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# Circulating Biomarkers, Fraction of Exhaled Nitric Oxide, and Lung Function in Patients With Human Immunodeficiency Virus and Tuberculosis

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***Background.*** Identification of proinflammatory factors responding to *Mycobacterium tuberculosis* is important to reduce long-term sequelae of pulmonary tuberculosis (TB).

***Methods.*** We examined the association between plasma biomarkers, the fraction of exhaled nitric oxide (FeNO), and lung function among a prospective cohort of 105 adults newly diagnosed with TB/human immunodeficiency virus (HIV) in South Africa. Participants were followed for 48 weeks from antiretroviral therapy (ART) initiation with serial assessments of plasma biomarkers, FeNO, lung function, and respiratory symptoms. Linear regression and generalized estimating equations were used to examine the associations at baseline and over the course of TB treatment, respectively.

***Results.*** At baseline, higher FeNO levels were associated with preserved lung function, whereas greater respiratory symptoms and higher interleukin (IL)-6 plasma levels were associated with worse lung function. After ART and TB treatment initiation, improvements in lung function were associated with increases in FeNO (rate ratio [RR] = 86 mL, 95% confidence interval [CI] = 34–139) and decreases in IL-6 (RR = −118 mL, 95% CI = −193 to −43) and vascular endothelial growth factor ([VEGF] RR = −178 mL, 95% CI = −314 to −43).

***Conclusions.*** Circulating IL-6, VEGF, and FeNO are associated with lung function in adults being treated for TB/HIV. These biomarkers may help identify individuals at higher risk for post-TB lung disease and elucidate targetable pathways to modify the risk of chronic lung impairment among TB survivors.

**Keywords.** HIV; fraction of exhaled nitric oxide; interleukin-6; pulmonary function; tuberculosis.

With approximately 10 million cases diagnosed annually, tuberculosis (TB) remains one of the leading causes of infectious disease deaths worldwide. In recent years, however, there has been growing recognition of pulmonary sequelae of TB, whereby 30%–50% of those cured have impaired lung function despite successful treatment completion [1, 2]. Although mechanisms associated with post-TB lung disease remain unclear, studies have implicated various factors as potentially important, including neutrophilic inflammation, extracellular matrix degradation by matrix metalloproteinases (MMPs), eicosanoid and cytokine imbalances, and altered cell death pathways, including necrosis and autophagy [3, 4].

Host immune responses to TB have been implicated in lung injury, with higher levels of plasma cytokines such as interleukin (IL)-1β, interferon (IFN)-γ, tumor necrosis factor-α, IL-6, and IFN-inducible protein 10 measured early during TB treatment being linked with bacterial burden, lung pathology, and TB disease severity [5–7]. However, few studies have evaluated associations between biomarkers and respiratory impairment over the course of TB treatment, despite the fact that both host responses and bacterial burden are highly dynamic after TB treatment initiation [7–9]. Relating host responses to respiratory outcomes is important, because reduced lung function is associated with decreased survival in various pulmonary diseases [10, 11], and host-directed therapies targeting relevant pathways could decrease long-term morbidity in these individuals. The relationship between host inflammation and lung tissue damage is also particularly relevant to patients infected with

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human immunodeficiency virus (HIV) who initiate antiretroviral therapy (ART) after TB diagnosis, because these patients may develop immune reconstitution inflammatory syndrome (IRIS), which we have previously shown to be associated with local lung inflammation and loss of lung function [12].

Although most studies examining the relationship between lung function and immune responses have assessed plasma biomarkers, more direct measures of airway inflammation may also be useful. For example, the fraction of exhaled nitric oxide (FeNO) in breath is a commonly used point-of-care biomarker for eosinophilic airway inflammation in asthma [13, 14]. Production of nitric oxide has been linked with host defense against *Mycobacterium tuberculosis* (*Mtb*); therefore, either abnormally low or high levels of this biomarker could plausibly relate to lung pathology in TB. This relationship was reported in a study of 200 TB patients in Indonesia among whom a lower FeNO was associated with more severe TB disease, lower odds of culture conversion, and lower odds of successful treatment [15]. However, to date, no studies have assessed the relationship between FeNO levels and lung function in those with TB or with TB and HIV coinfection. Because point-of-care FeNO measurement is available in many respiratory clinics, investigation of the relationship between FeNO and lung function in TB disease may inform strategies of clinical care.

We performed serial measurements of plasma cytokines and FeNO in a well characterized cohort of people newly diagnosed with HIV and TB who were initiating ART and TB treatment. We then leveraged longitudinal models to evaluate the association between trajectories of biomarker levels and changes in lung function during and after TB treatment.

## METHODS

### Settings and Design

Between 2016 and 2019, we recruited ART-naive, HIV-infected adults with drug-sensitive pulmonary TB in Gauteng, South Africa as part of a prospective observational cohort entitled the Lung Function after TB-IRIS (LIFT-IRIS) study. In this study, we investigated the relationship between immune recovery after ART initiation and pulmonary function [16]. Study participants were followed for up to 48 weeks after ART initiation. Data before ART initiation (baseline), then at 4, 12, 24, and 48 weeks thereafter, are reported here. CD4 counts and HIV viral loads were measured at baseline and 4 weeks after ART initiation. Plasma was collected at baseline and then at 4 and 12 weeks after ART initiation, when inflammation is typically greatest during TB treatment. Lung function and FeNO were measured at all study visits (ie, baseline, weeks 4, 12, 24, and 48) because these measurements are noninvasive and do not require phlebotomy. All participants were treated with standard first-line tuberculosis treatment (isoniazid, rifampin, ethambutol, and pyrazinamide) and ART (ie, efavirenz, tenofovir, emtricitabine) as per South African guidelines. Participants were clinically screened for additional opportunistic infections and other clinical events at all follow-up visits by study physicians. In the current analysis, we included study participants who had at least 1 follow-up measurement of lung function and biomarkers after baseline.

