treatment modalities pursued.

This study has several limitations. It is a single-center study with a high prevalence of smoking and drug use in the cohort and may not be applicable to the entire HIV population, although smoking and drug use are quite common in HIV-infected individuals.[36] We did not evaluate other diseases that could affect diffusing capacity and have been reported more frequently in HIV such as primary pulmonary hypertension or lymphocytic interstitial pneumonia.[1] However, these diseases are still rare even in HIV infection, and they would be unlikely to be responsible for the great degree of impaired diffusing capacity seen. We have cross-sectional data on CD4+ T-cell and plasma HIV RNA levels, but peak and nadir levels or changes over time may be important factors in the development of impaired diffusing capacity. Pre-therapy HIV RNA levels, HIV specific immune responses, host genetic factors, or immune activation/chronic inflammation may also be important. We have a relatively small sample of never smokers which may limit the power to detect meaningful associations. Additionally, the degree and magnitude of secondhand smoke exposure is difficult to quantify, and with over 70% of never smokers reporting some secondhand smoke exposure it raises the question as to whether they are truly without smoke-related injury.

In conclusion, abnormal diffusing capacity is common in HIV-infected persons, even in non-smokers. While smoking and illicit drug use are associated with abnormal diffusing capacity, independent contributions of obstructive lung

disease, primary pulmonary vascular disease, and inflammation suggest either a multifactorial cause of diffusing capacity impairment in HIV-infected individuals or a common, underlying pathway linking these processes. Given that reduced DL_{CO} also predicts mortality,[8] the driving forces resulting in accelerated emphysema, pulmonary vascular disease, and inflammation in HIV infection are important to understand in order to ameliorate this significant impairment in lung function.

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Figure Legends

Figure 1. Distribution of DL_{CO} % predicted. Dashed line represents 60% predicted DL_{CO}. Solid line represents the mean. (light gray) = never smokers; (dark gray) = ever smokers.

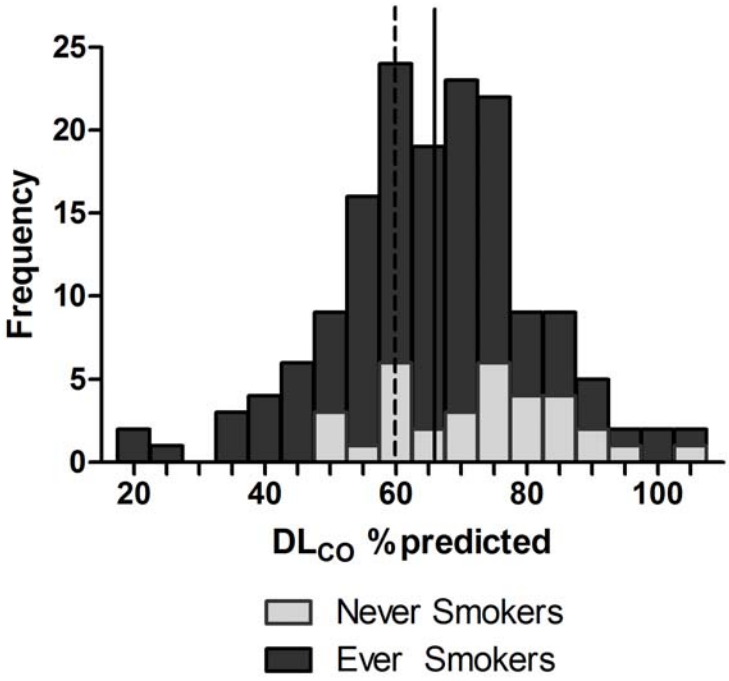

Figure 2. Scatterplots and regression lines for DL_{CO} % predicted by a) post-bronchodilator (BD) FEV_1 % predicted, b) Tricuspid regurgitant velocity, c) sputum neutrophil percent, d) post-BD FEV_1/FVC, and e) natural logarithm (ln) of the fraction of lung <-950 for ever smokers (positive symbols,+, and solid lines) and never smokers (open squares, □, and dashed lines). Pearson correlation coefficients (r) and significance values (p) are shown nearest to the regression lines of smokers (bottom) and never smokers (top) for correlations between the independent variable in each graph and DL_{CO} % predicted.

