# Chronic lung disease in HIV-infected children established on antiretroviral therapy

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## RUNNING TITLE

Chronic lung disease in HIV-infected children

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## ABSTRACT

### Objective

Respiratory disease is a major cause of morbidity and mortality in HIV-infected children. Despite antiretroviral therapy (ART), children suffer chronic symptoms. We investigated symptom prevalence, lung function, and exercise capacity among older children established on ART, and an age-matched HIV-uninfected group.

### Design

A cross-sectional study in Zimbabwe of: 1) HIV-infected children aged 6-16 years receiving ART for over six months; 2) HIV-uninfected children attending primary health clinics from the same area.

### Methods

Standardised questionnaire, spirometry, Incremental Shuttle Walk Testing (ISWT), CD4 count, HIV viral load, and sputum culture for tuberculosis were performed.

### Results

202 HIV-infected and 150 uninfected participants (median age 11.1 years in each group) were recruited. Median age at HIV diagnosis and ART initiation was 5.5 (IQR 2.8-7.5) and 6.1 years (IQR 3.6-8.4) respectively. Median CD4 count was 726 cells/μl, and 79% had HIV viral load<400copies/ml. Chronic respiratory symptoms were rare in HIV-uninfected children (n=1 [0.7%]), but common in HIV-infected participants (51 [25%]), especially cough (30 [15%]) and dyspnoea (30 [15%]). HIV-infected participants were more commonly previously treated for tuberculosis (76 [38%] versus 1 [0.7%], p<0.001), had lower exercise capacity (mean ISWT distance 771m versus 889m respectively, p<0.001), and more frequently abnormal spirometry (43 [24.3%] versus 15 [11.5%], p=0.003) compared to HIV-uninfected participants. HIV diagnosis at

an older age was associated with lung function abnormality (p=0.025). No participant tested positive for *M. tuberculosis*.

## Conclusions

In children, despite ART, HIV is associated with significant respiratory symptoms and functional impairment. Understanding pathogenesis is key, as new treatment strategies are urgently required.

## KEYWORDS

HIV, Sub-Saharan Africa, Lung Function, Chronic lung disease, Antiretroviral therapy (ART)

## INTRODUCTION

Respiratory disease is the most common manifestation of HIV/AIDS among children, accounting for more than 50% of HIV-associated mortality.[1-4] The use of antiretroviral therapy (ART) and co-trimoxazole prophylaxis has contributed to a reduction in the rate of acute respiratory tract infections and mortality among HIV-infected children in both high- and low-resource settings.[5] In the pre-ART era, 30-40% of HIV-infected children also developed chronic lung disease, most commonly due to lymphoid interstitial pneumonitis (LIP), but this condition responds well to ART and is now uncommon in clinical practice, except in children under 5 years old.[6-8]

Nevertheless, recent studies in southern Africa have demonstrated that about 30% of African HIV-infected older children have chronic respiratory symptoms, classically a chronic cough (often leading to presumptive treatment for tuberculosis (TB)) and reduced exercise tolerance.[3, 4] In these studies, even participants with pronounced respiratory impairment looked well at rest, not all had cough, and plain radiological abnormalities were subtle, and not consistent with LIP.[9] However, these studies did not include HIV-uninfected controls, and included a mix of children who were ART naïve as well as those taking ART. The aim of this study was to investigate the burden and features of chronic lung disease among HIV-infected children established on ART and in an age-matched HIV-uninfected group.

## METHODS

The study was carried out between September 2014 and June 2015 at the Harare Children's Hospital HIV clinic in Harare, Zimbabwe, a public sector clinic that provides HIV care for more than 4000 children. Children were eligible for the study if they were aged between 6 and 16 years, had been taking ART for at least 6 months, were not acutely unwell and were not taking TB treatment. We consecutively recruited up to five eligible participants per day, restricted to this number due to logistical constraints. A comparison group of HIV-uninfected children in the same

age group was also recruited from 7 clinics which provided HIV testing and counselling to all attendees regardless of the reason for attendance, and which served the same population as that of Harare Hospital (high population density suburbs, with small dwellings being typical). We enrolled children who had tested HIV-negative and who were not acutely unwell and who were not receiving treatment for respiratory infection or tuberculosis. A sample size of 200 HIV-1 infected children was selected to provide a precision of ±6% around an estimated 25% prevalence of chronic lung disease (CLD), defined as any one of: unexplained chronic cough for ≥3 months; hypoxaemia (SpO2 <90%) at rest or desaturation on exertion by more than 4% from baseline; abnormal spirometry without another medical explanation e.g. asthma; chronic dyspnoea (MRC breathlessness score >1)[10].

## Data collection

A nurse-administered questionnaire was used to collect details of socio-demographic indices, clinical history and current symptoms. A standardised examination was performed including WHO staging of HIV infection, measurement of height and weight, spirometry and exercise testing.