### Lung Function and Fraction of Exhaled Nitric Oxide

Pulmonary function tests (PFTs) were conducted using an EasyOne Pro spirometer (New Diagnostic Designs Medical Technologies, Andover, MA) at all study visits. Data were interpreted according to American Thoracic Society/European Respiratory Society guidelines [17]. Forced expiratory volume in 1 second ($FEV_1$) and forced vital capacity (FVC) values were obtained as absolute volumes (milliliter) and percentage-predicted values, which are standardized based on height, age, race, and sex [18]. Respiratory symptoms, which are associated with survival in multiple lung diseases [19, 20], were assessed using the chronic obstructive pulmonary disease (COPD) Assessment Test (CAT). The CAT is a standardized questionnaire that assesses the impact of respiratory symptoms (cough, sputum, dyspnea, chest tightness, activity limitation, confidence performing activities, sleep, and energy) on health status. Each item scores from 0 (none) to 5 (severe) and sums to calculate a total CAT score [21]. The FeNO measurements began after approximately one third of the cohort had completed a baseline visit and were collected at all visits thereafter with a Niox Vero device (Circassia, Morrisville, NC).

### Quantitation of Plasma Analyses

Concentrations of various plasma proteins were measured using custom-made multianalyte bead kits (R&D Systems, Minneapolis, MN). Multiplex analyses were set up according to manufacturer's recommendations.

### Pulmonary Inflammation

$^{18}$F-fluorodeoxyglucose positron emission tomography-computed tomography ($^{18}$F-FDG PET/CT) was used to assess active lung inflammation at baseline and week 4 in a consecutively enrolled subset of a planned study with 48 participants, as previously described [16]. In brief, scans were done using an integrated 40-detector PET/CT scanner (Biograph 40 Truepoint PET/CT; Siemens Medical, IL, Hoffman Estates). We applied a threshold of >1 standardized uptake value (SUV) within the segmented lung area. The total FDG signal for this 3-dimensional area was multiplied by the region's volume, and the aggregate activity in both lungs is referred to as the total glycolytic activity (TGA).

### Statistical Analysis

Categorical data were described as frequency and percentages, and continuous data were described as means and standard deviations. Plasma levels were $\log_{10}$-transformed for analysis.

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Linear regression models were used to analyze the cross-sectional association between baseline predictors and the baseline $FEV_1$ or FVC percentage predicted (which is standardized by height, age, race, and sex), with adjustment for baseline CD4 count. Next, generalized estimating equation (GEE) analyses were conducted using longitudinal data from all available study visits, using plasma biomarkers and FeNO as the predictors and the absolute value of $FEV_1$ (milliliter) or FVC as the outcome. Each lung function, plasma biomarker, and FeNO were fitted into each longitudinal GEE model and adjusted for age, sex, and the change in CD4 count between baseline and week 4, given demonstrated associations between these variables and lung function in this cohort previously [12]. Finally, for the participants who underwent $^{18}$F-FDG PET/CT scans at baseline, linear regression was used to explore the association between biomarkers and pulmonary inflammation, as determined by quantitative assessment of $^{18}$F-FDG activity expressed either as SUVs or TGAs [16]. In addition, as a supplementary analysis, we explored baseline relationships between FeNO (parts per billion [ppb]) and plasma biomarkers using linear regression and longitudinal relationships using GEE analyses. Statistical analyses were performed using R, version 3.6.1.

#### Ethics

The clinical protocol was approved by the Human Research Ethics Committee of the University of Witwatersrand in South Africa and the University of Pennsylvania Institutional Review Board in the United States of America. All study participants provided written informed consent before participation in this study.

## RESULTS

There were 105 participants with plasma biomarker measurements and PFTs at baseline and at least 1 follow-up visit available for inclusion in this analysis. The average age of the cohort was 37.7 years (±8.3), 63 (60%) of whom were male and 38 (36%) were ever smokers, with an average pre-ART CD4 count of 132 cells/μL (±112) at baseline and 237 cells/μL (±172) at week 4 (Table 1). Over the course of the study period, participants typically had improvement in respiratory symptoms, as assessed by the CAT score, moving from a baseline average of 7.4 (±6.2) (with cough, sleeplessness, and breathlessness as the 3 most common symptoms) to 0.3 (±0.8) at week 48. During the same period, both $FEV_1$ and FVC percentage predicted increased from 77.2% to 83.7% and 83.8% to 91.7%, respectively, but FeNO values did not change substantially. Seventeen and 24 participants experienced a decrease in $FEV_1$ and FVC percentage predicted measurement at week 48 compared to baseline (−10% ± 9% and −8% ± 6%, respectively). At baseline among the 48 patients completing FDG PET-CT scans, one third had cavitation and approximately 19% of the lung was involved (Table 1).