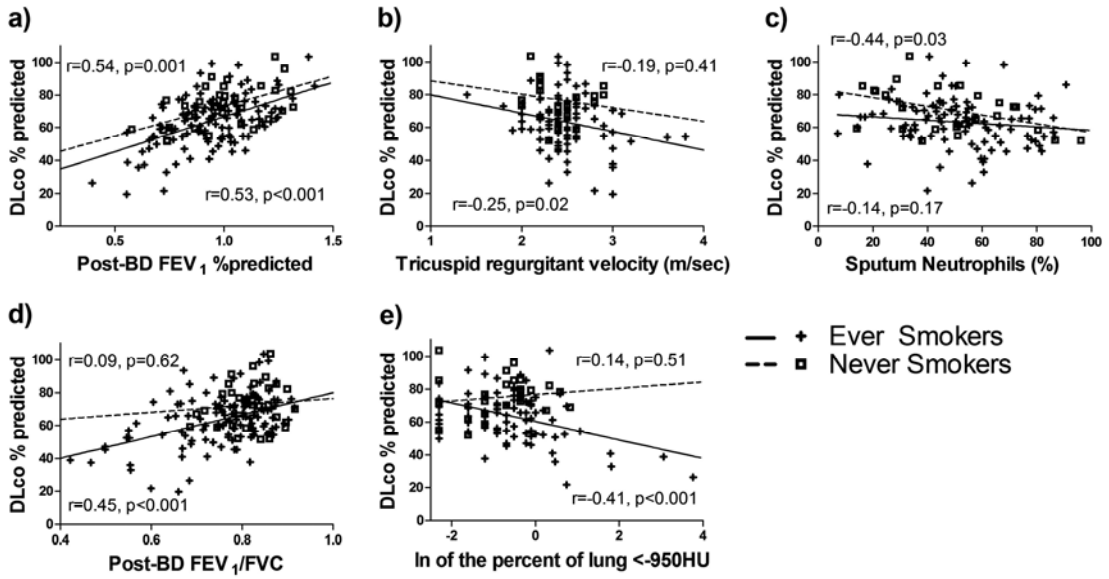
a)
r=0.54, p=0.001
r=0.53, p<0.001
DLco % predicted
Post-BD FEV 1 %predicted
b)
r=-0.19, p=0.41
r=-0.25, p=0.02
DLco % predicted
Tricuspid regurgitant velocity (m/sec)
c)
r=-0.44, p=0.03
r=-0.14, p=0.17
DLco % predicted
Sputum Neutrophils (%)
d)
r=0.09, p=0.62
r=0.45, p<0.001
DLco % predicted
Post-BD FEV 1/FVC
e)
r=0.14, p=0.51
r=-0.41, p<0.001
DLco % predicted
ln of the percent of lung <-950HU
Ever Smokers
Never Smokers

Table 1.

	Ever Smokers (n=125)	DL_{CO} >60% predicted (n=78)	DL_{CO} ≤60% predicted (n=47)	p-value	Never Smokers (n=33)	DL_{CO} >60% predicted (n=25)	DL_{CO} ≤60% predicted (n=8)	p-value
Age, mean (SD)	46.1 (9.2)	45.4 (10.0)	47.3 (7.6)	0.25	48.8 (10.7)	48.4 (11.6)	50.0 (8.2)	0.71
Female, n (%)	38 (30.4)	25 (32.1)	13 (26.7)	0.61	7 (21.2)	4 (16.0)	3 (37.5)	0.32
African American, n (%)	70 (56.0)	43 (55.1)	27 (57.5)	0.37	10 (30.3)	7 (28.0)	3 (37.5)	0.61
HIV risk factor, n (%)				0.26				0.28
MSM	60 (48.4)	39 (50.0)	21 (44.7)		18 (54.6)	15 (60.0)	3 (37.5)	
Heterosexual contact	41 (30.1)	28 (35.9)	13 (27.7)		10 (30.3)	7 (28.0)	3 (37.5)	
Intravenous drug use	15 (12.1)	6 (7.7)	9 (19.1)		0 (0)	--	--	
Blood transfusion/unknown/refused	9 (7.2)	5(6.4)	3 (6.4)		5 (15.2)	3 (12.0)	2 (20.0)	
Body mass index, mean (SD)	26.7 (5.7)	27.5 (5.7)	25.4 (5.5)	0.02	27.3 (5.2)	26.6 (4.9)	29.3 (6.1)	0.21
Smoking Status, n (%)				0.14				na
Never	--	--	--		33 (100)	25 (100)	8 (100)	
Former	39 (31.2)	28 (35.9)	11 (23.4)					
Current	86 (68.8)	50 (64.1)	36 (76.6)					
Pack-years smoked, median (range)	14.2 (0-75)	10 (0-75)	17.4 (0-70)	0.03	0 (0-0)	0 (0-0)	0 (0-0)	na
Secondhand smoke exposure, n (%)*								
Childhood home	77 (78.6)	40 (69.0)	37 (92.5)	0.01	19 (70.4)	14 (70.0)	5 (71.4)	0.94
Adult home	83 (84.7)	48 (82.8)	35 (87.5)	0.52	20 (74.1)	14 (70.0)	6 (85.7)	0.63
Adult outside of home	85 (86.7)	50 (86.2)	35 (87.5)	0.85	18 (66.7)	13 (65)	5 (71.4)	0.76
Marijuana use ever, n (%)	80 (64.0)	51 (65.4)	29 (61.7)	0.68	8 (24.2)	5 (20.0)	3 (37.5)	0.37
Cocaine use ever, n (%)	34 (27.2)	16 (20.5)	18 (38.3)	0.03	3 (9.1)	2 (8.0)	1 (12.5)	1.0
Hepatitis C, n (%)	19 (15.2)	11 (14.1)	8 (17.2)	0.68	5 (15.2)	4 (16.0)	1 (12.5)	1.0
Occupational exposure, n (%)	30 (24.0)	18 (23.1)	12 (25.5)	0.76	6 (18.2)	5 (20.0)	1 (12.5)	0.63
Using pneumonia prophylaxis, n (%)	38 (30.4)	23 (29.5)	15 (31.9)	0.85	7 (21.2)	6 (24.0)	1 (12.5)	0.65
Prior pneumonia, n (%)	32 (25.6)	21 (20.6)	17 (31.5)	0.13	6 (18.2)	4 (16.0)	2 (25.0)	0.62
Prior *Pneumocystis* pneumonia, n (%)	3 (2.4)	2 (2.6)	1 (2.1)	0.81	0	--	--	na

