Received: 18 April 2023 | Accepted: 26 September 2023

DOI: 10.1111/hiv.13565

ORIGINAL ARTICLE

Exhaled nitric oxide is associated with inflammatory biomarkers and risk of acute respiratory exacerbations in children with HIV-associated chronic lung disease

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Funding information
Norges Forskningsråd; Global Health and Vaccination Research (GLOBVAC) programme; Helse Nord RHF, Grant/Award Numbers: HNF 1539-20, HNF 1387-17; Wellcome Trust PhD Programme in Genomic Medicine and Statistics, Grant/Award Number: 108 861/B/15/Z; Wellcome Trust, Grant/Award Number: 206 316/Z/17/Z; UK Medical Research Council; UK Department for International Development (DFID); European Union, Grant/Award Number: MR/R010161/1

Abstract

Objectives: Chronic lung disease is a recognized complication in children with HIV. Acute respiratory exacerbations (ARE) are common among this group and cause significant morbidity. Exhaled nitric oxide (eNO) is a known marker of local airway inflammation. We investigated the association between eNO and ARE, biomarkers of systemic inflammation, and the effect of azithromycin on eNO levels.

Methods: Individuals aged 6–19 years with HIV-associated chronic lung disease in Harare, Zimbabwe, were enrolled in a placebo-controlled randomized trial investigating the effect of 48-week azithromycin treatment on lung function and ARE. eNO levels and biomarkers were measured at inclusion and after treatment in a consecutively enrolled subset of participants. Linear regression and generalized linear models were used to study associations between eNO and ARE, biomarkers, and the effect of azithromycin on eNO levels.

Results: In total, 172 participants were included in this sub-study, 86 from the placebo group and 86 from the azithromycin group. Participants experiencing

at least one ARE during follow-up had significantly higher eNO levels at baseline than participants who did not (geometric mean ratio 1.13, 95% confidence interval [CI] 1.03–1.24, $p = 0.015$), adjusted for trial arm, age, sex and history of tuberculosis. Matrix metalloproteinase (MMP)-3, -7, and -10 were significantly associated with higher baseline eNO levels. At 48 weeks, azithromycin treatment did not affect eNO levels (geometric mean ratio 0.86, 95% CI 0.72–1.03, $p = 0.103$).

Conclusion: Higher baseline eNO levels were a risk factor for ARE. eNO was associated with proinflammatory biomarkers previously found to contribute to the development of chronic lung disease. The potential use of eNO as a marker of inflammation and risk factor for ARE in HIV-associated chronic lung disease needs further investigation.

KEYWORDS

Africa, biomarkers, chronic lung disease, exhaled nitric oxide, HIV, inflammation

INTRODUCTION

The scale-up of antiretroviral therapy (ART) has led to increasing numbers of children perinatally infected with HIV surviving into adolescence and adulthood. Data show that ~30% of children and adolescents with HIV in sub-Saharan Africa present with chronic respiratory symptoms and/or airway obstruction [1–3]. Obliterative bronchiolitis and bronchiectasis are the most common forms of HIV-associated chronic lung disease (HCLD) [2, 4]. These children are at high risk of developing acute respiratory exacerbations (ARE); respiratory infections are among the most common causes of hospitalizations and therefore a significant cause of morbidity in this group [5]. The pathogenesis of HCLD is multifactorial and not completely understood. Understanding the aetiology of chronic HIV-associated comorbidities such as HCLD [6, 7] is a growing research priority to establish optimal treatment strategies and tools to monitor disease progression.

Recent studies have suggested that systemic immune activation associated with HIV infection persists despite effective ART and may play a role in in the development of chronic HIV-related comorbidities, including HCLD [8, 9]. Moreover, persistent inflammation due to repeated respiratory infections and a high prevalence of tuberculosis (TB) in this group may also contribute to lung damage [10]. In adults, data show that HCLD is associated with elevated levels of systemic proinflammatory biomarkers such as C-reactive protein, interleukin-6, tumour necrosis factor-α, and T-cell activation [11]. We previously described how matrix metalloproteinases (MMP)-1, -7, and -10, as well as biomarkers of inflammation, interferon (IFN)-γ, C-reactive protein, and interferon gamma-induced protein-10 were associated with HCLD in children and adolescents [12].