At baseline, models adjusting for CD4 count demonstrated that $FEV_1$ percentage predicted was inversely associated with total CAT score (−0.91%, 95% confidence interval [CI] = −1.48% to −0.34%) and log-scaled IL-6 (−5.22%, 95% CI = −8.24% to −2.20%) (Table 2). In contrast, higher FeNO values were associated with higher $FEV_1$ percentage predicted values (4.72%, 95% CI = 1.37% to 8.07%). Models evaluating the association between baseline parameters and FVC percentage predicted that were adjusted for baseline CD4 count also found significant associations between total CAT score and IL-6 levels, in addition to significant inverse associations with MMP-1 and CXCL-10 (Supplementary Table 1). There was no significant association between FeNO and FVC percentage predicted at baseline.

In the longitudinal GEE models, a total of 105 participants with a mean of 3.8 visits per participant (equivalent to 270 observations) were included among patients with at least 1 follow-up measurement of lung function and plasma biomarkers available for analysis. Between baseline and week 12, increases in inflammatory biomarkers were generally associated with declines in $FEV_1$, with statistically significant associations for every log-scale increase in IL-6 over time (−134 mL, 95% CI = −220 mL to −47 mL) and vascular endothelial growth factor ([VEGF] −146 mL, 95% CI = −284 mL to −8 mL). These associations remained significant after adjusting for age, sex, and changes in CD4 counts from baseline to week 4 (IL-6, −118 mL and 95% CI = −193 mL to −43 mL; VEGF, −178 mL and 95% CI = −314 mL to −43 mL) (Table 3). The $FEV_1$ and CAT score were measured from baseline up to week 48, yielding 439 observations for inclusion in the GEE model. For every 1-unit increase in CAT score, $FEV_1$ decreased by an average of 33 mL (95% CI = −46 mL to −20 mL) after adjusting for age, sex, and changes in CD4 counts from baseline to week 4. In longitudinal models with FVC as the outcome, there were significant associations between FVC and IL-6 and CAT score, although not with VEGF (Supplementary Table 2).

We next evaluated the association between lung function and FeNO using 321 observations across all visits between baseline and week 48. For every 10-ppb increase in FeNO, $FEV_1$ increased by an average of 86 mL (95% CI = 34 mL to 139 mL) after adjusting for age, sex, and changes in CD4 counts from baseline to week 4 (Table 3). Similar associations were found between changes in FeNO and FVC over time (Supplementary Table 2). At baseline, cigarette smoking was associated with FeNO in a linear regression model adjusted for age, sex, and baseline CD4 count, such that participants who had ever smoked had a lower FeNO (−9.14 ppb, 95% CI = −16.15 to −2.12) (Supplementary Table 3). However, there was no association between circulating biomarkers and FeNO at baseline. Given that NO is a biological mediator of innate immune function, we also explored associations between FeNO and circulating immune biomarkers. In a series of longitudinal GEE models with



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**Table 1. Characteristics of Study Population Over 48 Weeks of Antiretroviral Treatment**

| | Baseline (n = 105) | Week 4 (n = 83) | Week 12 (n = 77) | Week 24 (n = 79) | Week 48 (n = 79) |
|---|---|---|---|---|---|
| Sociodemographic and Clinical Characteristics | | | | | |
| Age (years) | 37.7 (8.3) | - | - | - | - |
| Male sex[a] | 63 (60%) | - | - | - | - |
| CD4 count (cells/μL) | 132 (112) | 237 (172) | - | - | - |
| Viral load (copies/mL) median, IQR | 177 082 (68 934–637 789) | 375 (197–1066) | … | … | … |
| Ever cigarette smoker[a] | 38 (36%) | - | - | - | - |
| Body mass index (kg/m$^2$)[b] | 20.0 (3.1) | - | - | - | - |
| CAT score | 7.4 (6.2) | 3.9 (4.9) | 1.4 (1.9) | 0.6 (1.7) | 0.3 (0.8) |
| Cough | 1.50 (1.15) | 0.74 (0.90) | 0.29 (0.51) | 0.10 (0.34) | 0.10 (0.34) |
| Phlegm | 0.943 (0.897) | 0.573 (0.770) | 0.338 (0.620) | 0.127 (0.463) | 0.0380 (0.192) |
| Tight chest | 0.619 (0.955) | 0.280 (0.614) | 0.0260 (0.160) | 0 (0) | 0 (0) |
| Breathlessness | 1.09 (1.22) | 0.573 (0.994) | 0.143 (0.451) | 0.0886 (0.398) | 0 (0) |
| Limited activities | 0.886 (1.30) | 0.463 (0.984) | 0.143 (0.506) | 0.0380 (0.192) | 0.0380 (0.338) |
| Lung condition | 0.333 (0.884) | 0.207 (0.680) | 0.0260 (0.160) | 0.0127 (0.113) | 0 (0) |
| Unenergetic | 0.74 (1.29) | 0.37 (0.92) | 0.12 (0.36) | 0.04 (0.19) | 0.03 (0.16) |
| Sleepless | 1.27 (1.18) | 0.70 (1.09) | 0.27 (0.72) | 0.20 (0.84) | 0.05 (0.35) |
| Time between ART and ATT (d)[b] | 30.0 (18.4) | - | - | - | - |
| Lung Function and Airway Measures | | | | | |
| $FEV_1$ (liters) | 2.4 (0.7) | 2.4 (0.7) | 2.4 (0.7) | 2.6 (0.7) | 2.6 (0.7) |
| $FEV_1$ percentage predicted (%) | 77.2 (19.3) | 78.1 (19.3) | 79.0 (17.5) | 82.6 (15.8) | 83.7 (17.5) |
| FVC (liters) | 3.1 (0.9) | 3.2 (0.8) | 3.2 (0.8) | 3.4 (0.8) | 3.4 (0.8) |
| FVC percentage predicted (%) | 83.8 (18.7) | 87.1 (19.0) | 86.8 (14.4) | 90.7 (13.9) | 91.7 (15.7) |
| FeNO (ppb) | 23.5 (14.2) | 21.2 (8.8) | 21.7 (11.2) | 21.5 (12.0) | 23.1 (17.8) |
| Plasma Biomarkers (pg/mL$^{-1}$) | | | | | |
| Collagen-1a | 2700 (1880) | 2760 (1770) | 5270 (2600) | - | - |
| TIMP1 | 182 000 (117 000) | 184 000 (115 000) | 156 000 (107 000) | - | - |
| CCL-2 | 229 (105) | 180 (74.7) | 189 (92.4) | - | - |
| CXCL11 | 111 (87.4) | 75.5 (83.5) | 61.0 (78.7) | - | - |
| IL-8 | 13.9 (8.2) | 13.0 (9.2) | 9.01 (4.3) | - | - |
| MMP-1 | 1230 (1240) | 1090 (706) | 907 (699) | - | - |
| VCAM1 | 2 340 000 (1 360 000) | 1 930 000 (1 470 000) | 1 170 000 (667 000) | - | - |
| CXCL10 | 555 (636) | 470 (697) | 228 (190) | - | - |
| IL-6 | 7.9 (10.5) | 10.4 (28.3) | 3.9 (7.8) | - | - |
| MCSF | 192 (123) | 174 (129) | 117 (86.7) | - | - |
| uPA | 960 (356) | 905 (298) | 760 (235) | - | - |
| VEGF | 17.1 (9.1) | 16.2 (10.4) | 14.5 (10.6) | - | - |
| $^{18}$F-fluorodeoxyglucose Positron Emission Tomography-Computed Tomography[c] | | | | | |
| Percentage of lung involved, median (IQR) | 19% (6%–31%) | … | … | … | … |
| Cavitation on CT scan, n (%) | 18 (33%) | … | … | … | … |
| Mean SUV, median (IQR) | 1.6 (1.3–2.2) | … | … | … | … |
| Maximum SUV, median (IQR) | 8.7 (5.3–13.9) | … | … | … | … |
| CT hard volume, median mL (IQR) | 37 (24–113) | … | … | … | … |
| Region of interest volume, median mL (IQR) | 464 (148–718) | … | … | … | … |
| Total lung glycolytic activity, median (IQR) | 768.8 (197.8–1585.1) | … | … | … | … |

