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Original article

Plasma cysteine/cystine and glutathione/glutathione disulfide redox potentials in HIV and COPD patients

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is prevalent in patients infected with HIV. The purpose of this study was to test the hypothesis that systemic oxidation correlates with loss of lung function in subjects with COPD, and that HIV infection can contribute to creating such an environment. Subjects were recruited at the University of Louisville in the following groups: HIV-infected (n = 36), COPD (n = 32), HIV and COPD (n = 28), and uninfected controls with normal lung function (n = 34). HIV infection was assessed by viral load and CD4 cell counts. Pulmonary function was determined by spirometry, and plasma was collected for measurement of cysteine (Cys), cystine (CySS), glutathione (GSH) and GSH disulfide (GSSG) by HPLC followed by estimation of redox potentials (E_h) using the Nernst equation. Results showed that patients with COPD had more oxidized plasma E_h Cys/CySS than patients with normal lung function, but plasma E_h GSH/GSSG was unaltered. In addition, there was a correlation between the extent of plasma E_h Cys/CySS oxidation and loss of lung function, and this correlation remained even after correcting for age, sex, race and body mass index. HIV infection *per se* was not associated with increased oxidation of plasma E_h Cys/CySS, but plasma E_h Cys/CySS was more oxidized in patients with lower CD4-positve T cell counts. In patients with both HIV infection and COPD, there was a significant correlation between CD4 cell counts and lung function. Thus, systemic oxidation correlated with decreased lung function in subjects with COPD and decreased CD4 counts in subjects infected with HIV. Thus, factors contributing to plasma E_h Cys/CySS may represent novel mechanisms underlying the increased prevalence of COPD in people living with HIV.

1. Introduction

Lung diseases are common in individuals infected with the Human Immunodeficiency Virus (HIV). Prior to the introduction of current antiretroviral therapy (ART), opportunistic infections affecting the lung were among the major causes of morbidity and mortality in these patients [1]. The widespread use of ART has decreased opportunistic infections and transformed HIV into a chronic disease. In this context, non-infectious lung disorders such as chronic obstructive pulmonary disease (COPD) are emerging as important causes of illness [2,3].

COPD is a progressive chronic respiratory illness characterized by limited airflow that is not reversible by bronchodilators. It is not

Abbreviations: COPD, Chronic obstructive pulmonary disease; Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential; HIV, human immunodeficiency virus; ART, antiretroviral therapy; FEV, forced expiratory volume; FVC, forced vital capacity; OLS, ordinary least squares; BMI, body mass index

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entirely clear what drives the progressive loss of lung function in COPD, but oxidative stress appears to be one of the critical factors [4,5]. Intriguingly, signs of oxidative stress are frequently observed in people infected with HIV, providing a clue to the relationship between COPD and HIV infection [6,7].

Glutathione (GSH) is one of the most important endogenous antioxidants. It is a tripeptide synthesized from glutamate, cysteine (Cys) and glycine, with Cys providing the redox-active thiol group that is essential for its many functions [8]. Because Cys concentrations are relatively low inside cells where GSH is produced, the rate of GSH synthesis is usually limited by the amount of Cys available [9]. When demand for GSH synthesis increases (e.g., during oxidative stress), Cys is transported into the cell from the extracellular space where it is much more abundant [10,11]. The majority of extracellular Cys is actually found in its oxidized form, cystine (CySS), which is a disulfide-linked dimer of 2 molecules of Cys. Once inside the cell, CySS can be reduced to Cys and used for GSH or protein synthesis [12]. Because Cys and CySS are recognized by distinct transporters, the relative concentrations of these 2 redox forms in the plasma can impact the ability of cells to import Cys or CySS as a function of which transporters they express [13].

GSH synthesized within cells can be oxidized to its disulfide GSSG, and this happens at an accelerated rate during oxidative stress. GSH and GSSG can be exported. Thus, plasma contains detectable concentrations of not only Cys and CySS, but also GSH and GSSG. Calculation of the redox potentials (E_h) from plasma concentrations of Cys, GSH, and their corresponding oxidized forms provides a more comprehensive picture of the reducing power of these 2 redox couples [14]. In plasma, the Cys/CySS redox couple is more abundant than GSH/GSSG, whereas the opposite is true in cells and tissues [15].