Administration of macrolide antibiotics, such as azithromycin, is thought to be beneficial in patients with chronic airway diseases [13, 14]. Azithromycin has bacteriostatic activity and anti-inflammatory properties, reducing the expression of several pro-inflammatory cytokines [13–15]. Macrolide treatment can reduce the number of exacerbations and have immunomodulatory effects in patients with a variety of chronic lung diseases (CLDs) [5, 13, 14, 16, 17]. A recent randomized controlled trial [18] demonstrated that children with HCLD who received once-weekly azithromycin for 48 weeks had a significantly lower risk of ARE than children who received placebo [5]. In the same participants, reduced levels of plasma-soluble biomarkers of inflammation were observed in the azithromycin arm [19].

No studies to date have investigated the role of local airway inflammation in this group of patients and the potential effects of treatment. Exhaled nitric oxide (eNO) is by far the most widely studied biomarker of local inflammation in CLD. Elevated levels of eNO have been used as a marker of eosinophilic airway inflammation in asthma and are suggested to be useful in diagnosing and monitoring of asthmatic disease [20–22]. eNO can easily be measured in exhaled air by non-invasive, standardized methods [21]. These features could make the use of eNO as a clinical marker of inflammation and disease progression relevant in resource-limited settings if prices and availability beyond clinical trials improve.

The aim of this study was to investigate the effect of azithromycin treatment on levels of eNO, a marker of

local inflammation in the lungs, in children and adolescents with HCLD. We also assessed the association between eNO and the risk of ARE in children with HCLD and described the association between eNO and plasma-soluble biomarkers of systemic inflammation.

MATERIALS AND METHODS

Study participants

This study was nested within the BREATHE trial; a double-blinded, randomized, placebo-controlled trial of weekly weight-adjusted azithromycin for 48 weeks in individuals aged 6–19 years with HCLD in Harare, Zimbabwe, and Blantyre, Malawi (BREATHE trial, clinicaltrials.gov, identifier NCT02426112). The inclusion criteria for the BREATHE trial were age 6–19 years, perinatally acquired HIV, taking ART for at least 6 months, having CLD (defined as forced expiratory volume in 1 second [FEV_1] z-score lower than −1.0), no history or symptoms of active TB or any acute respiratory tract infection. All participants were screened for TB using the Xpert MTB/RIF assay upon enrolment and excluded upon a diagnosis of active TB. Data collection was performed between April 2017 and June 2019. The detailed study protocol and trial main results are published elsewhere [18, 23]. Participants were randomized 1:1 using block randomization stratified by trial site.

In this study, only participants from Harare, Zimbabwe, were included. Measurement of eNO started after the first 68 participants were enrolled to the main study (following an ethics amendment). Thereafter, 173 consecutively enrolled and randomized participants underwent eNO measurements in addition to blood samples and spirometry at baseline and after 48 weeks of treatment.

All participants completed interview-administered questionnaires to ascertain demographic and clinical history. Both self-reported and physician-diagnosed lung diseases and atopic disorders, including rhinitis and atopic dermatitis and asthma, were recorded. Participants were encouraged to attend an unscheduled visit if they developed any acute respiratory symptoms. ARE was defined as new or worsening respiratory symptoms, with or without fever or symptoms of infection as assessed by a clinician. Those presenting with incident acute respiratory symptoms had nasal swabs and sputum samples taken and were treated with co-amoxiclav for 10 days. If they did not show any improvement after treatment, chest X-rays and TB culture on expectorated sputum was performed. A more detailed description is published elsewhere [5].

Ethical approvals

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, and the Regional Committee for Medical and Health Research Ethics in Norway (2015/1650). Guardians gave written informed consent, and participants aged <18 years provided assent. Those aged >18 years gave independent consent.

Exhaled NO

Levels of eNO were measured using an electrochemical analyser (NIOX VERO, Circassia, UK) according to American Thoracic Society guidelines, recorded in parts per billion [21]. Repeated exhalations of eNO were made to obtain at least two measurements that agreed within 10%. Details of measurements are described elsewhere [24]. The mean eNO value was calculated from the two measurements with the least difference between them. Where the differences between three measurements were the same, we calculated the mean of all three measurements.