Abbreviations: ART, antiretroviral therapy; ATT, antitubercular therapy; CT, computed tomography; CAT, COPD Assessment Test; FeNO, fraction of exhaled nitric oxide; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukin; IQR, interquartile range; MCSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; ppb, parts per billion; SUV, standardized uptake value; uPA, urokinase-type plasminogen activator; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

NOTE: Continuous variable data are presented as mean (standard deviation), exceptional to viral load and $^{18}$F-fluorodeoxyglucose positron emission tomography-computed tomography (median and IQR) and categorical data as frequency (percentage). '-'dash means either not applicable or not measured.

[a]Categorical variables expressed as frequency (percentage).

[b]Eleven percent missing in smoke, body mass index, time between ART and ATT, and viral load.

[c]Available for 48 subset participants.

FeNO as an outcome, higher plasma levels of CCL-2 and vascular cell adhesion molecule (VCAM) were significantly associated with increased FeNO over time (CCL-2, 4.38 ppb and 95% CI = .33 ppb to 8.42 ppb; VCAM, 2.3 ppb and 95% CI = .24 ppb to 4.35 ppb). Higher plasma VCAM levels remained significantly associated with increased FeNO (2.4 ppb, 95% CI = .26 ppb to



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**Table 2. Baseline Linear Regression Model of $FEV_1$ Expressed as a Percentage-Predicted Value**

| | | $FEV_1$ Percentage Predicted | | | |
|---|---|---|---|---|---|
| | | Unadjusted Linear Regression | | Adjusted for CD4 | |
| | n | Coefficient | 95% CI | Coefficient | 95% CI |
| Sociodemographic and Clinical Characteristics | | | | | |
| Age (years) | 105 | 0.35 | −.09 to .80 | 0.36 | −0.08 to 0.80 |
| Male sex | 105 | −3.98 | −11.59 to 3.63 | −4.85 | −12.30 to 2.59 |
| CD4 count (cells/μL)[a] | 105 | **−4.02** | **−7.29 to −.76** | … | … |
| Ever cigarette smoker | 100 | 0.21 | −8.07 to 8.48 | −0.19 | −8.25 to 7.87 |
| Body mass index (kg/$m_2$) | 100 | 0.71 | −.60 to 2.01 | 0.45 | −0.84 to 1.75 |
| CAT score | 105 | **−0.99** | **−1.56 to −.41** | **−0.91** | **−1.48 to −0.34** |
| Time between ART and ATT (d)[a] | 99 | −0.08 | −2.34 to 2.18 | −0.21 | −2.41 to 1.99 |
| Airway Measures | | | | | |
| FeNO (ppb)[a] | 80 | **5.44** | **2.12 to 8.77** | **4.72** | **1.37 to 8.07** |
| Plasma Biomarkers (pg/$mL^{-1}$)[b] | | | | | |
| Collagen-1a | 105 | 3.51 | −1.79 to 8.81 | 3.36 | −1.82 to 8.54 |
| TIMP-1 | 105 | 1.56 | −3.80 to 6.92 | 1.58 | −3.65 to 6.82 |
| CCL-2 | 105 | 6.02 | −1.55 to 13.59 | 3.79 | −3.96 to 11.53 |
| CXCL-11 | 105 | −0.27 | −3.14 to 2.60 | −0.93 | −3.78 to 1.92 |
| IL-8 | 105 | 0.03 | −6.83 to 6.89 | −1.35 | −8.14 to 5.43 |
| MMP-1 | 105 | −4.05 | −8.96 to .86 | −3.10 | −8.00 to 1.80 |
| VCAM-1 | 105 | 2.71 | −4.01 to 9.44 | −0.18 | −7.23 to 6.87 |
| CXCL-10 | 105 | −3.31 | −8.30 to 1.68 | −3.78 | −8.65 to 1.09 |
| IL-6 | 105 | **−5.57** | **−8.63 to −2.51** | **−5.22** | **−8.24 to −2.20** |
| MCSF | 105 | −2.50 | −7.48 to 2.49 | −3.04 | −7.91 to 1.83 |
| uPA | 105 | −3.78 | −14.61 to 7.04 | −6.18 | −16.86 to 4.51 |
| VEGF | 105 | −4.18 | −10.15 to 1.80 | −5.05 | −10.90 to .79 |