There is some evidence that oxidative stress may be playing a causal role in both HIV and COPD. Oxidation of E_h Cys/CySS has been shown to stimulate lung fibroblasts to produce fibronectin (a matrix glycoprotein involved in tissue injury and repair) and transforming growth factor β (a pro-fibrotic growth factor) [16]. It also stimulates proliferation and transdifferentiation of lung fibroblasts, processes implicated in aberrant tissue remodeling that might influence lung function [17]. A variety of oxidative stress markers are elevated in plasma of patients with HIV, including 8-oxo-deoxyguanine and lipid peroxidation products [18]. Some groups have reported that plasma concentrations of the Cys and/or GSH are lower in people infected with HIV [19–21]. It is unclear whether these systemic changes contribute directly to HIV infection or progression, but a number of studies have reported links between cell surface redox changes or intracellular oxidative stress and viral entry, viral replication, or reactivation of latency in macrophages or lymphocytes [22–24].

We hypothesized that the increase in oxidative stress associated with HIV could exacerbate loss of lung function and contribute to the development of COPD. To begin to address this hypothesis, we measured the redox potentials of plasma Cys/CySS and GSH/GSSG in patients with HIV, COPD or both, to determine whether these values correlated with loss of lung function.

2. Methods

2.1. Study design and participants

Patients were recruited into this study based on criteria that placed them in 1 of 4 different categories: COPD (−) and HIV (−); COPD (−) and HIV (+); COPD (+) and HIV (−); and COPD (+) and HIV (+). COPD was confirmed by spirometry, characterized by airflow limitation as defined by a post-bronchodilator FEV1/FVC ratio < 0.72. All HIV patients were being treated in the HIV or general clinics of the University of Louisville. The study was approved by the Institutional Review Board, and all participants gave written informed consent.

2.2. Pulmonary function tests

Lung function of all study participants was assessed by spirometry [25]. The primary outcome variables were the maximum volume of air exhaled in the first second of effort (forced expiratory volume in 1 s; FEV1), the amount of air that could be forcibly exhaled after taking the deepest breath possible (forced vital capacity; FVC), and the ratio of the two (FEV1/FVC). At least 3 FEV1 and FVC measurements were recorded before and 30 min after inhalation of albuterol in accordance with American Thoracic Society guidelines. The highest values pre- and post-bronchodilator were used to calculate the pre- and post-FEV1/FVC ratio. Subjects were designated as having COPD if their FEV1/FVC ratio was less than 0.72 and did not improve after administration of bronchodilator.

2.3. Collection of blood and plasma

Blood was collected by venipuncture of the antecubital vein. A portion of blood was immediately processed to prepare plasma for measurement of Cys, CySS, GSH and GSSG while avoiding oxidation, as described previously [26]. Briefly, blood was transferred to an “N-tube” containing a preservation solution gamma-glutamylglutamate as an internal standard, centrifuged to obtain plasma, and then the plasma was combined with an equal volume of buffered perchloric acid in an “S-tube.” The acidified plasma was stored at −80 °C until it could be derivatized for HPLC analysis. Other portions of the whole blood were used for CD4 counts and determination of blood urea nitrogen concentrations.

2.4. Measurement of Cys, CySS, GSH and GSSG and calculation of redox potentials

Deproteinized plasma was S-carboxymethylated and N-dansylated prior to separation and quantification by HPLC with fluorescence detection, as described previously [26,27]. Redox potentials were calculated by the equations: E_h Cys/CySS = $-250 + 30*\log([CySS]/[Cys]^2)$ and E_h GSH/GSSG = $-264 + 30*\log([GSSG]/[GSH]^2)$, where −250 and −264 were the midpoint potentials (in millivolts) for Cys/CySS and GSH/GSSG, respectively, and concentrations were expressed in moles/liter.

2.5. Statistical analysis

Descriptive statistics were performed, split by study group. Frequencies with percentages and medians with interquartile ranges were used to describe categorical and continuous data, respectively. To compare categorical data between groups, Chi-squared tests or Fisher exact tests were used. To compare continuous data between groups, Mann-Whitney U-tests and Kruskal-Wallis One Way ANOVAs were used. For analysis, CD4 count was log transformed to reduce skewed distribution.

To assess the impact of demographic and laboratory values on redox states, LASSO regression was performed with plasma E_h Cys/CySS and plasma E_h GSH/GSSG as the response variables for predictor variable elimination. LASSO regression uses a shrinkage term to penalize beta coefficients, potentially to the point of total elimination, and is more robust to multicollinearity than a normal ordinary least squares (OLS) regression. We selected an optimal shrinkage parameter based on minimizing the mean squared error for the model based on 10- and 20-fold cross-validation. Variables remaining in the LASSO model were then entered into an OLS multivariable regression. LASSO regression was performed for the full cohort, as well as an HIV + subgroup.

