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BRIEF REPORT

HIV Infection and Circulating Levels of Prosurfactant Protein B and Surfactant Protein D

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Prosurfactant protein B (pro-SFTPB) and surfactant protein D (SFTPD) are markers of lung inflammation and damage. We estimated geometric mean pro-SFTPB and SFTPD levels in 500 human immunodeficiency virus (HIV)-infected and 300 HIV-uninfected injection drug users, adjusting for smoking and other covariates. Pro-SFTPB levels were significantly higher among people with HIV (PWH) (adjusted geometric mean, 21.4 vs 18.1 ng/mL; P = .03), and were higher with lower CD4 counts (P trend = .001), higher HIV RNA (P trend = .05), and without highly active antiretroviral therapy (P = .03). These associations were not observed for SFTPD. Serum levels of pro-SFTPB are elevated among PWH and are associated with immunosuppression and uncontrolled viremia.

Keywords. HIV; lung; inflammation; surfactant protein.

Among people with human immunodeficiency virus (PWH) in the United States, lung cancer rates are double those in the general population [1]. The high smoking prevalence has contributed to elevated lung cancer rates in PWH: the prevalence of current smoking is 54% in PWH overall and 74% among human immunodeficiency virus (HIV)-infected injection drug users (IDUs), compared with 20% in the general population [2]. However, the association between HIV and lung cancer risk remains after controlling for cigarette smoking [3]. Though residual confounding by smoking remains a possible explanation, another hypothesis is that chronic pulmonary inflammation due to HIV-related pulmonary infections contributes to elevated lung cancer risk.

In the general population, lung cancer risk is increased in the presence of inflammatory lung conditions or elevated circulating levels of proinflammatory markers [4, 5]. In addition, circulating levels of two surfactant proteins produced in the lung, prosurfactant protein B (pro-SFTPB; a hydrophilic precursor to SFTPB) and surfactant protein D (SFTPD) play a role in inflammation regulation [6], and are associated with lung cancer risk in prospective studies [7, 8].

In the current study, we evaluated serum levels of pro-SFTPB and SFTPD among HIV-infected and HIV-uninfected IDUs. We hypothesized that higher circulating levels of these proteins in PWH would provide indirect support for a role of inflammation in the etiology of lung cancer in this population. In addition, we compared levels by clinical severity of HIV disease and HIV treatment.

METHODS

Study Population

The Study of HIV Infection in the Etiology of Lung Disease (SHIELD) enrolled participants aged ≥18 years from 2 established HIV cohort studies in Baltimore, MD: the AIDS Link to the Intravenous Experience (ALIVE) Study (a cohort study of HIV-infected and HIV-uninfected IDUs) and the Johns Hopkins HIV Clinical Cohort (JHHCC) Study (an open clinical cohort of PWH treated at the Johns Hopkins HIV Clinic) [9, 10]. Informed consent was obtained from all participants. SHIELD was approved by the Institutional Review Board of Johns Hopkins University.

At baseline and at semiannual follow-up visits, SHIELD participants provided information on demographics, lifetime and recent tobacco and drug use, and pulmonary symptoms and diseases via questionnaire. Participants additionally underwent blood collection and spirometry to characterize pulmonary function. A subset of participants also received additional pulmonary evaluation as part of a chronic obstructive pulmonary disease (COPD) substudy.

We selected 500 HIV-infected and 300 HIV-uninfected SHIELD participants with an injection drug use history and spirometry data. Participants in the COPD substudy had blood samples collected within 1 year of their substudy visit; blood samples from the most recent SHIELD visit were utilized for the remaining participants. Questionnaire and HIV treatment data were utilized from the visit associated with the selected blood sample, supplemented with baseline data.

