

History of tuberculosis is associated with lower exhaled nitric oxide levels in HIV-infected children

Evgeniya Sovershaeva^a, Katharina Kranzer^{b,c,d}, Grace Mchugh^d,
Tsitsi Bandason^d, Edith D. Majonga^{b,d}, Omar S. Usmani^e,
Sarah Rowland-Jones^f, Tore Gutteberg^g, Trond Flægstad^{h,i},
Rashida A. Ferrand^{b,d} and Jon Ø. Odland^{a,j,k}

Objective: HIV disrupts host defense mechanisms and maintains chronic inflammation in the lung. Nitric oxide is a marker of lung inflammation and can be measured in the exhaled air. We investigated the relationship between exhaled nitric oxide (eNO), HIV status and airway abnormalities in perinatally HIV-infected children aged 6–19 years.

Design: A cross-sectional study.

Methods: HIV-infected individuals on antiretroviral therapy and HIV-uninfected children with no active tuberculosis (TB) or acute respiratory tract infection were recruited from a public hospital in Harare, Zimbabwe. Clinical history was collected and eNO testing and spirometry was performed. The association between eNO and explanatory variables (HIV, FEV1 z-score, CD4⁺ cell count, viral load, history of TB) was investigated using linear regression analysis adjusted for age, sex and time of eNO testing.

Results: In total, 222 HIV-infected and 97 HIV-uninfected participants were included. Among HIV-infected participants, 57 (25.7%) had a history of past TB; 56 (25.2%) had airway obstruction, but no prior TB. HIV status was associated with lower eNO level [mean ratio 0.79 (95% confidence interval, 95% CI 0.65–0.97), $P=0.03$]. Within the HIV-infected group, history of past TB was associated with lower eNO levels after controlling for age, sex and time of eNO testing [0.79 (95% CI 0.67–0.94), $P=0.007$].

Conclusion: HIV infection and history of TB were associated with lower eNO levels. eNO levels may be a marker of HIV and TB-induced alteration in pulmonary physiology; further studies focused on potential causes for lower eNO levels in HIV and TB are warranted.

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^aDepartment of Community Medicine, Faculty of Health Sciences, UiT-The Arctic University of Norway, Tromsø, Norway, ^bClinical Research Department, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK, ^cNational Tuberculosis Reference Laboratory, Research Centre Borstel, Borstel, Germany, ^dBiomedical Research and Training Institute, 10 Seagrave Road, Harare, Zimbabwe, ^eNational Heart and Lung Institute, Imperial College London, London, ^fNuffield Department of Medicine, University of Oxford, Oxford, UK, ^gDepartment of Microbiology and Infection Control, ^hDepartment of Pediatrics, University Hospital of North Norway, ⁱPediatric Research Group, Department of Clinical Medicine, Faculty of Health Sciences, UiT-The Arctic University of Norway, Tromsø, ^jDepartment of Public Health and Nursing, Faculty of Medicine and Health Sciences, NTNU The Norwegian University of Science and Technology, Trondheim, Norway, and ^kSchool of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria, South Africa.

Correspondence to Evgeniya Sovershaeva, Department of Community Medicine, Faculty of Health Sciences, UiT-The Arctic University of Norway, Hansine Hansens veg 18, Tromsø 9019, Norway.

Tel: +4746310426; e-mail: evgeniya.rovershaeva@uit.no

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Background

Access to antiretroviral therapy (ART) has substantially increased survival of children with HIV, with increasing numbers now reaching adolescence and adulthood. However, it is becoming increasingly apparent that HIV infection is associated with chronic comorbidities in the ART era [1,2].

In recent years, several studies from sub-Saharan Africa have shown that chronic respiratory symptoms and airway obstruction are common among children taking ART [3–6], with obliterative bronchiolitis and bronchiectasis being common noncommunicable causes of chronic respiratory symptoms [7–12]. Furthermore, ART is not fully protective against tuberculosis (TB) and individuals with HIV on ART have an estimated 10% four-year risk of developing TB compared with a 10% lifetime risk in the general population of South Africa [13,14]. Moreover, children with HIV experience more rapid TB progression, poorer response to treatment and are at a higher risk of TB recurrence [15,16]. The long-term consequences of TB often persist despite successful treatment, adding to the burden of chronic lung disease [17].