R v3.4.2 (R Foundation for Statistical Computing, Vienna Austria) was used for all analysis. P-values of < 0.05 were considered statistically significant.

Table 1
Clinical characteristics of patients in the 4 categories included in this study.

n	COPD(+)/HIV(+)	COPD(+)/HIV(−)	COPD(−)/HIV(+)	COPD(−)/HIV(−)	p
	28	34	36	34	
Age (median [IQR])	54 [51–59]	57 [51–64]	50 [47–54]	36 [32–54]	< 0.001
Male Sex (%)	22 (79)	18 (53)	26 (72)	17 (50)	0.045
Race (%)					0.177
White	14 (50)	17 (50)	15 (42)	9 (26)	
Black	14 (50)	17 (50)	20 (56)	25 (74)	
Other	0 (0)	0 (0)	1 (3)	0 (0)	
Tobacco Use (%)					0.209
Current	20 (71)	27 (79)	29 (81)	28 (82)	
Previous	6 (21)	6 (18)	4 (11)	1 (3)	
Never	2 (7)	1 (3)	3 (8)	5 (15)	
Pack-years smoked (median [IQR])	28 [15–43]	38 [19–54]	20 [10–36]	6 [2–17]	< 0.001
Illicit drug use (%)	12 (43)	10 (29)	20 (56)	12 (35)	0.141
Alcohol abuse (%)	1 (4)	2 (6)	5 (14)	0 (0)	0.092
Height, cm (median [IQR])	175 [170–182]	169 [161–179]	172 [167–179]	170 [164–176]	0.117
Weight, kg (median [IQR])	73 [64–84]	90 [70–100]	79 [66–88]	81 [73–93]	0.089
BMI, kg/m2 (median [IQR])	23 [21–27]	31 [25–35]	26 [22–30]	29 [24–32]	0.003
Years since COPD diagnosis (median [IQR])	5.5 [3.2–13.2]	7.0 [4.0–8.0]			0.834
FEV1 (median [IQR])	2.2 [1.5–2.8]	1.7 [1.1–2.2]	2.8 [2.4–3.3]	3.0 [2.2–3.5]	< 0.001
FVC (median [IQR])	3.7 [2.9–4.2]	2.6 [1.9–3.5]	3.8 [3.3–4.3]	3.5 [2.8–4.4]	< 0.001
FEV1/FVC (median [IQR])	0.6 [0.5–0.7]	0.6 [0.5–0.7]	0.8 [0.7–0.8]	0.8 [0.8–0.8]	< 0.001
Post FEV1 (median [IQR])	2.4 [1.7–3.0]	1.9 [1.2–2.6]	3.0 [2.6–3.5]	3.0 [2.4–3.7]	< 0.001
Post FVC (median [IQR])	3.8 [3.3–4.3]	2.9 [2.3–3.7]	3.9 [3.2–4.3]	3.5 [3.1–4.4]	0.001
Post FEV1/FVC (median [IQR])	0.7 [0.5–0.7]	0.7 [0.6–0.7]	0.8 [0.8–0.8]	0.8 [0.8–0.9]	< 0.001
Years since HIV diagnosis (median [IQR])	16 [14–21]		14 [6–22]		0.236
CD4 Count (median [IQR])	494 [318–744]		540 [429–799]		0.543
Viral Load (median [IQR])	15 [0–348]		10 [0–136]		0.839
ART use (%)	25 (89)		33 (92)		> 0.999

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in first second; FVC, forced vital capacity; ART, antiretroviral therapy. Pack years were calculated as packs per day multiplied by number of years smoked.

3. Results

3.1. Demographics

A total of 130 subjects were recruited from patients and family members entering University of Louisville clinics over a 2.5 year period. Table 1 shows the clinical characteristics of these subjects. Given the demographic differences between our COPD and HIV patients, we were not able to recruit a sample population that was homogenous in terms of age, sex, race, smoking or weight in the context of this single center study. Age at enrollment ranged from 22 to 78 years, with a mean age across all groups of 50 years. COPD patients tended to be older than patients without COPD, and the control group (COPD (−)/HIV (−)) was about 10 years younger than the overall average. The control group was 74% African American, whereas the other 3 groups were between 50 and 56% African American. The majority of people enrolled in the study were current or former smokers, but those in the two COPD (+) groups were heavier smokers than those in the COPD (−) groups, as evidenced by the greater number of pack-years smoked. The average body mass index (BMI) was in the overweight to obese range for all groups except for the COPD (+)/HIV (+) group with an average BMI of 23 which is in the range for normal, healthy adults. In contrast to the HIV (−) groups, the majority of patients in both HIV (+) groups were male. This is consistent with the makeup of the overall population of HIV patients seen at the University of Louisville clinics. Use of alcohol and illicit drugs was similar in all groups. All patients with COPD had a low FEV1/FVC ratio that did not significantly improve after use of bronchodilator, but there was no difference in lung function between the 2 COPD (+) groups. The majority (47 out of 60, or 78%) of COPD patients had airflow limitation classified as moderate based on FEV1% predicted (GOLD 2), 12 (20%) were classified as severe (GOLD 3), and only 1 (2%) was classified as very severe (GOLD 4). Between the 2 HIV (+) groups there were no differences in years since diagnosis, CD4 counts, viral load or ART use.