Abbreviations: ART, antiretroviral therapy; ATT, antitubercular therapy; CAT, COPD Assessment Test; CI, confidence interval; FeNO, fraction of exhaled nitric oxide; $FEV_1$, forced expiratory volume in 1 second; IL, interleukin; MCSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; ppb, parts per billion; uPA, urokinase-type plasminogen activator; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

NOTE: Significant data *P* resented in bold.

[a]The regression results are interpreted as per 100 CD4 cell counts, per 10 days between ATT and ART, per 10 ppb of fractional exhaled nitric oxide.

[b]Plasma biomarkers: log-transformed and regression coefficients interpreted as per log-fold change.

4.53 ppb) after adjusting for age, sex, and changes in CD4 counts (Supplementary Table 4).

Finally, in the subset of 48 participants in whom PET-CT scans were performed, we measured the association between total lung glycolytic activity on $^{18}$F-FDG PET-CT imaging and both FeNO and circulating biomarkers. At baseline, higher glycolytic activity in the lungs, suggesting higher lung inflammation, was associated with lower levels of collagen-1a (−1106.7, 95% CI = −1727.7 to −485.6) and higher levels of MMP-1 (604.07, 95% CI = 59.7 to 1148.4) and IL-6 (528.58, 95% CI = 165.0 to 892.1) (Supplementary Table 5).

## DISCUSSION

In this prospective cohort study of adults with newly diagnosed HIV and TB, we evaluated the association between plasma biomarkers and FeNO with lung function after the initiation of ART and TB treatment. We found that higher plasma levels of IL-6 and VEGF, worse respiratory symptoms, and lower FeNO values were associated with worse lung function both before and after ART initiation. These findings highlight an important link between dynamic host inflammatory profiles and patient-oriented respiratory outcomes in people with HIV and TB coinfection.

Numerous epidemiologic studies indicate that up to half of the adults treated for pulmonary TB have objective evidence of respiratory impairment that persists after cure [1, 2]. However, mechanisms of lung tissue damage in TB remain unclear, and few investigations have included people with HIV. Previous analyses from this cohort demonstrated that more robust adaptive immune responses, assessed as *Mtb*-specific CD4 T cells, were associated with greater radiographic lung inflammation, which was, in turn, associated with worse lung function [16]. The present study extends these findings by linking higher levels of IL-6, an innate immune biomarker, and VEGF, which drives angiogenesis, with worse lung function at baseline and during treatment.

Our results are broadly consistent with a purported role of IL-6 in lung diseases including COPD [22], asthma [23, 24], and TB-IRIS [25, 26]. Interleukin-6 induces protective anti-*Mtb* responses including IFN-γ [27]; however, IL-6 may also drive TB pathology, including local tissue necrosis, thereby



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**Table 3. Longitudinal Analysis of the Marginal Association Between the $FEV_1$ and Other with Time-Varying Variables**

| | | $FEV_1$ mL | | | |
|---|---|---|---|---|---|
| | | Unadjusted | | Adjusted for Age, Sex, and Change in CD4 Count[e] | |
| | N | Rate Ratio | 95% CI | Rate Ratio | 95% CI |
| Sociodemographic and Clinical Characteristics | | | | | |
| CD4 count (cells/μL)[a,b] | 190 | −44 | −122 to 33 | - | - |
| CAT score | 439 | **−35** | **−49 to −22** | **−33** | **−46 to −20** |
| Airway Measure | | | | | |
| FeNO (ppb) | 321 | **105** | **38 to 172** | **86** | **34 to 139** |
| Plasma Biomarkers ($pg/mL^{-1}$)[b,c,d] | | | | | |
| Collagen-1a | 270 | 65 | −53 to 183 | 89 | −21 to 198 |
| TIMP-1 | 270 | 44 | −89 to 177 | 20 | −113 to 154 |
| CCL-2 | 270 | **234** | **18 to 451** | 129 | −66 to 325 |
| CXCL-11 | 270 | −49 | −112 to 14 | −40 | −95 to 15 |
| IL-8 | 270 | −122 | −287 to 42 | −58 | −205 to 89 |
| MMP-1 | 270 | −108 | −266 to 50 | −136 | −290 to 18 |
| VCAM-1 | 270 | 14 | −146 to 174 | 26 | −103 to 155 |
| CXCL-10 | 270 | −79 | −187 to 30 | −82 | −186 to 21 |
| IL-6 | 270 | **−134** | **−220 to −47** | **−118** | **−193 to −43** |
| MCSF | 270 | −68 | −180 to 44 | −84 | −200 to 31 |
| uPA | 270 | −98 | −370 to 174 | −69 | −279 to 141 |
| VEGF | 270 | **−146** | **−284 to −8** | **−178** | **−314 to −43** |

Abbreviations: CAT, COPD Assessment Test; CI, confidence interval; FeNO, fraction of exhaled nitric oxide; $FEV_1$, forced expiratory volume in 1 second; MCSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; ppb, parts per billion; uPA, urokinase-type plasminogen activator; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

NOTE: Significant data are presented in bold.