Even though the pathogenesis of chronic lung disease in HIV-infected individuals is not completely understood, chronic airway inflammation and oxidative stress are thought to play a distinct role [12,18]. One of the markers of airway inflammation is nitric oxide that is produced by various cells in the body, including the respiratory tract. Under normal physiological conditions, there is constant production of NO in the lung that regulates bronchodilation, neurotransmission, mucous secretion and the inflammatory response [19,20]. The presence of NO can be easily measured in exhaled air using a noninvasive, quantitative, standardized method [21]. Increased levels of exhaled nitric oxide (eNO) are a well known marker of eosinophilic airway inflammation in asthma, while reduced eNO levels have been reported in patients with active lung TB, cystic fibrosis and in smokers [22–24]. Increased eNO levels are explained by overexpression of the inducible NO synthase (iNOS), which often correlates with systemic and lung inflammation, while decreased eNO levels may be due to lack of the substrate (L-arginine); reduced activity of iNOS in the airways (which may be directly inhibited as an immune evasion mechanism by *Mycobacterium tuberculosis*) or thick mucus that may inhibit the NO diffusion into exhaled air (e.g. in cystic fibrosis) [25–27].

Current data suggest that eNO may be a useful tool to guide asthma management in both children and adults [28,29], though its clinical application for other lung disorders is unclear. A recent study showed that increased eNO levels may predict clinical response to inhaled corticosteroids in adults with nonspecific respiratory symptoms [30].

Data investigating the association between HIV infection and eNO levels are scarce [31]. Although lower levels of eNO were found in one study among 36 HIV-infected patients [31] compared with HIV-uninfected healthcare workers, no significant difference in eNO levels were reported in patients with HIV-TB coinfection compared with HIV-uninfected patients with TB [22,32]. No studies so far have investigated the level of eNO in children with HIV-associated chronic lung disease.

In this study, we aimed to compare the levels of eNO in children with and without HIV and investigated the association between eNO and history of TB and airway obstruction in HIV-infected children on ART.

Materials and methods

Study population

This cross-sectional study was conducted between April 2017 and August 2018 as a substudy of a randomized controlled clinical trial investigating the effect of azithromycin in children with HIV-associated chronic lung disease (BREATHE trial, clinicaltrials.gov identifier NCT02426112). The detailed study protocol has been published elsewhere [33]. Participants were eligible for the trial if they were aged 6–19 years, had perinatally acquired HIV and had been taking ART for at least 6 months, and had no evidence of active TB or acute respiratory tract infection. The trial is being conducted in Malawi and Zimbabwe, but the substudy was conducted in Zimbabwe only at the Harare Central hospital paediatric HIV clinic, the largest public sector HIV clinic in Harare.

TB may cause lung tissue changes (bronchovascular distortion, fibrotic lesions and cavitation) persisting beyond the acute episode [17]; these morphological changes may influence eNO levels. We expected to find a high prevalence of past TB because of the high incidence of TB in people living with HIV. Thus, we divided HIV-infected participants into three groups on the basis of clinical history and spirometry results: normal lung function and no TB history; history of TB irrespective of airway obstruction; and airway obstruction and no TB history. Airway obstruction was defined as forced expiratory volume in 1 s (FEV₁) z-score less than -1.64 with no reversibility (<12% improvement in FEV₁ after salbutamol 200 µg inhaled using a spacer).

In addition, a group of HIV-uninfected participants was recruited from the same catchment area as the HIV-infected group. HIV-uninfected children (were tested for HIV at enrolment) aged 6–16 years with no prior history of heart/lung diseases (including history of TB), no reported chest pain after exercise, shortness of breath

during exercise or chronic cough and normal lung function were eligible for the study as a control group.

Ethical approval

The study was approved by the London School of Hygiene and Tropical Medicine Ethics Committee; the Harare Central Hospital Ethics Committee; the Medical Research Council of Zimbabwe; Regional Committee for Medical and Health Research Ethics in Norway (2015/1650). Written informed consent by guardian and assent by participants aged less than 18 years were obtained. Those aged 18 years and older gave independent consent.