3.2. LASSO regression for variable selection

Input variables for the full cohort model were sex, age, race, body mass index (BMI), post-bronchodilator FVC, post-bronchodilator FEV1/FVC, HIV status, history of alcohol abuse, history of tobacco use, and pack-years smoked. For the HIV (+) subgroup LASSO regression, HIV status was replaced with log CD4 count. LASSO variable selection for plasma E_h Cys/CySS is shown in Table 2. All variables were eliminated from both the full cohort model and HIV (+) subgroup for plasma E_h GSH/GSSG, so no multivariable regression was performed for this response.

Table 2
LASSO regression model selection for E_h Cys/CySS.

Predictor Variable	Full cohort	HIV (+) subgroup
Age	X	X
Male sex	X	X
African-American race		X
BMI	X	X
Post-bronchodilator FVC		
Post-bronchodilator FEV1/FVC	X	X
History of alcohol abuse		
History of tobacco use (current/previous/never)		
Pack-years smoked		X
HIV (+)	X	N/A[a]
Log CD4 count	N/A[b]	X

Predictor variables selected from LASSO regression for E_h Cys/CySS are shown above. An X in the column corresponds to the model the variable was selected in. No X indicates the variable was eliminated.

[a] HIV (+) status was not considered for the sub-analysis.

[b] CD4 count was not considered for the full cohort.

Table 3
Multivariable regression results for E_h Cys/CySS in the full cohort.

Variable	β coefficient (95% CI)	p-value
Age	0.13 (−0.09, 0.36)	0.232
Male sex	8.63 (3.82, 13.43)	**0.001**
BMI	0.55 (0.24, 0.86)	**0.001**
Post-bronchodilator FEV1/FVC (%)	−0.20 (−0.38, −0.02)	**0.026**
HIV (+)	−2.71 (−7.43, 2.01)	0.258

3.3. Multivariable regression results

In the full population, sex, BMI and post-bronchodilator FEV1/FVC were significant predictors of plasma E_h Cys/CySS. Controlling for age, sex, BMI and HIV status, a decrease of 1% FEV1/FVC indicated an increase in plasma E_h Cys/CySS of 0.20 mV (i.e., 0.20 mV more oxidizing). Controlling for age, sex, BMI and post-bronchodilator FEV1/FVC, HIV status was not found to be significantly associated with plasma E_h Cys/CySS. Regression results can be seen in Table 3.

In the HIV (+) subgroup analysis, sex, race, BMI, post-bronchodilator FEV1/FVC and log CD4 count were significant predictors of plasma E_h Cys/CySS. In this population, controlling for age, sex, race, BMI, pack-years smoked, and log CD4 count, a decrease of 1% FEV1/FVC indicated an increase in E_h Cys/CySS of 0.31 mV. Controlling for age, sex, race, BMI, pack-years smoked, and post-bronchodilator FEV1/FVC, a decrease of 1 log CD4 count (corresponding to a decrease of CD4 count by a factor of 10) indicated an increase in E_h Cys/CySS of 13.82 mV. Regression results for the HIV (+) subgroup can be seen in Table 4.

3.4. Plasma redox potentials in HIV and COPD

Oxidation of plasma E_h Cys/CySS was associated with lower FEV1/FVC and lower CD4 counts, even after correcting for BMI, sex, race and age (Tables 2–4). However, there were no differences in plasma E_h Cys/CySS when averages of the individual groups were compared (Fig. 1A). Consistent with the regression analysis, plasma E_h GSH/GSSG was similar in all groups examined (Fig. 1B). When HIV status was ignored, COPD subjects showed a small but significant 6 mV oxidation of E_h Cys/CySS when compared to non-COPD subjects. In contrast, there were no differences when HIV subjects were compared to non-HIV subjects (Fig. 2).