Laboratory tests

All participants provided blood samples for full blood count, HIV viral load, and $CD4^+$ T-cell count, as previously described [24]. Soluble biomarkers were measured from cryopreserved plasma, stored at −80°C before use, using a Luminex multiplex bead assay on a MagPix instrument according to manufacturer's protocol (Luminex technology, Hertogenbosch, Netherlands). A detailed description is published elsewhere [12]. Anaemia was defined according to World Health Organization criteria [25].

Data collection/storage

Electronic record forms (for questionnaires) were collected on Google Nexus tablets (Google, Mountain View, CA, USA) with OpenDataKit software. Paper forms were used for data collection of clinical tests. Data from the paper forms were extracted using CARDIFF TELEFORM character optical mark recognition software (version 10.9). Data were managed in a Microsoft Access database (Microsoft, Redmond, WA, USA).

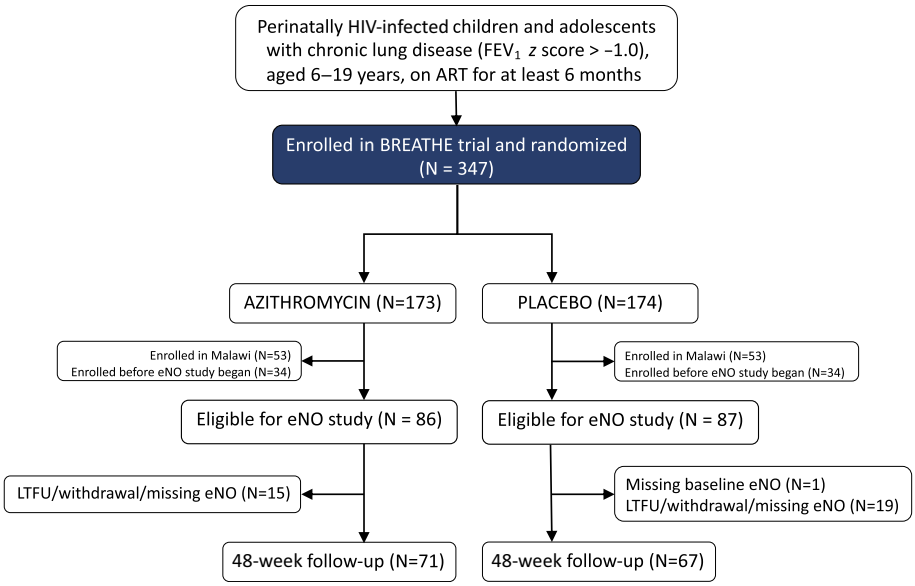

FIGURE 1 Study flow chart. ART, antiretroviral therapy; eNO, exhaled nitric oxide; LTFU, lost to follow-up.

Statistical analysis

Statistical analyses were performed using R (version 4.2.0) and IBM SPSS (version 28.0.0). Characteristics between participants in the azithromycin and placebo groups were compared using the Wilcoxon rank-sum test for continuous parameters and Fisher's exact test for categorical parameters. Data are shown as medians with interquartile ranges or geometric means with 95% confidence intervals (CIs). eNO values were natural log-transformed to approximate normality for statistical analysis and back transformed to geometric means with 95% CIs for presentations. Weight-for-age and height-for-age z-scores were calculated using British 1990 growth reference curves [26]; those with z-scores lower than −2 were characterized as being underweight and stunted, respectively.

T-test and paired *T*-test were used to compare eNO levels between trial groups and time points. Plots were made using the *ggplot2* package in R studio [27]. A generalized linear regression model (GLM) was used to describe the effect of azithromycin on eNO levels. GLM was adjusted for age, sex, history of TB, and baseline eNO levels. The same model was used to determine whether there was an association between baseline levels of eNO and having at least one ARE during the study period. Adjustments were made for trial group, age, sex, and previous history of TB. There were no significant interactions to include in the model. The association between eNO and markers of systemic inflammation was estimated in linear regression analysis.

As history of TB has previously been associated with lower eNO levels [24], we conducted a subgroup analysis comparing eNO levels between participants treated for TB and those without TB. Biomarkers and MMPs included in regression analyses were chosen based on previously confirmed associations with HCLD or response to azithromycin treatment in the same group of participants [19].