Study procedures

A detailed questionnaire regarding demographic and clinical history was administered to all participants. Participants were explicitly asked about current and previous respiratory symptoms (chronic cough, wheezing and dyspnoea) in the past 3 months. Self-reported and/or physician-diagnosed lung diseases and/or atopic disorders including rhinitis and atopic dermatitis were recorded. Participants were classified as having asthma based on clinical history of diagnosed asthma or self-reported symptoms of asthma (episodes of wheezing, nocturnal tightness in the chest, attacks of shortness of breath following strenuous activity, at rest or at night-time) or treatment with asthma-specific medications (short-term/long-term β_2 agonists; inhaled corticosteroids; leukotriene receptor antagonists; methylxanthines).

Measurement of exhaled nitric oxide

The level of eNO was measured by the electrochemical analyser (NIOX VERO, Circassia, UK) according to American Thoracic Society (ATS) guidelines [21] and expressed in parts per billion (ppb). The calibration of the machine and quality control were performed according to the manufacturer's instructions. The participants were asked to sit and rest for a minimum of 5 min before the measurement. Repeated exhalations were performed in order to obtain at least two measurements that agreed within 10%. Measurements were taken with a minimum of 30 s rest time in between. Up to six eNO measurement attempts were made and the mean eNO value was calculated from two eNO measurements with minimal difference between them. All eNO measurements were performed between 0800 and 1400 h and exact time of the testing was recorded.

Spirometry

Spirometry was performed after eNO measurement using EasyOne spirometer (ndd Medical Technologies Inc., Andover, Massachusetts, USA) according to the ATS guidelines. FEV1 z -scores were calculated using prediction equations from the Global Lung Function Initiative [34].

Laboratory tests

All participants provided blood samples for full blood count, and for participants with HIV, also for HIV

viral load and CD4⁺ cell count testing. HIV viral load was measured using the Gene Xpert assay, with a limit of detection of 40 copies/ml (XpertTM HIV-1 Viral Load; Cepheid, Sunnyvale, California, USA) and CD4⁺ cell count was measured as a point of care test using a PimaTM Analyser (Alere, Orlando, Florida, USA). Anaemia was defined according to WHO criteria (haemoglobin <11.5 g/dl for children 6–11.9 years; haemoglobin <12 g/dl for children 12–14.9 years; haemoglobin <12 g/dl for girls aged ≥ 15 years; haemoglobin <13 g/dl for boys aged ≥ 15 years) [35].

Data collection

Electronic record forms (for questionnaires) collected on Google Nexus tablets (Google, Mountain View, California, USA) with OpenDataKit software and paper forms (for clinical tests) were used for data collection. Data from paper forms were extracted using CARDIFF TELEFORM character optical mark recognition software (Version 10.9). Data were stored in Microsoft Access database (Microsoft, Redmond, Washington, USA).

Statistical analysis

All statistical analyses were performed using STATA Version 14 (StataCorp LLC, College Station, Texas, USA). Values for eNO were presented as geometric mean with 95% confidence interval. Weight-for age and height-for-age z -scores were calculated using British 1990 Growth Reference Curves [36], with z -scores less than -2 representing wasting and stunting, respectively.

Characteristics between study groups were compared using Fisher's exact test (for categorical parameters) and Kruskal–Wallis or Wilcoxon rank sum test (for continuous parameters). The values of eNO were not normally distributed and therefore were log transformed to approximate normality. The analyses were performed with log-transformed eNO data and back transformed to present geometric mean. The association between eNO levels and *a priori* defined explanatory variables was studied using linear regression analysis. Explanatory variables included HIV status, age, sex, anthropometric parameters, haemoglobin level, haematocrit, white blood cell count, neutrophil count, eosinophil count, FEV1 z score, atopic status, passive smoking, for all participants; CD4⁺ cell count, viral load, history of TB and presence of airway obstruction, for participants with HIV only. Age, sex and exact time of eNO testing were adjusted for *a priori*. Parameters were included into multivariable linear regression model if they showed a significant effect on the prediction of eNO level in age, sex and time of eNO testing adjusted models at P value less than 0.05. Adjustment for unbalanced parameters (for HIV-infected group) was also performed. Variance inflation factor was used to detect multicollinearity in the multivariable models. Height was not included into multivariable regression models due to high collinearity with age. The linear association between eNO and continuous variables

was estimated graphically. Residual analysis with residual plots and normal probability plots of residuals confirmed no violation of the linear regression assumptions.