The linear relationship between plasma E_h Cys/CySS and lung function is shown in Fig. 3A); lower FEV1/FVC ratios were associated with more oxidized plasma E_h Cys/CySS. This was specific for the Cys/CySS redox couple, as plasma E_h GSH/GSSG did not change with loss of lung function (Fig. 3B).

3.5. CD4 counts, redox potentials and lung function

Among HIV-infected patients, CD4 cell counts correlated with plasma E_h Cys/CySS (Fig. 4A), but not plasma E_h GSH/GSSG (Fig. 4B); the more oxidized the E_h Cys/CySS, the lower the CD4 cell counts. Similarly, the ratio of CD4 cells to CD8 cells decreased as plasma E_h Cys/CySS became more oxidized (not shown). Because plasma E_h Cys/CySS correlated with both CD4 counts and lung function, we investigated whether CD4 counts were related to lung function. When all HIV-infected patients were included in the analysis (including those with and without COPD), there was no correlation between CD4 counts and lung function (Fig. 5A). However, when the analysis was restricted to those patients that were COPD-positive, the correlation became significant (Fig. 5B). Therefore, low CD4 counts do not contribute directly to lower lung function, but there appears to be a relationship between CD4 counts and lung function in patients with both COPD and HIV infection.

Table 4
Multivariable regression results for E_h Cys/CySS in the HIV (+) subgroup.

Variable	β coefficient (95% CI)	p-value
Age	0.27 (−0.12, 0.65)	0.173
Male sex	9.11 (2.11, 16.11)	**0.012**
African-American race	8.66 (2.70, 14.62)	**0.005**
BMI	0.67 (0.25, 1.08)	**0.002**
Post-bronchodilator FEV1/FVC (%)	−0.31 (−0.54, −0.07)	**0.012**
Pack-years smoked	−0.07 (−0.18, 0.04)	0.227
Log CD4 count	−13.82 (−23.63, −4.02)	**0.007**

4. Discussion

In the era of antiretroviral therapy, infectious lung diseases are being replaced by chronic lung diseases like COPD as major causes of morbidity and mortality in patients infected with HIV. Because oxidative stress has been implicated in the pathogenesis of both HIV and COPD, we speculated that oxidative stress would correlate with decreased lung function in patients with COPD, and that the presence of HIV would increase oxidative stress and contribute to even lower lung function in these patients. Consistent with these expectations, COPD subjects were under oxidative stress, demonstrated by an oxidation of their plasma E_h Cys/CySS redox potential when compared to non-COPD subjects. However, the presence of HIV infection did not alter either plasma E_h Cys/CySS or lung function, suggesting that our initial hypothesis may have been too simplistic. Indeed, when the analyses were extended to consider the extent of HIV infection as opposed to just whether an individual was infected or not infected, we found that patients with lower CD4 counts had more oxidized plasma E_h Cys/CySS than those with higher CD4 counts. These results support the concept that an oxidizing environment promotes worsening of lung function in chronic lung diseases like COPD, and that HIV infection can contribute to the development of such an environment. It is difficult to establish whether a disease causes oxidative stress, or whether oxidative stress contributes to the pathogenesis of a disease. A case can be made that cigarette smoke exposure is an independent cause of oxidative stress. Cigarette smoking is a major risk factor for COPD, and it can impact viral load and life expectancy in people with HIV infection [28]. Following this logic, oxidative stress can be seen as promoting both disease processes. Therefore, the reverse could also be true and COPD may be impacting HIV viral load or CD4 counts.

The finding that lung function in patients with COPD correlated with plasma E_h Cys/CySS is consistent with previous studies examining other markers of oxidative stress. A clinical study performed in India found a positive correlation between plasma TBARS (a measure of lipid peroxidation) and severity of COPD [29]. That study and another conducted in Australia reported that total protein thiols in plasma decreased in association with lung function [30]. Some studies have found either no change or an increase in total GSH in patients with COPD [31], but in those patients with a history of recent acute exacerbation events there was a decrease in GSH [5,32]. The significant correlation between plasma redox and lung function is consistent with the idea that the extracellular redox environment can influence cellular processes that contribute to the pathogenesis of COPD. For example, *in vitro* studies have shown that primary lung fibroblasts grown in media approximating oxidized plasma will upregulate expression of tissue remodeling genes [17].