RESULTS

Study population

A subgroup of 173 participants of the total 347 recruited in the BREATHE trial was included in this study (Figure 1). One participant missed baseline eNO measurement and was therefore excluded. In total, 86 participants from the azithromycin group and 86 from the placebo group had eNO measured. A total of 15 participants in the azithromycin group and 19 in the placebo group were lost to follow-up, withdrew during the study, or did not provide an eNO test at 48 weeks. This left 71 participants in the azithromycin group and 67 in the placebo group with 48-week follow-up samples (Figure 1).

Baseline characteristics between the groups are presented in Table 1. The groups did not differ regarding age, eNO levels, or prevalence of TB.

Association between eNO and acute respiratory exacerbations

Participants who experienced at least one ARE during the study period had significantly higher levels of eNO at

TABLE 1 Baseline characteristics of participants by trial group.

Variable	Azithromycin ($N = 86$)	Placebo ($N = 86$)
Age	15.04 (12.65–17.73)	16.45 (13.15–18.45)
Female sex	35 (41)	45 (52)
eNO level (ppb), geometric mean (95% CI)	15.45 (13.69–17.44)	16.72 (14.69–19.02)
Wasted (weight for age z-score lower than −2)	45 (52)	41 (48)
Stunted (height for age z-score lower than −2)	45 (52)	37 (43)
Passive smoking	25 (29)	24 (28)
History of TB	28 (33)	21 (24)
Using cotrimoxazole	80 (93)	76 (88)
Years on ART[a]	5.91 (4.02–8.4)	6.59 (3.93–8.18)
Prescence of atopy (asthma, eczema, or hay fever)	15 (17)	9 (10)
FEV_1 z-score	−1.67 (−2.47 to −1.33)	−1.74 (−2.09 to −1.25)
Obstructive (FEV_1 z-score lower than −1.64)	45 (52)	45 (52)
Anaemia[b]	24 (28)	32 (37)
White blood cell count ($*10^9$/L)[b]	4.08 (3.36–4.83)	4.32 (3.71–5.34)
Eosinophil count ($*10^9$/L)[b]	0.06 (0.03–0.13)	0.08 (0.04–0.13)
Basophil count ($*10^9$/L)[b]	0.02 (0.01–0.03)	0.02 (0.01–0.03)
Neutrophil count ($*10^9$/L)[b,c]	1.5 (1.27–2.02)	1.83 (1.35–2.48)
Lymphocyte count ($*10^9$/L)[b]	1.98 (1.56–2.45)	2.02 (1.6–2.48)
Monocyte count ($*10^9$/L)[b]	0.36 (0.29–0.47)	0.41 (0.3–0.51)
Virally supressed (VL <1000 copies/mL)	56 (65)	52 (60)
CD4 T-cell count (cells/μL)	554 (363–661)	540.5 (323.5–789.5)
CD4 T-cell count <200 cells/μL	11 (13)	11 (13)
Acute respiratory exacerbations during study period	11 (13)	15 (17)
Hospitalization during study period[d]	2 (2)	3 (3)

Note: Azithromycin group: one admitted for pneumonia, one unknown cause of admission. One in the azithromycin group used salbutamol, no-one used corticosteroids.

Note: Data are presented as *N* (%) or median (interquartile range) unless otherwise indicated.

Abbreviations: ART, antiretriviral therapy; CI, confidence interval; eNO, exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; ppb, parts per billion; TB, tuberculosis; VL, viral load.

[a]One missing data on duration of ART in placebo group.

[b]Two missing data on haemoglobin and white blood cell count in azithromycin group.

[c]One outlier with neutrophil count of 50.80 in placebo group.

[d]Placebo group: one participant admitted for TB treatment, one for ovarian cancer, and one for anaemia.

baseline (geometric mean 20.8, 95% CI 16.5–26.1) than those who did not experience ARE (geometric mean 15.4, 95% CI 14.0–16.9, $p = 0.017$) (Figure 2, Table 2). This difference remained significant in both adjusted linear regression (geometric mean ratio 1.35, 95% CI 1.07–17, $p = 0.013$) and adjusted GLM (geometric mean ratio 1.13, 95% CI 1.03–1.24, $p = 0.015$). We found similar results when adding viral suppression, $CD4^+$ T-cell count, and MMPs associated with HCLD or response to azithromycin treatment to the GLM (Table 2). Baseline characteristics based on having an episode of ARE are presented in Table S1.