Results

In total, 227 HIV-infected and 104 HIV-uninfected children were enrolled. Five participants ($n=1$ HIV-infected, $n=4$ HIV-uninfected) aged 6–7 years did not understand the procedure and were excluded from the study. Of the remaining participants, acceptable eNO measurements were obtained from 225 HIV-infected and 98 HIV-uninfected children. A total of four participants were excluded from the study for the following reasons: abnormal spirometry ($n=1$, HIV-uninfected) and missing spirometry data ($n=3$, HIV-infected).

Baseline characteristics of study participants and their distribution across the four groups are presented in Table 1. The median time on ART among HIV-infected children was 6.6 years (IQR 4.0–8.4) and comparable across the three different groups. HIV-infected children overall were more likely to be wasted and stunted compared with HIV-uninfected children (wasted: 41.9 vs. 3.1%, $P<0.001$; stunted: 42.8 vs. 4.1%, $P<0.001$). Anaemia was more common in HIV-infected than HIV-uninfected children (31.2 vs. 9.3%, $P<0.001$). Exposure to passive smoking was more common in HIV-infected than HIV-uninfected participants.

Among HIV-infected participants, those with a history of prior TB had significantly higher prevalence of wasting than participants with no airway abnormalities (52.6 vs. 28.4%, $P=0.001$, respectively). In addition, participants with a history of prior TB received a protease inhibitor based regimen more often than other two groups. The prevalence of both stunting and wasting was higher in participants with airway obstruction when compared with those with no airway abnormalities (stunted: 58.9 vs. 33.0%, $P<0.001$; wasted: 57.1 vs. 28.4%, $P<0.001$).

HIV status was associated with lower eNO levels after adjusting for age, sex and time of eNO testing [geometric mean ratio 0.79 (95% CI 0.65–0.97), $P=0.03$]. Among HIV-infected participants, those with a history of TB had significantly lower eNO levels than those without TB whether they had airway obstruction [geometric mean 13.7 (95% CI 12.2–15.5) vs. 16.9 (95% CI 14.3–19.8), $P=0.03$] or had no airway abnormalities [geometric mean 17.9 (95% CI 16.0–20.0), $P=0.003$] (Fig. 1). Linear regression analysis controlled for age, sex and time of eNO testing confirmed the association between past TB and lower eNO levels (Table 2).

The association between participant characteristics and eNO levels in HIV-infected and HIV-uninfected participants are presented in Tables 2 and 3, respectively. The level of eNO increased with increasing age and height in HIV-infected children. Higher haemoglobin level and neutrophil count were associated with higher eNO level in adjusted analysis. Female sex was associated

Table 1. Characteristics of study participants.