The present study confirmed that patients infected with HIV are under oxidative stress, but only when the severity of the infection is considered. In our HIV cohort, lower CD4 counts were associated with higher (more oxidizing) plasma E_h Cys/CySS. Consistent with this finding, others have reported that lower CD4 counts correlated with higher levels of oxidative damage biomarkers [18,33]. Others found no differences in BAL fluid GSH and Cys levels between HIV and HIV-uninfected individuals, although higher levels were found in ART-treated subjects [34]. In plasma, some groups found lower concentrations of

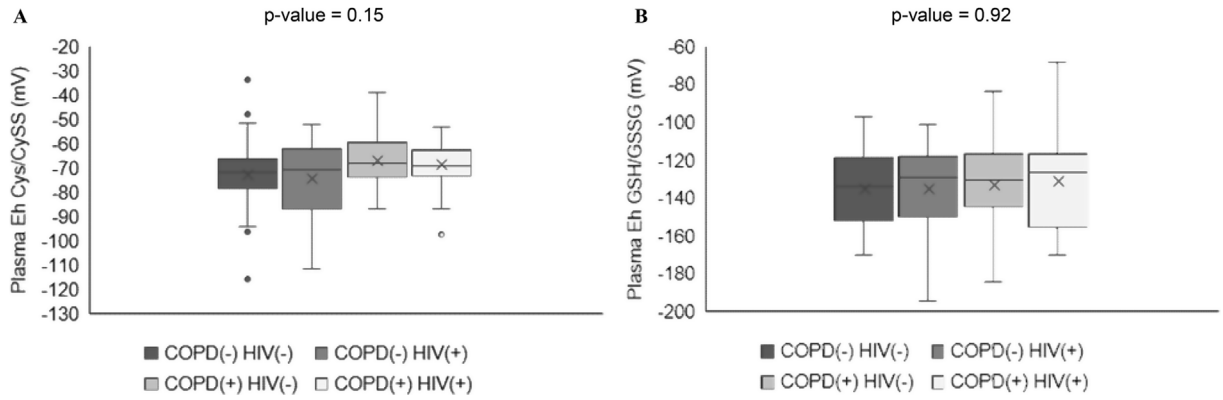

Fig. 1. Average values for plasma E_h Cys/CySS and E_h GSH/GSSG are not different among the 4 groups of patients. (A) Plasma E_h Cys/CySS and (B) plasma E_h GSH/GSSG were calculated from the Nernst equation using concentrations measured by HPLC. Data are represented as box and whisker plots. P-values were calculated by Kruskal-Wallis One Way ANOVA.

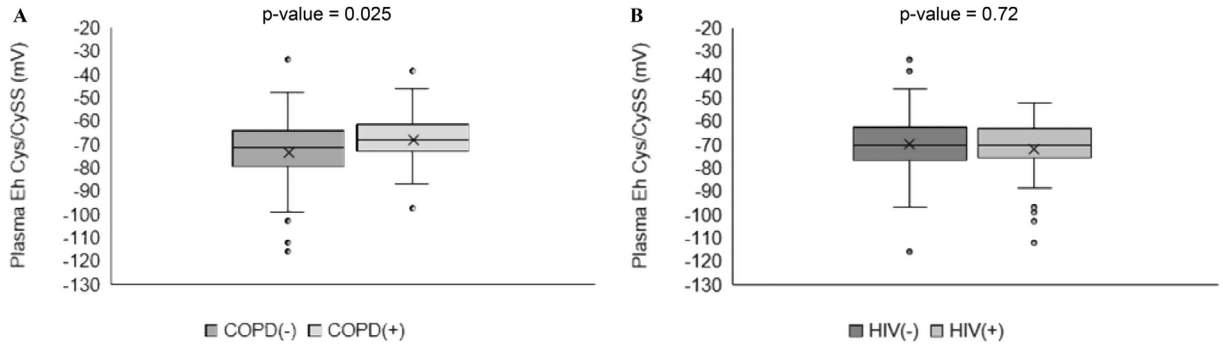

Fig. 2. Patients with COPD (both with and without HIV) have oxidized plasma E_h Cys/CySS, whereas E_h Cys/CySS is unaffected by HIV infection. Data are represented as box and whisker plots. Significance was determined by Mann-Whitney U tests.