Levels of eNO and plasma soluble biomarkers

MMP-3 ($p = 0.024$), MMP-7 ($p = 0.016$), and MMP-10 ($p = 0.005$) were significantly associated with higher levels of eNO at baseline in multivariable linear regression. IFN-γ ($p = 0.039$) was associated with lower baseline eNO levels in multivariable linear regression. MMP-12 was significantly associated with lower eNO levels ($p = 0.034$). However, MMP-12 did have a high proportion of samples (13%) where values were under the limit of detection, so the results are uncertain [12]. None of the

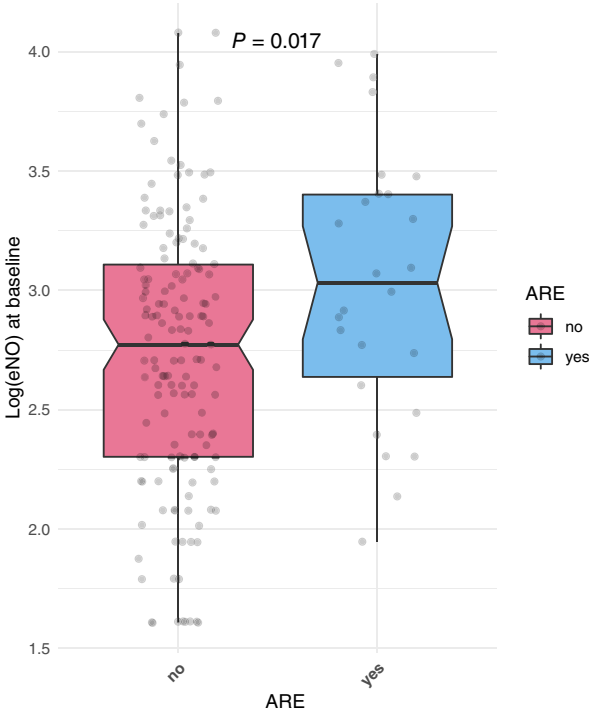

FIGURE 2 Box plot of log-transformed exhaled nitric oxide (eNO) at baseline by experience of at least one acute respiratory exacerbation (ARE) during the study period.

TABLE 2 Association between levels of log eNO at baseline and at least one acute respiratory exacerbation during follow-up, using generalized linear models.

Model	Geometric mean ratio of log eNO at baseline (95% CI)	*p*-value
Unadjusted	1.34 (1.05–1.71)	0.017
Adjusted for trial group, age, sex, and TB history	1.13 (1.03–1.24)	0.015

Abbreviations: CI, confidence interval; eNO, exhaled nitric oxide; TB, tuberculosis; VL, viral load.

other biomarkers investigated were found to be significantly associated with eNO (Table 3).

Effect of azithromycin on levels of eNO

There was no evidence to suggest that azithromycin affected eNO levels after 48 weeks of treatment ($p = 0.189$). Participants in the azithromycin group had a tendency towards lower eNO levels after treatment (geometric mean 14.5, 95% CI 12.6–16.8) compared with participants in the placebo group (geometric mean 17.3,

TABLE 3 Linear regression analyses testing associations of risk factors with eNO at baseline; adjusted for age, sex, and history of TB.