Variables	HIV-infected			HIV-uninfected ($N=97$)
	History of TB ($N=57$)	Airway obstruction, but no history of TB ($N=56$)	No TB, no obstruction ($N=109$)	
Age (years), Median (IQR)	15 (12–18)	15 (13–18)	16 (12–18)	10 (7–12)
Female sex, N (%)	26 (45.6)	18 (32.1)	71 (65.1)	47 (48.4)
Wasting (weight for age z-score <-2), N (%)	30 (52.6)	32 (57.1)	31 (28.4)	3 (3.1)
Stunted (height-for-age z-score <-2), N (%)	26 (45.6)	33 (58.9)	36 (33.0)	4 (4.1)
Passive smoking, N (%)	21 (36.8)	15 (26.8)	25 (22.9)	3 (3.1)
Living in high-density area, N (%)	53 (94.6)	49 (96.1)	100 (93.5)	95 (97.9)
eNO level (ppb), geometric mean (95% CI)	13.7 (12.2–15.5)	16.9 (14.3–19.8)	17.9 (16.0–20.0)	16.5 (14.8–18.5)
FEV1 z-score, Median (IQR)	-1.8 (-2.3 to -1.3)	-2.1 (-2.7 to -1.9)	-1.1 (-1.3 to 0.4)	-0.2 (-0.6 to 0.3)
Presence of atopy (asthma, eczema, allergic rhinitis), N (%)	12 (21.0)	12 (21.4)	13 (11.9)	10 (10.3)
Haemoglobin (g/dl), Median (IQR)	13 (11.9–13.7)	12.7 (11.6–13.6)	12.8 (11.7–14)	13.2 (12.4–13.9)
Haematocrit, %, Median (IQR)	36.7 (34.0–38.4)	35.2 (33.3–37.9)	36 (33.4–38.6)	36.3 (34.8–39)
White blood cell count ($\times 10^9/l$), Median (IQR)	4.3 (3.8–5.4)	4.6 (3.3–5.3)	4.2 (3.6–5)	5.3 (4.5–6.3)
Eosinophil count ($\times 10^9/l$), Median (IQR)	0.075 (0.04–0.12)	0.065 (0.02–0.13)	0.06 (0.03–0.16)	0.1 (0.06–0.2)
Neutrophil count ($\times 10^9/l$), Median (IQR)	1.7 (1.3–2.2)	1.7 (1.1–2.5)	1.8 (1.4–2.4)	2.2 (1.8–2.9)
Lymphocyte count ($\times 10^9/l$), Median (IQR)	2.0 (1.6–2.6)	2.1 (1.6–2.7)	2.0 (1.6–2.3)	2.3 (2.0–2.8)
Monocyte count ($\times 10^9/l$), Median (IQR)	0.39 (0.32–0.48)	0.39 (0.30–0.5)	0.35 (0.27–0.46)	0.47 (0.36–0.63)
Anaemia, N (%)	15 (26.8)	17 (32.7)	35 (32.7)	9 (9.3)
Viral load (\log_{10} copies/ml), Median (IQR)	2.7 (1.6–4.0)	2.5 (1.6–3.7)	2.1 (1.6–3.4)	–
CD4 ⁺ cell count (cells/ μ l), Median (IQR)	509 (339–702)	559 (326–728)	624 (355–779)	–
Years on ART, Median (IQR)	6.8 (4.6–9.1)	5.9 (3.6–7.8)	6.5 (4.0–8.4)	–
PI-based regimen, N (%)	25 (43.9)	12 (21.4)	12 (11.0)	–

ART, antiretroviral therapy; IQR, interquartile range; eNO, exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; PI, protease inhibitor.

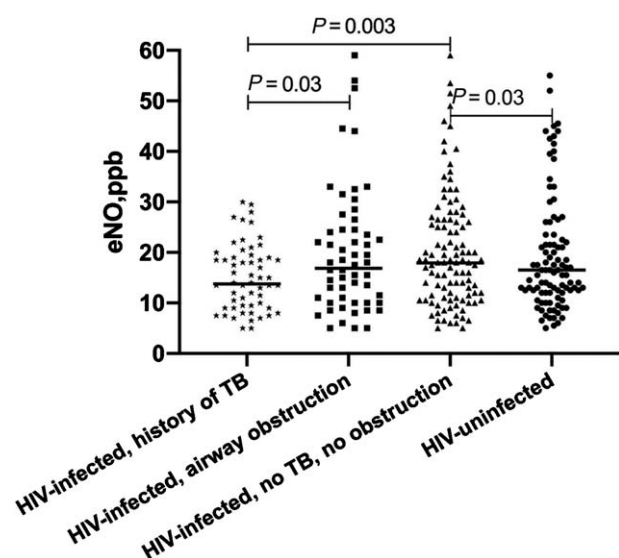


Fig. 1. Level of exhaled nitric oxide in HIV-infected and HIV-uninfected participants. Horizontal lines indicate geometric mean values.

with lower eNO level in adjusted analysis. Due to imbalances in wasting, stunting and use of protease inhibitor based regimen by HIV-infected groups, these variables were adjusted for in multivariable analysis. On multivariable linear regression, haemoglobin, neutrophil count, history of TB and age remained significantly associated with eNO levels in HIV-infected participants.