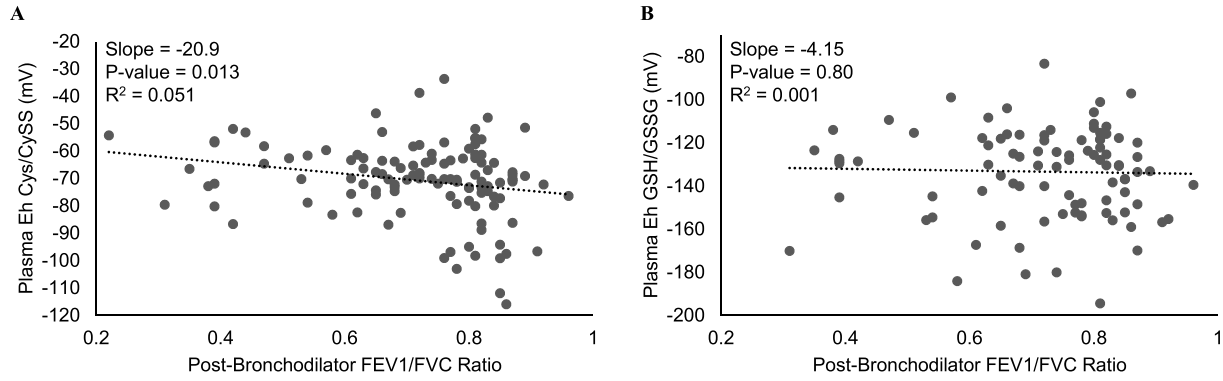

Fig. 3. Lung function correlates with plasma E_h Cys/CySS, but not plasma E_h GSH/GSSG. Linear regression analysis of (A) plasma E_h Cys/CySS and (B) plasma E_h GSH/GSSG as a function of post-FEV1/FVC ratio presented as the percentage of the value expected for a healthy control.

Cys and/or GSH in people infected with HIV [19–21], although other studies have found no difference between patients and controls [35]. We did not observe differences in absolute concentrations of Cys, CySS, GSH or GSSG in patients with HIV. The basis for the discrepancies among these studies is unclear, but it could be related differences in sample collection, analytical methods, study populations or the use of different combinations of antiretroviral therapy. Indeed, some studies have shown that certain types of ART can impose an oxidative stress beyond that seen with HIV infection alone [18,36]. In laboratory studies, reverse transcriptase inhibitors and protease inhibitors can induce production of reactive oxygen species or deplete antioxidant defenses [37,38]. However, recent studies have found no direct impact of ART use on COPD or loss of lung function [39].

Mean plasma E_h Cys/CySS was about −70 mV in all 4 groups examined, whereas E_h GSH/GSSG was about −130 mV. This difference between the redox potentials of the 2 couples is consistent with previous findings [26]. However, the value of −70 mV for E_h Cys/CySS is about 10 mV more oxidizing than has been reported for young healthy individuals [26], suggesting that our controls (HIV (−)/COPD (−)) may have been different from previously-studied control populations. Compared to our controls, neither HIV nor COPD was associated with overt oxidation of the plasma redox potential. The majority of patients in all 4 groups (including controls) were either smokers or former smokers, which may account for the overall increase in E_h Cys/CySS relative to other studies [40]. Thus, the high percentage of smokers may have obscured some of the effects of COPD and HIV on plasma redox potentials. Among smokers in our study, the number of pack-years was not associated with changes in either plasma E_h Cys/CySS or plasma E_h

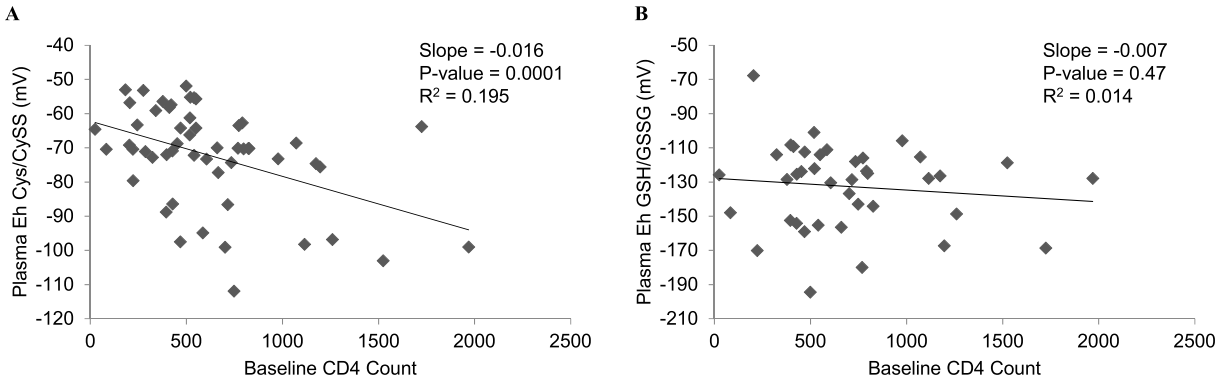

Fig. 4. Severity of HIV infection correlates with plasma E_h Cys/CySS but not plasma E_h GSH/GSSG. Linear regression analysis of (A) plasma E_h Cys/CySS and (B) plasma E_h GSH/GSSG as a function of CD4 cell count.