Laboratory Methods

Serum was stored at −70°C. Pro-SFTPB and SFTPD were measured using enzyme-linked immunosorbent assays produced in house [8] or commercially (Immuno-Biological Laboratories, Japan), respectively. Samples blinded regarding HIV status were measured in duplicate and averaged for analysis. Values

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of pro-SFTPB were multiplied by a plate-specific normalization factor, computed by dividing the median internal standard across plates by the internal standard of each plate. SFTPD levels were missing for 1 PWH. Quality control samples from healthy controls, and samples with known high and low values, were measured in each plate. Coefficients of variation measured across plates ranged from 10%–29% for SFTPD and 26%–47% for pro-SFTPB. HIV RNA was measured using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (version 2.0) (Roche Diagnostics, Basel, Switzerland). A lower limit of 50 copies/mL was used for this analysis to remain consistent with the parent cohorts over time.

Statistical Analysis

We compared geometric mean levels of pro-SFTPB and SFTPD by demographics, behaviors, and lung conditions, adjusting for age, race, sex, smoking status, and HIV with multivariable linear regression. Adjusted geometric means were estimated from regression models as exponentiated values of the least squares means of log-transformed marker levels. We then compared geometric mean levels of pro-SFTPB and SFTPD by HIV status, and among PWH, across categories of CD4 cell counts, serum HIV RNA levels, and current highly active antiretroviral therapy (HAART) use, adjusting for age, race, sex, smoking status, average number of cigarettes smoked per day in the last 6 months, and smoked drugs in the last 6 months. In additional models, we further adjusted for COPD present on spirometry, and histories of pneumonia and asthma. A sensitivity analysis was restricted to ALIVE Study participants.

RESULTS

Compared to HIV-uninfected participants, PWH were more likely to be black, ever smokers, and to have a history of pneumonia (Supplementary Table 1). No significant differences by HIV status were observed in the average number of cigarettes smoked per day, pack-years smoked at baseline, cigar use, recreational drug use, or history of asthma, tuberculosis, or COPD. For pro-SFTPB, adjusted geometric mean levels increased significantly with increasing age (P trend = .001), were higher in current smokers than never smokers (geometric mean, 33.1 vs 11.6 ng/mL; $P < .0001$) and increased with number of cigarettes smoked per day during the last 30 days ($P = .002$; Table 1). Participants with COPD present on spirometry also had higher levels of pro-SFTPB (geometric mean, 20.2 vs 17.2 ng/mL; $P = .05$). For SFTPD, levels increased with age (P trend = .05), but there were no significant differences between current and never smokers ($P = .09$) or by cigarettes smoked per day (P trend = .40). Additionally, whites had significantly higher levels of SFTPD than blacks (geometric mean, 114.2 vs 86.0 ng/mL; $P = .007$). Serum levels of pro-SFTPB and SFTPD were weakly correlated ($r = 0.20$; $P < .001$).

Pro-SFTPB levels were significantly higher among HIV-infected compared to HIV-uninfected participants (geometric mean, 21.4 vs 18.1 ng/mL; $P = .03$) after adjusting for age, race, sex, smoking status, cigarettes smoked per day, and smoked drugs in the past 6 months (Table 1). Further adjustment for COPD present on spirometry, history of pneumonia, and history of asthma did not impact the results (geometric mean, 21.6 vs 18.2 ng/mL; $P = .02$). However; no difference in adjusted SFTPD levels was observed by HIV status (88.8 vs 95.2 ng/mL; $P = .25$; 88.6 vs 96.2 with further adjustment for COPD, pneumonia and asthma; $P = .18$). Results were similar when restricted to ALIVE participants (geometric mean, 22.1 vs 17.6 ng/mL; $P = .004$).

Among PWH pro-SFTPB levels were higher among those with lower CD4 cell counts (geometric mean, 31.6 vs 19.0 ng/mL for CD4 <200 vs ≥500 cells/mm^3; P trend = .001), higher HIV RNA (29.4 vs 20.6 ng/mL for viral loads ≥10 000 copies/mL vs undetectable levels; P trend = .05), and among those not currently being treated with HAART (28.7 vs 19.7 ng/mL for untreated vs treated individuals; $P = .03$; Figure 1). Compared to HIV-uninfected people, only the most immunosuppressed PWH had significantly elevated pro-SFTPB (eg, CD4 count <200 cells/mm^3 [difference = 13.8 ng/mL; $P < .0001$], HIV RNA ≥10 000 copies/mL [difference = 11.7 ng/mL; $P < .0001$], and those not on HAART [difference = 10.6 ng/mL; $P = .005$]). In contrast, there was no significant trend in SFTPD levels across categories of CD4 cell count (P trend = .94), HIV RNA (P trend = .69), or current HAART use ($P = .74$), nor were there any differences in SFTPD levels between HIV-uninfected people and PWH in any of these categories (all $P > .05$; Figure 1).