Antiretroviral use, n (%)	110 (88.0)	67 (85.9)	43 (91.5)	0.35	27 (81.8)	20 (80.0)	7 (87.5)	1.0
CD4+ T-lymphocytes/μL, median (range)	561.5 (24-2094)	571 (33-2094)	528.5 (24-1798)	0.59	533 (179-1327)	513 (179-1140)	685.5 (265-1327)	0.48
Plasma HIV RNA - ln copies/mL, median (range)	UD (UD-13.2)	UD (UD-13.2)	UD (UD-12.1)	0.88	UD (UD-13.7)	UD (UD-13.7)	UD (UD-8.9)	0.65
DL_{CO} % predicted, mean (SD)	64.1 (15.0)	73.0 (9.5)	49.4 (10.0)	na	72.7 (13.4)	78.1 (10.5)	55.8 (3.5)	na

*Data on secondhand smoke exposure was collected for 125 participant, 98 smokers and 27 never smokers.
P-values represent comparisons between the participants with DL_{CO}>60% predicted and those with a DL_{CO}≤60% predicted.
n = number; SD = standard deviation; HIV = human immunodeficiency virus; MSM = men who have sex with men; RNA = ribonucleic acid; UD = undetectable; EGFR = estimated glomerular filtration rate; DL_{CO} diffusing capacity for carbon monoxide.

Table 2. Univariate associations of markers of airway obstruction,pulmonary hypertension, and airway inflammation with DL_{CO} % predicted

	Ever Smokers		Never Smokers	
	DL_{CO} % predicted	**p-value**	**DL_{CO} % predicted**	**p-value**
	β-coefficient		β-coefficient	
Obstructive lung disease				
Post-BD FEV_1 % predicted	0.4245	<0.001	0.3679	0.001
Post-BD FVC % predicted	0.3255	<0.001	0.4743	<0.001
Post-BD FEV_1/FVC	0.6748	<0.001	0.2084	0.62
Log Fraction <950 HU	-0.0564	<0.001	0.0191	0.52
Pulmonary vascular and LV disease				
TRV, per m/sec	-0.1123	0.02	-0.0827	0.41
TRV ≥3.0 m/sec	-0.1572	0.002	---	---
LV ejection fraction	0.0024	0.512	0.0205	0.02
LV diastolic dysfunction	-0.0290	0.46	0.0971	0.13
LV hypertrophy	-0.0182	0.65	-0.0113	0.91
NT-proBNP, per log units/mL	-0.0153	0.14	0.0036	0.91
Airway inflammation				
Sputum neutrophils	-0.1025	0.17	-0. 2684	0.03
Sputum eosinophils (detectable vs. none)	-0.0321	0.27	-0.0105	0.86
Square root - sputum lymphocytes	0.0546	0.75	1.3000	0.003

BD = bronchodilator; FEV_1 = forced expiratory volume in the first second; FVC = forced vital capacity; HU = Hounsfield units; TRV = tricuspid regurgitant jet velocity; LV = left ventricle; NT-proBNP = N-terminal pro-brain natriuretic peptide; DL_{CO} = diffusing capacity for carbon monoxide.

Table 3. Multivariable regression models showing independent associations for DL_{CO} % predicted in ever smokers and never smokers.

	Ever Smokers*		**Never Smokers**	
	β-coefficient	p-value	β-coefficient	p-value
Post-FEV_1 % predicted	0.3940	<0.001		
Post-BD FVC % predicted			0.3323	0.02
Log Fraction <-950 HU	-0.0423	0.001		
Sputum % Neutrophils			-0.1967	0.03
Sputum % Lymphocyte (square root)			0.9407	0.009

*Body Mass Index, pack-years smoking, and cocaine use were considered for model construction.

BD – bronchodilator; FEV_1 – forced expiratory volume in 1 second; TRV – tricuspid regurgitant jet velocity