In HIV-uninfected individuals, anthropometric parameters (height and weight) were significantly positively associated with eNO level in univariate analysis, though the association became nonsignificant after accounting for age, sex and time of eNO testing. Higher haemoglobin level and haematocrit were associated with higher eNO level, while wasting was associated with lower eNO level in univariate and adjusted analysis. On multivariable analysis, wasting, haemoglobin and age remained significantly associated with eNO levels in HIV-uninfected participants.

Discussion

Our study showed that children with HIV infection have lower eNO levels than HIV-uninfected children. HIV-infected children with a prior history of TB had significantly lower eNO levels than those without prior TB.

There are few data on the levels of eNO in HIV-infected adults [31], and none among children. Two studies reported lower eNO levels associated with HIV infection among adults with [32] and without TB [31], mirroring the results of our study. However, a study including 19 HIV-infected and 126 HIV-uninfected adults with TB did not find any difference in eNO, but was likely underpowered [22]. The observed low eNO levels in

Table 2. Analysis of factors associated with exhaled nitric oxide level in HIV-infected participants.

Variables	Unadjusted analysis		Adjusted for age, sex and time of eNO testing		Multivariable model	
	GMR ^a (95% CI)	P	GMR ^a (95% CI)	P	GMR ^a (95% CI)	P
Age (years)	1.03 (1.00–1.05)	0.004	1.03 (1.01–1.05)	0.003	1.03 (1.01–1.05)	0.005
Female sex	0.89 (0.77–1.03)	0.13	0.85 (0.74–0.99)	0.04	0.86 (0.74–1.01)	0.07
Weight (kg)	1.01 (1.00–1.01)	0.002	1.01 (1.00–1.02)	0.16		
Height (cm)	1.01 (1.00–1.02)	<0.001	1.02 (1.01–1.03)	<0.001		
Weight-for-age z-score	1.01 (0.96–1.06)	0.72	1.00 (0.94–1.05)	0.94		
Height-for-age z-score	1.06 (1.00–1.13)	0.07	1.05 (0.99–1.13)	0.12		
Wasting (weight for age z-score < -2)	0.97 (0.83–1.13)	0.68	0.99 (0.85–1.16)	0.95	1.01 (0.84–1.22)	0.89
Stunted (height-for-age z-score < -2)	0.93 (0.80–1.08)	0.33	0.94 (0.81–1.09)	0.41	0.94 (0.80–1.12)	0.51
Passive smoking (ref. no)	1.19 (1.00–1.40)	0.04	1.17 (0.99–1.38)	0.06		
History of tuberculosis	0.78 (0.67–0.90)	0.004	0.79 (0.67–0.94)	0.007	0.80 (0.68–0.95)	0.013
FEV1 z-score	1.01 (0.95–1.07)	0.73	0.98 (0.92–1.05)	0.59		
FEV1 z-score < -1.64 (airway obstruction)	0.92 (0.79–1.08)	0.33	0.92 (0.78–1.09)	0.33		
Presence of atopy	0.94 (0.77–1.15)	0.54	0.94 (0.77–1.15)	0.55		
Haemoglobin (g/dl ²)	1.07 (1.03–1.12)	0.002	1.06 (1.01–1.11)	0.009	1.07 (1.02–1.12)	0.004
Haematocrit, %	1.02 (1.00–1.03)	0.023	1.01 (1.00–1.02)	0.12		
White blood cell count (log transformed)	1.17 (0.92–1.49)	0.20	1.57 (0.90–2.71)	0.11		
Eosinophil count (log transformed)	1.03 (0.97–1.10)	0.33	1.04 (0.97–1.11)	0.26		
Neutrophil count (log transformed)	1.19 (1.05–1.35)	0.012	1.46 (1.07–1.99)	0.016	1.19 (1.05–1.36)	0.008
Lymphocyte count (log transformed)	0.91 (0.78–1.08)	0.28	0.96 (0.66–1.41)	0.84		
Monocyte count (log transformed)	1.16 (0.96–1.40)	0.12	1.36 (0.89–2.08)	0.15		
Anaemia (ref. no)	0.97 (0.82–1.14)	0.71	0.97 (0.83–1.15)	0.75		
HIV viral load (log ₁₀ copies/ml)	1.00 (0.95–1.05)	0.95	0.99 (0.95–1.05)	0.86		
CD4 ⁺ cell count (cells/μl)	1.00 (0.99–1.00)	0.07	1.00 (0.99–1.01)	0.34		
Years on ART	0.99 (0.97–1.02)	0.70	0.98 (0.95–1.00)	0.13		
PI-based regimen (NNRTI-based as ref.)	0.85 (0.71–1.02)	0.09	0.86 (0.72–1.03)	0.11	0.90 (0.75–1.08)	0.27