Variable	Geometric mean ratio of log eNO (95% CI)	*p*-value
Age	1.028 (1.003–1.053)	0.028
Sex = male	1.177 (0.992–1.395)	0.061
Study group = azithromycin	0.947 (0.797–1.126)	0.538
ARE during study period = Yes	1.345 (1.065–1.699)	0.013
Atopy = yes	1.148 (0.875–1.507)	0.316
Virally supressed (VL <1000 copies/mL)	1.029 (0.862–1.229)	0.749
CD4 T-cell count	0.998 (0.995–1.001)	0.348
TB treated = yes	0.769 (0.638–0.928)	0.006
FEV_1 z-score	1.021 (0.893–1.168)	0.761
Airway obstruction (FEV_1 z-score lower than −1.64)	1.014 (0.852–1.207)	0.874
Anaemia = yes	0.879 (0.728–1.061)	0.179
White blood cell count (log)	1.288 (0.995–1.666)	0.054
Neutrophils (log)	1.169 (1.006–1.228)	0.041
Eosinophils	1.194 (0.793–1.798)	0.393
Lymphocytes (log)	1.059 (0.884–1.269)	0.532
Monocytes (log)	1.223 (0.985–1.518)	0.068
Wasted (weight-for-age z-score lower than −2)	0.997 (0.828–1.200)	0.974
Stunted (height-for-age z-score lower than −2)	0.924 (0.776–1.100)	0.372
MMP-1 (log)	1.089 (0.974–1.218)	0.132
MMP-3 (log)	1.190 (1.024–1.384)	0.024
MMP-7 (log)	1.186 (1.032–1.363)	0.016
MMP-8 (log)	1.036 (0.963–1.114)	0.337
MMP-10 (log)	1.252 (1.073–1.461)	0.005
MMP-12 (log)	0.919 (0.849–0.993)	0.034
IFN-γ (log)	0.794 (0.638–0.989)	0.039
CRP (log)	1.038 (0.995–1.082)	0.081
sE-selectin (log)	1.146 (0.905–1.450)	0.256
Fas (log)	1.049 (0.795–1.386)	0.732
GCSF (log)	0.922 (0.762–1.115)	0.340

Note: Analyses were done on natural log-transformed values of eNO and back transformed to geometric mean ratio with 95% CI for reporting.
Abbreviations: ARE, acute respiratory exacerbation; CI, confidence interval; CRP, C-reactive protein; eNO, exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; GCSF, granulocyte colony-stimulating factor; IFN, interferon; MMP, matrix metalloproteinase; sE-Selectin, soluble E-selectin; TB, tuberculosis; VL, viral load.

95% CI 14.9–20.1) (Table S2, Figure S1). However, this was not statistically significant in adjusted linear regression analysis ($p = 0.538$) (Table 3) or in GLM (geometric mean ratio 0.86, 95% CI 0.72–1.03, $p = 0.103$) (Table S2).

History of TB

As previously described in the same cohort, participants with a history of TB had lower eNO levels at baseline ($p = 0.006$). The only significant change was higher levels of eNO at 48 weeks (geometric mean 18.8, 95% CI 14.6–24.3) than at baseline (geometric mean 12.6, 95% CI 10.2–15.5) in participants with a history of TB within the placebo group ($p = 0.002$).

DISCUSSION

Our study is the first to investigate the effect of long-term azithromycin treatment on eNO levels in children with HCLD. We found that participants who experienced at least one ARE during the study period had significantly higher baseline levels of eNO. eNO levels are known to be higher during asthmatic exacerbations [21]. Studies in other CLDs are few, and the results are conflicting. A meta-analysis found eNO to be mildly elevated in people with chronic obstructive pulmonary disease (COPD) experiencing an exacerbation, although this was not significant [28]. One study showed elevated eNO levels in COPD exacerbations, where participants with viral airway infections had the highest increase in eNO [29]. Another study found that eNO was elevated only in patients with asthmatic exacerbations [30]. Finally, a study of eNO measurements repeated annually in participants with severe asthma showed that higher eNO levels and large variations in eNO were associated with developing ARE [31]. This supports the evidence that increased airway inflammation is a key factor in ARE and emphasizes the potential role of eNO for both monitoring disease and predicting ARE. Although evidence to support the use of eNO as a risk factor for ARE in diseases other than asthma is scarce, our results suggest that its use in children with HCLD could be of interest, but further studies are warranted.

CLD is characterized by both local and systemic inflammation. Hameiri-Bowen et al. previously showed that systemic levels of IFN-γ, a pro-inflammatory cytokine, is associated with having HCLD in children [12]. Our study surprisingly demonstrated a negative association between IFN-γ and eNO in children with HCLD. This is despite previous studies showing that IFN-γ induces NO production through activation of inducible NO synthase (iNOS) in macrophages [32]. Other studies have linked the development of COPD to lung tissue injury promoted by IFN-γ-mediated release of MMPs [33]. Elevated levels of IFN-γ-producing $CD8^+$ T-cells correlating positively with levels of eNO in participants with COPD have also been described [34]. Considering these findings, eNO could potentially be used as a marker of lung injury in COPD. However, the exact signalling pathway and role of IFN-γ in lung tissue injury and development of HCLD need further investigation.