[a]CD4 count over time analysis was performed up to week 4.

[b]The regression results are interpreted as per 100 CD4 cell counts and per 10 ppb of fractional exhaled nitric oxide.

[c]$FEV_1$ and biomarkers over time analyses were performed up to week 12.

[d]Plasma biomarkers: log-transformed.

[e]A covariate of change in CD4 count was generated from the CD4 count at week 4 minus that at baseline.

propagating *Mtb* replication [28] and exacerbating pulmonary inflammation [26, 29]. Although IL-6 levels typically decline or stabilize during TB treatment [30, 31], sustained and robust associations between IL-6 and lung function over time in the present study further highlight its clinical relevance in TB. A recent study by Gupte et al [7] also found that higher levels of IL-6 were associated with poor treatment outcomes and death in people with TB and suggested IL-6 levels could be used as a clinical biomarker. Drivers of high IL-6 levels could include mycobacterial burden [31], genetic variability [32], or the synergistic effect of pathogens (HIV and *Mtb*) among HIV-TB-coinfected individuals [33]. Anti-IL-6 treatment has been successfully used in severe cases of TB-associated inflammation [29], and such treatment could be further investigated in pulmonary TB. Our findings that relate IL-6 levels to local lung inflammation suggest that FDG PET-CT could be a useful approach for the identification of patients who may benefit from anti-inflammatory therapies, for example, in clinical trials. Given consistent associations between impaired lung function and death [10, 11], interventions to dampen excessive elevations in IL-6 may provide an opportunity to improve long-term pulmonary outcomes for people with HIV and TB.

Higher plasma levels of VEGF were also associated with lower lung function at baseline and over the course of TB treatment, although this association was only significant for $FEV_1$ and not for FVC. Vascular endothelial growth factor, like IL-6, has been previously linked to the severity of TB disease; however, few such studies have included repeated measures of lung function or data from people with HIV coinfection [34, 35]. Vascular endothelial growth factor, a mediator of angiogenesis, is released in response to tissue inflammation, which likely underlies its expression in patients with more severe TB disease [36]. The association with $FEV_1$ but not FVC in our cohort may reflect a particular role for VEGF in airway remodeling, as has been shown in COPD [37–39]. Secretion of VEGF from *Mtb*-infected macrophages may also facilitate mycobacterial spread by inducing the formation of blood vessels [40, 41]. Thus, host-directed therapies targeting VEGF-mediated angiogenesis may also merit investigation in future trials [34, 35, 41].

We demonstrated that FeNO, an exhaled biomarker traditionally linked to eosinophilic inflammation in asthma, was associated with lung function during TB treatment. Specifically, we found that lower FeNO levels were associated with lower



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lung function, thus highlighting the potential for FeNO as a noninvasive biomarker of lung health during TB treatment. Although other studies have not shown an association between FeNO and clinical outcomes [42, 43], our findings are supported by Ralph et al [15] who found that a lower FeNO was associated with both more severe TB disease on chest x-ray (CXR) and lower lung function in a cohort of 200 people with pulmonary TB in Indonesia. Ralph et al [15] have also shown that a persistently low FeNO after treatment initiation was associated with delayed sputum culture conversion. Collectively, these data support the notion that pulmonary bioavailability of NO, as measured by FeNO, correlates with improved TB disease outcome, and further supports its potential role as a biomarker for tracking TB treatment response and respiratory health. The association between low levels of NO within the lungs and reduced lung function could reflect either ongoing damage in the context of delayed microbial clearance or impaired recovery and remodeling. It is interesting to note that we did not find an association between FeNO and IL-6 and VEGF, indicating that FeNO and the circulating biomarkers are independently associated with lung function and may represent distinct biological pathways of lung damage and healing during TB treatment. However, this analysis was exploratory and further data are needed. Given the ongoing uncertainty surrounding the drivers of NO production in TB, future studies could leverage cell-based and animal models to better delineate whether low NO reflects bacterial burden or a lower host response, whether innate or adaptive, to *Mtb*.

Our study has several limitations. First, our findings are limited to people with newly diagnosed HIV and TB. These patients have not been well characterized with respect to lung function, historically, but they may be immunologically distinct in that virologic declines lead to robust adaptive immune recovery during TB treatment. Second, in light of the hypothesis-generating nature of this study, we did not correct for multiple testing (eg, Bonferroni correction). Although such corrections can avoid false-positive findings in confirmatory studies, they can be overly conservative for hypothesis-generating studies and increase the chance of false-negative findings (ie, type II error). Third, we did not perform baseline CXRs as part of the study protocol, nor did we collect data on bacterial burden (eg, Xpert MTB/RIF cycle threshold). As a result, we were unable to evaluate the relationship between lung function, biomarkers, CXR findings, and bacterial load. Fourth, $CD4^+$ T-cell count and viral load are only monitored at baseline and week 4 because our goal of the study was primarily to evaluate how early response to ART related to early immune recovery and subsequent lung damage. Moreover, $CD4^+$ T-cell count and viral load are not routinely measured in South Africa; hence, our study was not able to evaluate complete viral suppression at the later time points. However, all participants were clinically screened at all follow-up visits by study physicians, and none of them were considered to have experienced ART failure at week 24 or week 48. Finally, the plasma biomarker assays were only collected during the first 12 weeks of TB treatment, when inflammation is highest, and relationships after treatment completion also may be clinically relevant. Although our study primarily aimed to evaluate early responses, future studies could incorporate plasma biomarker measurements over the full course of TB treatment, thus permitting insights into how biomarkers, such as IL-6 and VEGF, change over the course of treatment.