ART, antiretroviral therapy; CI, confidence interval; eNO, exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; PI, protease inhibitor.
^aGeometric mean ratio.

Table 3. Analysis of factors associated with exhaled nitric oxide level in HIV-uninfected participants.

Variables	Unadjusted analysis		Adjusted for age, sex and time of eNO testing		Multivariable model	
	GMR ^a (95% CI)	P	GMR ^a (95% CI)	P	GMR ^a (95% CI)	P
Age	1.09 (1.05–1.13)	<0.001	1.08 (1.05–1.12)	<0.001	1.07 (1.03–1.11)	0.001
Female sex	1.25 (1.01–1.56)	0.04	1.14 (0.93–1.40)	0.21	1.14 (0.92–1.41)	0.22
Weight (kg)	1.02 (1.01–1.03)	<0.001	1.01 (0.99–1.03)	0.42		
Height (cm)	1.02 (1.00–1.02)	<0.001	1.01 (0.99–1.03)	0.15		
Weight-for-age z-score	1.00 (0.88–1.13)	0.99	1.00 (0.88–1.13)	0.99		
Height-for-age z-score	1.04 (0.93–1.16)	0.50	1.04 (0.93–1.17)	0.50		
Wasting (weight for age z-score < -2)	0.45 (0.24–0.85)	0.014	0.45 (0.24–0.85)	0.015	0.52 (0.29–0.92)	0.03
Stunted (height-for-age z-score < -2)	0.70 (0.40–1.22)	0.21	0.70 (0.40–1.23)	0.21		
Passive smoking (ref. no)	1.52 (0.80–2.88)	0.20	1.12 (0.61–2.04)	0.71		
FEV1 z-score	1.01 (0.87–1.18)	0.87	1.01 (0.86–1.18)	0.90		
Presence of atopy	1.08 (0.75–1.56)	0.67	1.05 (0.75–1.47)	0.75		
Haemoglobin (g/dl ²)	1.13 (1.05–1.23)	0.003	1.09 (1.01–1.19)	0.031	1.09 (1.00–1.18)	0.04
Haematocrit, %	1.07 (1.04–1.11)	<0.001	1.05 (1.01–1.08)	0.009		
White blood cell count (log transformed)	0.89 (0.66–1.20)	0.45	0.94 (0.49–1.81)	0.86		
Eosinophil count (log transformed)	1.03 (0.92–1.14)	0.62	1.05 (0.95–1.16)	0.32		
Neutrophil count (log transformed)	0.97 (0.78–1.20)	0.76	1.06 (0.66–1.71)	0.79		
Lymphocyte count (log transformed)	0.94 (0.64–1.37)	0.74	1.04 (0.45–2.40)	0.92		
Monocyte count (log transformed)	0.81 (0.62–1.05)	0.12	0.78 (0.44–1.40)	0.41		
Anaemia (ref. no)	1.13 (0.77–1.66)	0.52	1.13 (0.77–1.67)	0.53		

CI, confidence interval; eNO, exhaled nitric oxide; FEV1, forced expiratory volume in 1 s.

^aGeometric mean ratio.