Our study also found a significant positive association between MMP-3, -7, and -10 and eNO levels. Previous studies reported elevated levels of MMPs measured in bronchoalveolar lavage and blood samples in patients with COPD and pulmonary fibrosis, suggesting their role as mediators in the development of CLD [35–37]. One study in adults with COPD found that several MMPs were elevated in bronchoalveolar lavage, including MMP-3, -7, and -10. These three MMPs were also associated with both radiological markers of small airway disease and severity of emphysema [35]. Further, they found that MMP-10 had the strongest association with emphysema. In our study, MMP-10 had the strongest association with elevated levels of eNO, suggesting that these participants may be at higher risk of CLD progression in the future.

It is also known that both MMP-3 and MMP-7 are associated with the development of lung fibrosis by increasing levels of profibrotic mediators [36]. It has further been suggested that MMP-7 measured in blood could be used as a biomarker for progression and disease activity of idiopathic pulmonary fibrosis. MMP-7 has been significantly associated with mortality and disease progression of pulmonary fibrosis [37]. These results highlight that MMPs might play a significant role in airway remodelling, an important part of the pathogenesis of both COPD and lung fibrosis [35, 38]. Our findings of an association between these MMPs and higher levels of eNO can indicate higher levels of local inflammation in participants with a higher degree of airway remodelling.

Published data regarding the effects of macrolides on eNO levels are conflicting. A study in rats found an effect of macrolide antibiotics on iNOS resulting in reduced levels of eNO, suggesting a favourable effect of macrolides on immune-mediated inflammation and lung injury [39]. In our study, we hypothesized that azithromycin would lead to lower eNO levels because of its antimicrobial and immunomodulatory properties. However, we did not find any evidence that azithromycin treatment affects eNO levels after 48 weeks of treatment. Our results are in line with a previously published randomized controlled trial by Diego et al. where azithromycin had no effect on eNO levels in adults with non-cystic

fibrosis bronchiectasis. However, they did find that azithromycin treatment reduced the frequency of ARE [40].

Two other studies found reduced eNO levels following macrolide treatment. Sadeghdoust et al. found that both azithromycin and prednisolone induced a significant reduction in eNO levels in participants with severe asthma, where those receiving prednisolone had the most reduction in eNO [41]. Another study in children with mild asthma found that a 4-week course of low-dose clarithromycin—another macrolide—in combination with fluticasone both improved lung function as measured by FEV_1 and induced a significant reduction in eNO levels [42]. Of note, these studies were carried out in participants with asthmatic disease, characterized mainly by type 2 inflammation and eosinophilic activity, to which eNO is most strongly linked [22]. In addition, most participants had a chronic and stable disease over time, with few participants experiencing acute inflammation. Hodgson et al. found similar results to ours in adults with treatment-resistant cough after 8 weeks of treatment with azithromycin. They found no significant difference in either symptoms of coughing or levels of eNO, also when controlling for asthma [43]. Our participants had HCLD, and few participants had a history of asthma or atopic disease. The pathogenesis of HCLD is multifactorial and not completely understood. Data suggest that these patients have a form of obliterative bronchiolitis or bronchiectasis. Obliterative bronchiolitis is thought to be a result of repeated injury to the airway epithelium, followed by inflammation and ultimately leading to fibrosis and narrowing of the small airways [2, 17, 44]. Bronchiectasis is characterized by injury and subsequent neutrophilic inflammation [45]. This difference in pathogenesis makes comparisons more difficult. Furthermore, most of the studies included treatment with corticosteroids in addition to macrolides. Corticosteroids are highly potent immunosuppressants, which may influence the results. In summary, corticosteroids together with macrolides are more potent in reducing local inflammation than are macrolides alone. Further investigations regarding the use of corticosteroids for improvement of lung function and reduction of inflammation in HCLD could be considered. However, particular caution is warranted due to the increased risk of infections, especially in a virally non-supressed population.