Our data add to a growing list of biomarkers linked to lung function in people with TB that may, in turn, reflect targetable pathways for therapeutic interventions to improve long-term lung health [41]. We have previously shown that a substantial number of people with TB and HIV coinfection had persistently impaired lung function 48 weeks after initiation of standard ART and antitubercular therapy [12]. Host-directed therapies that modulate the innate immune response may limit the extent of lung injury among TB patients, including those with HIV. Of note, Stek et al [44] recently conducted a randomized trial of a 28-day course of prednisone at the time of ART initiation in adults with advanced HIV and TB and found statistically significant associations between prednisone use and changes in lung function over time. Although the effect of prednisone on lung function was not sustained through 24 weeks, benefits were apparent early during treatment. These findings support ongoing efforts to modulate host inflammation to improve long-term lung health among those being treated for pulmonary TB.

## CONCLUSIONS

In conclusion, we found significant associations among IL-6, VEGF, and FeNO with lung function among people with HIV who initiated both ART and TB treatment. These circulating and exhaled biomarkers may point to potential therapeutic targets for reducing pulmonary morbidity among TB survivors.

### 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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## References

1. Pasipanodya JG, McNabb SJ, Hilsenrath P, et al. Pulmonary impairment after tuberculosis and its contribution to TB burden. BMC Public Health **2010**; 10:259.
2. Marais BJ, Chakaya J, Swaminathan S, et al. Tackling long-term morbidity and mortality after successful tuberculosis treatment. Lancet Infect Dis **2020**; 20:641–2.
3. Stek C, Allwood B, Walker NF, Wilkinson RJ, Lynen L, Meintjes G. The immune mechanisms of lung parenchymal damage in tuberculosis and the role of host-directed therapy. Front Microbiol **2018**; 9:2603.
4. Muefong CN, Sutherland JS. Neutrophils in tuberculosis-associated inflammation and lung pathology. Front Immunol **2020**; 11:962.
5. Ravimohan S, Kornfeld H, Weissman D, Bisson GP. Tuberculosis and lung damage: from epidemiology to pathophysiology. Eur Respir Rev **2018**; 27:170077.
6. Kumar NP, Moideen K, Banurekha VV, Nair D, Babu S. Plasma proinflammatory cytokines are markers of disease severity and bacterial burden in pulmonary tuberculosis. Open Forum Infect Dis **2019**; 6:ofz257.
7. Gupte AN, Kumar P, Araujo-Pereira M, et al. Baseline IL-6 is a biomarker for unfavourable tuberculosis treatment outcomes: a multisite discovery and validation study. Eur Respir J **2022**; 59:2100905.
8. Rambaran S, Naidoo K, Lewis L, et al. Effect of inflammatory cytokines/chemokines on pulmonary tuberculosis culture conversion and disease severity in HIV-infected and -uninfected individuals from South Africa. Front Immunol **2021**; 12:641065.
9. Gupte AN, Selvaraju S, Gaikwad S, et al. Higher interleukin-6 levels and changes in transforming growth factor-beta are associated with lung impairment in pulmonary tuberculosis. ERJ Open Res **2021**; 7:00390-2020.
10. Mannino DM, Buist AS, Petty TL, Enright PL, Redd SC. Lung function and mortality in the United States: data from the First National Health and Nutrition Examination Survey follow up study. Thorax **2003**; 58: 388–93.
11. Menezes AM, Perez-Padilla R, Wehrmeister FC, et al. FEV1 is a better predictor of mortality than FVC: the PLATINO cohort study. PLoS One **2014**; 9:e109732.
12. Auld SC, Maenetje P, Ravimohan S, et al. Declines in lung function after antiretroviral therapy initiation in adults with human immunodeficiency virus and tuberculosis: a potential manifestation of respiratory immune reconstitution inflammatory syndrome. Clin Infect Dis **2020**; 70: 1750–3.
13. Sandrini A, Taylor DR, Thomas PS, Yates DH. Fractional exhaled nitric oxide in asthma: an update. Respirology **2010**; 15:57–70.
14. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med **2011**; 184:602–15.
15. Ralph AP, Yeo TW, Salome CM, et al. Impaired pulmonary nitric oxide bioavailability in pulmonary tuberculosis: association with disease severity and delayed mycobacterial clearance with treatment. J Infect Dis **2013**; 208:616–26.
16. Ravimohan S, Auld SC, Maenetje P, et al. Lung injury on antiretroviral therapy in adults with human immunodeficiency virus/tuberculosis. Clin Infect Dis **2020**; 70: 1845–54.
17. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J **2005**; 26:948–68.
18. Auld SC, Kornfeld H, Maenetje P, et al. Pulmonary restriction predicts long-term pulmonary impairment in people with HIV and tuberculosis. BMC Pulm Med **2021**; 21:19.
19. Gupta N, Pinto LM, Morogan A, Bourbeau J. The COPD assessment test: a systematic review. Eur Respir J **2014**; 44:873–84.
20. Kon SS, Canavan JL, Jones SE, et al. Minimum clinically important difference for the COPD assessment test: a prospective analysis. Lancet Respir Med **2014**; 2:195–203.
21. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Leidy NK. Development and first validation of the COPD assessment test. Eur Respir J **2009**; 34:648.
22. Su B, Liu T, Fan H, et al. Inflammatory markers and the risk of chronic obstructive pulmonary disease: a systematic review and meta-analysis. PLoS One **2016**; 11:e0150586.
23. Poynter ME, Irvin CG. Interleukin-6 as a biomarker for asthma: hype or is there something else? Eur Respir J **2016**; 48:979–81.
24. Li X, Hastie AT, Peters MC, et al. Investigation of the relationship between IL-6 and type 2 biomarkers in patients with severe asthma. J Allergy Clin Immunol **2020**; 145: 430–3.
25. Narendran G, Andrade BB, Porter BO, et al. Paradoxical tuberculosis immune reconstitution inflammatory syndrome (TB-IRIS) in HIV patients with culture confirmed pulmonary tuberculosis in India and the potential role of IL-6 in prediction. PLoS One **2013**; 8:e63541.
26. Ravimohan S, Tamuhla N, Nfanyana K, et al. Robust reconstitution of tuberculosis-specific polyfunctional CD4+ T-cell responses and rising systemic interleukin 6 in