Discussion

Circulating levels of pro-SFTPB are elevated among PWH. Further, pro-SFTPB levels are higher among PWH with lower CD4 cell counts or higher HIV RNA, and among those who are not receiving HAART. Taken together, these results suggest that PWH have higher levels of pulmonary inflammation and lung damage than HIV-uninfected persons, which increases with more advanced HIV disease, including greater immunosuppression and uncontrolled viremia. In contrast, no association was observed between HIV infection and serum SFTPD, another surfactant protein.

SFTPB is produced by type 2 alveolar pneumocytes and nonciliated bronchiolar cells, and its main function is to reduce surface tension in the lung [6]. SFTPB also has anti-inflammatory and antioxidant properties [6]. Elevated circulating levels of pro-SFTPB, the hydrophilic precursor to SFTPB, may result from increased permeability of the lung due to tissue damage [6]. SFTPD also has a number of important functions in the lung, including defense against pathogens, immune modulation, inflammation regulation, and antioxidant properties [11].

Table 1. Associations of Prosurfactant Protein B (pro-SFTPB) and Surfactant Protein D (SFTPD) Levels

	Pro-SFTPB Level, Geometric Mean (95%CI), ng/mL	*P* value	SFTPD Level, Geometric Mean (95%CI), ng/mL	*P* value
Age, years		.001		.05
<47	14.4 (12.0, 17.1)		80.9 (70.2, 93.3)	
47–51.9	18.0 (15.1, 21.4)		90.5 (78.5, 104.4)	
52–56.9	19.5 (16.5, 23.0)		88.6 (77.5, 101.3)	
57+	20.3 (17.2, 23.9)		97.2 (85.1, 111.1)	
Sex		.27		.55
Male	17.2 (15.2, 19.5)		90.0 (81.3, 99.6)	
Female	18.7 (16.1, 21.8)		86.5 (76.4, 97.9)	
Race		.10		.007
White	21.2 (16.7, 27.0)		114.2 (94.1, 138.6)	
Black	17.3 (15.4, 19.5)		86.0 (78.0, 94.7)	
Smoking status[a]				
Never	11.6 (8.68, 15.5)		79.7 (63.1, 100.6)	
Former	14.8 (12.3, 17.7)	.14	89.9 (77.6, 104.1)	.39
Current	33.1 (30.6, 35.8)	<.0001	98.1 (92.0, 104.5)	.09
Cigarettes per day in the last 30 days[b]		.002		.40
<5	26.5 (22.3, 31.5)		93.3 (80.1, 108.6)	
5–9	30.1 (26.0, 34.9)		107.6 (94.6, 122.4)	
10–14	36.7 (32.1, 41.9)		99.9 (88.9, 112.2)	
15+	36.2 (31.5, 41.7)		89.9 (79.5, 101.6)	
Smoking duration at baseline, years[b]		.90		.25
<30	30.5 (25.3, 36.8)		98.9 (84.0, 116.4)	
30–34	36.1 (30.7, 42.5)		111.3 (96.7, 128.2)	
35–39	33.6 (29.0, 39.0)		95.7 (84.1, 108.9)	
40+	32.2 (26.7, 38.9)		88.6 (74.3, 104.2)	
Pack-years smoked at baseline[b]				
<10	25.8 (21.1, 31.6)	.10	90.6 (75.9, 108.1)	.98
10–19.9	34.0 (29.9, 38.6)		103.8 (92.9, 116.0)	
20–29.9	33.8 (28.6, 40.0)		92.9 (80.2, 107.5)	
30+	34.4 (30.2, 39.3)		97.2 (86.6, 109.0)	
Smoked other drugs in the past 6 months		.21		.41
Yes	19.2 (16.2, 22.8)		85.0 (74.1, 97.6)	
No	17.3 (15.3, 19.5)		89.9 (81.5, 99.3)	
Ever diagnosed with asthma		.86		.59
Yes	17.5 (14.8, 20.8)		91.3 (79.5, 104.8)	
No	17.8 (15.7, 20.1)		87.9 (79.5, 97.1)	
Ever diagnosed with pneumonia		.33		.29
Yes	19.1 (15.8, 23.1)		94.7 (81.3, 110.4)	
No	17.5 (15.5, 19.7)		87.8 (79.7, 96.6)	
Ever diagnosed with tuberculosis		.46		.36
Yes	15.5 (10.6, 22.6)		77.1 (56.4, 105.4)	
No	17.9 (15.9, 20.1)		89.4 (81.3, 98.3)	
COPD on spirometry		.05		.36
Yes	20.2 (17.0, 24.1)		84.5 (73.3, 97.3)	
No	17.2 (15.3, 19.4)		89.7 (81.5, 98.8)	
HIV				
Infected	21.4 (18.2, 25.3)	.03	88.8 (77.5, 101.6)	.25
Uninfected	18.1 (15.3, 21.5)		95.2 (83.0, 109.3)	