Few studies on eNO have been conducted in people living with HIV. Previous studies have focused on HIV-TB co-infection or history of prior TB [24, 46, 47]. The results are conflicting, but studies have shown that eNO levels are lower in patients with active or prior TB and cystic fibrosis [48, 49]. This can be explained by the fact that *Mycobacterium tuberculosis* reduces the activity of iNOS, as an immune evasion strategy, thereby decreasing levels of NO in expired air [48]. In cystic fibrosis, diffusion of eNO to the exhaled air and expression of iNOS is reduced by the thick mucus [50]. The local antimicrobial effects of NO can therefore be compromised in these patients, leading to increased lung inflammation, although confirming data are scarce [50].

Two studies found lower eNO levels in adults and children with HIV [24, 46], and prior history of TB was associated with lower eNO levels in one. Another study found eNO to be reduced in participants with TB infection, independent of HIV status [47]. A large study in well-treated adults with HIV in Copenhagen, Denmark, found that eNO levels were higher in adults with HIV than in healthy controls [51]. This study looked at HIV infection alone. Participants had no history of TB or active TB at sample collection and no CLD, defined as no obstruction on spirometry and absence of asthma and airway medications. These results indicate that HIV alone may result in elevated eNO levels and that active lung TB or history of TB may explain the decreased eNO levels seen in other studies in people with HIV. This study was conducted in a western setting in virally supressed adults, whereas our participants were sub-Saharan African children, and almost 40% had HIV viral load >1000 copies/mL, making comparisons complicated.

The strengths of this study are the prospective design and randomization of participants to azithromycin or placebo treatment. The groups were quite similar at enrolment. We have a high rate of follow-up and completeness of data, and no participants used corticosteroids that could influence study results. Limitations include difficulties in adherence to medication for a whole year and the restriction of eNO measurements to enrolment and 48 weeks.

In conclusion, we found that a higher eNO level at baseline was a risk factor for ARE in children with HCLD. eNO was associated with MMPs previously found to be proinflammatory and to play a role in the development of CLD and airway remodelling. We observed no effect of azithromycin on eNO levels. Measurement of eNO at the time of ARE could be beneficial in the quest to further understand the role of eNO in HCLD progression. Further studies on the potential implications of eNO as a tool to monitor local airway inflammation and risk of ARE in patients with HCLD are warranted.

AUTHOR CONTRIBUTIONS

Study conception and design: Trym Thune Flygel, Evgeniya Sovershaeva, Rashida Abbas Ferrand, Trond Flægstad, Jon Øyvind Odland, and Sarah Rowland-Jones. Data collection and management: Dan Hameiri-Bowen, Louis-Marie Yindom, Victoria Simms, Tsitsi Bandason, and BREATHE study team. Data analysis: Trym Thune

Flygel and Victoria Simms. Manuscript preparations and writing: Trym Thune Flygel and Evgeniya Sovershaeva. Review: All authors. All authors have read and approved the final version.

ACKNOWLEDGEMENTS

The authors are grateful to the BREATHE study team, the clinic staff, and the study participants for making this study possible.

FUNDING INFORMATION

The study was funded by the Norwegian Research Council through the Global Health and Vaccination Research (GLOBVAC) programme. Helse Nord RHF offered additional funding (HNF 1387-17). Trym Thune Flygel's PhD scholarship is funded by Helse Nord RHF (HNF 1539-20). Dan Hameiri-Bowen is funded by the Wellcome Trust PhD Programme in Genomic Medicine and Statistics (108 861/B/15/Z). Rashida Abbas Ferrand is funded by the Wellcome Trust through a Senior Fellowship in Clinical Science (206 316/Z/17/Z). Victoria Simms is partially supported by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement, which is also part of the EDCTP2 programme supported by the European Union Grant, ref: MR/R010161/1.

CONFLICT OF INTEREST STATEMENT

There were no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Flygel TT, Hameiri-Bowen D, Simms V, et al. Exhaled nitric oxide is associated with inflammatory biomarkers and risk of acute respiratory exacerbations in children with HIV-associated chronic lung disease. *HIV Med*. 2023;1-10. doi:10.1111/hiv.13565