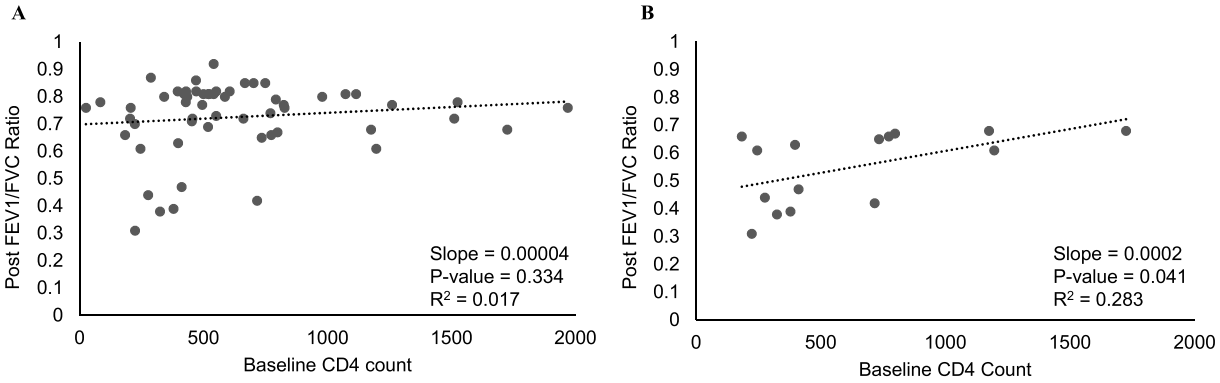

Fig. 5. Lung function correlates with CD4 counts in COPD patients with HIV infection. (A) Lung function does not correlate with CD4 counts when patients with and without COPD are grouped together. (B) In COPD patients (those with post FEV1/FVC less than 0.7), there was a significant correlation with CD4 counts among patients infected with HIV.

GSH/GSSG (not shown). However the number of pack-years was significantly correlated with loss of lung function as measured by the FEV1/FVC ratio.

The correlation between lung function and plasma E_h Cys/CySS remained significant after correcting for age, sex, race and BMI. In the population studied here, sex and BMI were independently correlated with plasma E_h Cys/CySS, consistent with a recent report [41]. A previous study found both plasma E_h Cys/CySS and plasma E_h GSH/GSSG became progressively more oxidized with age [40]. It is not clear why we did not see oxidation of plasma redox couples in older individuals; differences in other demographic factors such as smoking rates in the 2 populations may have contributed to the different findings. A number of studies have found decreased concentrations of GSH in plasma and other tissue in old animals or humans relative to their young counterparts (reviewed in Ref. [42]), but we did not see lower plasma GSH in the older individuals in this study. It has been proposed that the aging process itself could be the result of a decline in the reduced form of cysteine [43], which is another way of looking at a shift in the balance between reduced and oxidized forms. We found a trend correlating age with oxidation of E_h Cys/CySS ($r^2 = 0.034$; $p < 0.052$), but this relationship became non-significant after controlling for BMI, sex and race. The current study also revealed a difference in E_h Cys/CySS in females when compared to males. This confirms a recent report [41], and it may have contributed to some of the variability observed between groups. For example, both of the HIV-infected groups included in this study had a much higher proportion of males than females.

Limitations of this study include small sample number, differences in gender and racial distributions among the groups, variable BMI and high prevalence of smoking. Many of these limitations stem from the fact that this was a single center study. Smoking and high BMI are both prevalent within the population served by the University of Louisville, and there are more men than women in our cohort of HIV patients. The results presented here should be replicated in other populations to determine whether they are more broadly applicable.

Taken together, the results of the present study identify a role for oxidative stress in the etiology or progression of COPD in patients infected with HIV. The observation that plasma E_h Cys/CySS is more oxidized in patients with low lung function and with low CD4 counts points to a novel way to think about oxidative stress in chronic diseases. Whereas oxidative stress is a general term that is often used to express a shift in the balance between oxidants and antioxidants, the changes in extracellular redox potentials observed here are indicative of a specific alteration that can have profound effects on intracellular signal transduction and tissue function. Therefore, investigations into the mechanisms that control plasma E_h Cys/CySS and the specific pathways that respond to changes in E_h Cys/CySS should be useful in future studies of both HIV and COPD.

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