All models are adjusted for age, race, sex, smoking status, and HIV. HIV model was adjusted for age, race, sex, smoking status, cigarettes smoked per day, and smoked drugs in the past 6 months. Geometric means are predicted values from models assuming mean levels of each covariate.

[a]*P* values for smoking status compare former and current smokers to never smokers.

[b]Analysis is restricted to current smokers.

Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus.

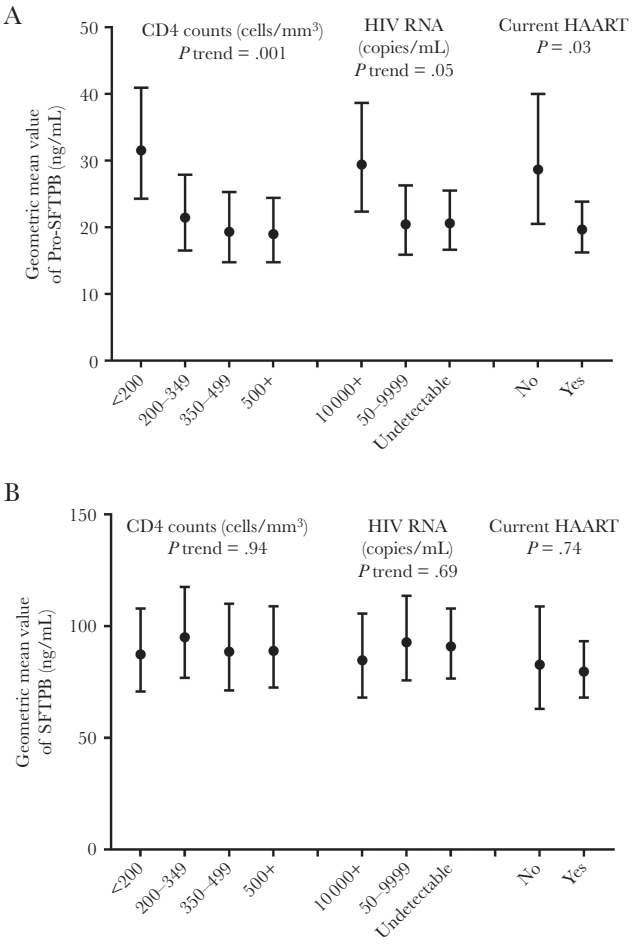

Figure 1. Associations of (*A*) prosurfactant protein B (pro-SFTPB) and (*B*) surfactant protein D (SFTPD) levels with CD4 cell count, human immunodeficiency virus (HIV) RNA, and highly active antiretroviral therapy (HAART) use among HIV-infected individuals. Points represent adjusted geometric mean values and lines represent confidence intervals. *P* values for trend were estimates by including categorical variables for CD4 count and viral load as continuous variables in the model. All models were adjusted for age, race, sex, smoking status, cigarettes smoked per day in the last 30 days, and smoked drugs in the past 6 months.