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paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. Clin Infect Dis **2016**; 62:795–803.
27. Saunders BM, Frank AA, Orme IM, Cooper AM. Interleukin-6 induces early gamma interferon production in the infected lung but is not required for generation of specific immunity to Mycobacterium tuberculosis infection. Infect Immun **2000**; 68:3322–6.
28. Mesquita ED, Gil-Santana L, Ramalho D, et al. Associations between systemic inflammation, mycobacterial loads in sputum and radiological improvement after treatment initiation in pulmonary TB patients from Brazil: a prospective cohort study. BMC Infect Dis **2016**; 16:368.
29. Boni FG, Hamdi I, Koundi LM, Shrestha K, Xie J. Cytokine storm in tuberculosis and IL-6 involvement. Infect Genet Evol **2022**; 97:105166.
30. Chowdhury IH, Ahmed AM, Choudhuri S, et al. Alteration of serum inflammatory cytokines in active pulmonary tuberculosis following anti-tuberculosis drug therapy. Mol Immunol **2014**; 62:159–68.
31. Vivekanandan MM, Adankwah E, Aniagyei W, et al. Plasma cytokine levels characterize disease pathogenesis and treatment response in tuberculosis patients. Infection **2023**; 51:169–79.
32. Zhang G, Zhou B, Wang W, et al. A functional single-nucleotide polymorphism in the promoter of the gene encoding interleukin 6 is associated with susceptibility to tuberculosis. J Infect Dis **2012**; 205:1697–704.
33. Nosik M, Ryzhov K, Rymanova I, et al. Dynamics of plasmatic levels of pro- and anti-inflammatory cytokines in HIV-infected individuals with M. tuberculosis co-infection. Microorganisms **2021**; 9:2291.
34. Matsuyama W, Hashiguchi T, Matsumuro K, et al. Increased serum level of vascular endothelial growth factor in pulmonary tuberculosis. Am J Respir Crit Care Med **2000**; 162:1120–2.
35. Harding JS, Herbath M, Chen Y, et al. VEGF-a from granuloma macrophages regulates granulomatous inflammation by a non-angiogenic pathway during mycobacterial infection. Cell Rep **2019**; 27:2119–31.e6.
36. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med **2003**; 9:669–76.
37. Kranenburg AR, de Boer WI, Alagappan VK, Sterk PJ, Sharma HS. Enhanced bronchial expression of vascular endothelial growth factor and receptors (Flk-1 and Flt-1) in patients with chronic obstructive pulmonary disease. Thorax **2005**; 60:106–13.
38. Wang L, Xu Z, Chen B, et al. The role of vascular endothelial growth factor in small-airway remodelling in a rat model of chronic obstructive pulmonary disease. Sci Rep **2017**; 7:41202.
39. Poto R, Loffredo S, Palestra F, Marone G, Patella V, Varricchi G. Angiogenesis, lymphangiogenesis, and inflammation in chronic obstructive pulmonary disease (COPD): few certainties and many outstanding questions. Cells **2022**; 11:1720.
40. Polena H, Boudou F, Tilleul S, et al. Mycobacterium tuberculosis exploits the formation of new blood vessels for its dissemination. Sci Rep **2016**; 6:33162.
41. Saghazadeh A, Rezaei N. Vascular endothelial growth factor levels in tuberculosis: a systematic review and meta-analysis. PLoS One **2022**; 17:e0268543.
42. Yhi JY, Park DW, Min JH, et al. Measurement of levels of fractional exhaled nitric oxide in patients with pulmonary tuberculosis. Int J Tuberc Lung Dis **2016**; 20:1174–80.
43. López JW, Loader MI, Smith D, et al. Exhaled nitric oxide is not a biomarker for pulmonary tuberculosis. Am J Trop Med Hyg **2018**; 98:1637–9.
44. Stek C, Allwood B, Du Bruyn E, et al. The effect of HIV-associated tuberculosis, tuberculosis-IRIS and prednisone on lung function. Eur Respir J **2020**; 55:1901692.

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