HIV-infected children may be explained by immunological and pathophysiological mechanisms. Alveolar macrophages play a central role in the defense against bacterial and mycobacterial infection; they may become infected with HIV and constitute the primary reservoir of the virus in the lung [37,38]. During HIV infection, alveolar macrophage function is compromised [37], with evidence of oxidative stress [39] and reduced ability to phagocytose and kill bacteria [40,41]. HIV-associated chronic activation of inflammatory cells in the alveolar space may further compromise the host response against infectious stimuli [42,43]. Thus, low levels of eNO detected in patients with HIV may reflect the inability of alveolar macrophages to produce eNO as part of the lung innate immune system. This may contribute to persistent lung immune dysfunction.

eNO levels are known to increase with age. This needs to be taken into consideration when interpreting eNO levels measured in children. Values of eNO increase linearly between 6 and 16 years in parallel with the somatic growth [44]. The ATS guidelines recommend adjustment for age when interpreting the eNO levels in children younger than 12 years of age [45]. In addition, the majority of published studies have reported an association between height and eNO levels [46–49]. Total airway mucosal surface area for NO diffusion increases with increasing age and height, thus leading to higher eNO levels [50]. In our study, both age and height were significantly associated with eNO levels in HIV-infected children. Given that HIV-infected children are generally smaller than their HIV-uninfected peers, this might partly explain the lower eNO levels among HIV-infected children observed in our study.

An interesting finding of our study was that history of TB in HIV-infected participants was associated with lower eNO levels. No studies have investigated the level of eNO in individuals with a history of TB, though a number of studies were conducted in adult patients with active lung TB [22,32,51]. At least two studies found that eNO levels were lower in patients with newly diagnosed untreated lung TB than healthy controls [22,32]. Moreover, one of these studies reported an association between low eNO levels at the time of TB diagnosis and severity of disease [22].

M. tuberculosis (Mtb) may persist in alveolar macrophages for a prolonged period of time [52,53]. Nonreplicating persistence of *Mtb* may maintain chronic airway inflammation and may lead to lung fibrosis [17]. There is some evidence from in-vitro studies that nitric oxide has a protective role in the progression of lung fibrosis [54]. Thus, lower levels of eNO in patients treated for TB in our study may reflect altered lung immune response mediating the development of chronic lung complications.

No association was found between airway obstruction measured as FEV1 z score less than -1.64 and eNO levels in HIV-infected children. This is in line with several other studies reporting no association between FEV1 and eNO in adults with chronic obstructive lung disease [55,56], implying that eNO has probably no pathophysiological role in the obstruction, but rather a marker of the underlying condition, as seen in eosinophilic inflammation in asthma patients [57].

In our study, we found significant positive associations between several blood parameters and eNO levels. The

associations between haemoglobin level, haematocrit and eNO may reflect physiological processes at the level of endothelium. Increased haematocrit results in increased shear stress and production of NO. The positive association between eNO and blood neutrophils observed in HIV-infected participants may be explained by the fact that neutrophils can produce nitric oxide in response to foreign stimuli [58]. However, in cystic fibrosis patients, an inverse correlation between blood neutrophils and eNO levels was reported, which was thought to be due to the ability of neutrophils to produce superoxide that downregulates nitric oxide production [24]. The observed associations emphasize the need to interpret eNO results with caution. eNO levels are influenced by a multitude of factors, including demographic, anthropometric and biological factors, thus questioning the utility of eNO as a clinical tool in conditions beyond asthma. Although noninvasive nature and measurement simplicity make eNO testing an attractive tool for resource-limited settings, it is too early to forecast its applicability and clinical usefulness among HIV-infected children with airway abnormalities in a given setting.

The strengths of the study are its prospective design, relatively large sample size, use of standardized protocol for eNO testing and spirometry performed by well trained nurses. Study limitations include self-report of TB with no information regarding TB localization (pulmonary or extrapulmonary), raising concerns of recall bias. Among other determinants that may have an impact on eNO levels and that were not assessed in the present study are genetic factors (affecting the activity of nitric oxide synthase), environmental factors (air pollution) and socioeconomic factors.

In summary, our study shows that both HIV infection and history of TB are associated with lower eNO levels, with no association found between eNO and airway obstruction. The low eNO levels may be a marker of abnormal pulmonary physiology due to perinatally acquired HIV infection and post-TB lung changes. The role of nitric oxide in the pathogenesis of HIV infection needs further investigation.

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Conflicts of interest

There are no conflicts of interest.

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