Higher levels of circulating SFTPD are seen among people with lung scarring on chest X-rays [7].

We believe that elevated levels of pro-SFTPB in the circulation of PWH indicate an association between HIV infection and increased pulmonary inflammation and damage. While the observed absolute difference in pro-SFTPB levels was modest (difference = 3.3 ng/mL), differences were 3 times larger when comparing the most immunosuppressed PWH with HIV-uninfected people. Moreover, among PWH, higher levels of pro-SFTPB were found to increase with lower CD4 counts and more marked HIV viremia, and were higher among individuals who were not being treated with HAART, indicating an association of immunosuppression and HIV viremia with lung damage. Importantly, whereas pro-SFPTB levels were associated with smoking intensity, the relationship of pro-SFTPB and HIV markers was observed independent of cigarette smoking. HIV has been shown to disrupt the physical barrier properties of the lung epithelium and promote local inflammation [12]. Indeed, HIV induces proinflammatory cytokine and chemokine secretion in the lung. HIV is associated with increased risk of COPD and other pulmonary function abnormalities [13]. Furthermore, HAART results in a strong decline in interferon-γ, interleukin-6, and proinflammatory chemokines in bronchoalveolar lavage. Although we did not see associations between HIV and SFTPD in the current study, another small study showed that SFTPD levels in PWH declined with HAART initiation [14].

In the United States, lung cancer incidence rates are doubled among PWH compared to the general population [1]. Though this increase is partly driven by a higher prevalence of smoking in PWH [2], there also appears to be an excess independent of cigarette smoking [3]. The effect of immunosuppression on lung cancer risk is less clear than for other cancers, with some studies reporting associations between decreasing CD4 cell count and lung cancer risk [15]. Increased pulmonary inflammation may contribute to the increased lung cancer risk among PWH, as pulmonary inflammation plays a role in the etiology of lung cancer in the general population [4, 8]. Furthermore, some prior studies have shown associations between inflammatory lung conditions (ie, pneumonia and COPD) and lung cancer risk in PWH [3, 15]. Finally, elevated circulating levels of pro-SFTPB and SFTPD are associated with increased risk of lung cancer in the general population [7, 8]. Thus, our results support a model whereby HIV infection is associated with pulmonary inflammation and lung damage, which may contribute to the development of lung cancer, and at least some of this effect is independent of cigarette smoking. This evidence is admittedly indirect, and future studies assessing these and other markers of pulmonary inflammation as mediators of the HIV and lung cancer association are needed.

The main strength of this study is the use of data from the SHIELD Study, which collects detailed questionnaire data and biospecimens. We also measured two surfactant proteins that are produced in the lung, allowing us to assess aspects of pulmonary inflammation and damage. A limitation of our study was its lack of direct assessment of pro-SFTPB and SFTPD levels in relation to lung cancer risk among PWH. We were unable to directly measure inflammation in the lung, as SHIELD collected bronchoalveolar lavage samples on too few participants. This study sample may not be representative of all PWH, as it was limited to IDUs, and the vast majority of participants were black. Finally, we cannot rule out residual confounding by smoking and other unmeasured confounding.

In summary, PWH manifest elevated levels of pro-SFTPB compared to HIV-uninfected people, and levels are further increased in association with declining CD4 cell count, high HIV RNA, and in the absence of HIV treatment. These associations with HIV disease were independent of tobacco use. As

pro-SFTPB is a predictor of lung cancer risk in the general population, this study provides indirect support for a role of pulmonary inflammation and lung damage in explaining increased lung cancer risk among PWH, though future studies are needed to elucidate this mechanism.

Supplementary Data

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

Notes

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