Spirometry was performed according to ATS standards using EasyOne World spirometers (ndd Medical Technologies, Inc., Andover, MA, USA).[11] Up to 8 forced exhalations were recorded while sitting. We analysed only data from individuals who produced three consistent traces which met ATS quality criteria. The highest $FEV_1$ and FVC measurements for each individual were used, with other indices recorded from the best trace (largest total of $FEV_1$ and FVC). Obstruction was defined as a reduced $FEV_1$:FVC. For clarity, we use the term "reduced FVC" where FVC was low with a normal $FEV_1$:FVC. We avoided the term "restriction" as we were unable to measure lung volumes. Where any spirometric abnormality was found, participants underwent repeat spirometry 15 minutes after administration of 2.5mg nebulised salbutamol. An improvement in$FEV_1$ of ≥12% was considered to represent significant reversibility.

An incremental shuttle walk test was performed as a measure of cardiorespiratory fitness. This was devised for adults,[12] but has been validated for use in children with chronic respiratory disease.[13] Participants were not tested if, at rest: $SpO_2$ <88%; heart rate >110; respiratory rate >30 breaths/min. On flat ground, participants were instructed to walk between two markers placed 10 metres apart. A pre-recorded series of "bleeps" were played, which determined how quickly each 10 metre segment should be completed. The standardised protocol demanded that the participant walked 30 metres in the first minute, with each subsequent minute escalating the distance by 10 metres (i.e. 40, 50 and 60 metres in the 2nd, 3rd and 4th minutes respectively). The test was terminated when participants were unable to reach the next marker by the time the beep was issued. The respiratory rate, heart rate and oxygen saturations were measured before and immediately after the end of the test. Only the first 82 participants in the HIV-uninfected group underwent SWT due to limited staffing. Predicted maximal heart rates were calculated by the Tanaka equation: 208 - 0.7*age.[14]

## Laboratory investigations

Where possible, all HIV positive participants had sputum samples obtained by spontaneous expectoration or by induction using nebulised hypertonic saline. Sputum smears were examined by Ziehl-Neelson stain microscopy and a single mycobacterial culture was performed on Lowenstein-Jensen media. Sputum from HIV uninfected participants was sought only if the WHO TB symptom screen was positive.[15] HIV viral load was measured with COBAS Ampliprep/Taqman 48 Version 2.0 and CD4 count was measured using an Alere PIMA™ CD4 machine (HIV-positive participants only).

## Data Analysis

Data were extracted from paper forms using optical character recognition software (Cardiff TELEFORM Intelligent Character, Version 10.7). Data analysis was carried out using Stata v12

(StatCorp, TX) and GraphPad Prism v6 (GraphPad, CA). Height-for-age and BMI-for-age z-scores were calculated using the WHO reference standards.[16] Normal spirometric ranges were defined using the GLI 2012 equation which determines race and sex specific reference values, taking account of height and age.[17] The lower limit of normal (LLN) is defined as 1.64 standard deviations below the mean expected value (which describes the lowest 10 centiles as "abnormal").

Shuttle walk and CD4 results were treated as parametric data, with Student t test used to compare means between HIV-infected and non-infected groups. Other continuous variables were non-parametric: central tendency was reported by the median and interquartile range (IQR), using the Mann-Whitney U test for equivalence testing between groups. Frequencies of categorical data - symptoms, past medical complaints and rates of abnormal spirometry and growth indices - were compared between HIV-infected and non-infected by chi squared test. Results were considered statistically significant at $p<0.05$. The association of abnormal lung function with a priori defined clinical data were investigated using univariable logistic regression, and reporting Odds ratios with a Wald 95% confidence interval. Stepwise backward multivariate logistic regression was then used; this incorporated age and sex, and those variables with individual $p<0.1$ on univariate testing.

Ethical approval was granted by the Medical Research Council of Zimbabwe, the Harare Hospital Ethics Committee, the Biomedical Research and Training Institute Institutional Review Board and the London School of Hygiene and Tropical Medicine Ethics Committee. All guardians gave written consent and participants gave assent to participate.in the study.

## RESULTS

### Participant characteristics

A total of 202 HIV-infected participants were recruited: median age 11.1 years (IQR 9.0 – 12.9), and 55% male. Summary statistics are given in Table 1, and a flow diagram of participation and testing is given in Figure 1. Of the 150 HIV-uninfected participants recruited as a comparison group, 42% were male and the median age was 11.0 (IQR 9.0-13.9). All but one HIV-infected participants were vertically-infected and the median age at HIV diagnosis was 5.5 (IQR 2.8 – 7.5)

155 years. The median duration of ART was 4.7 (IQR 2.6 – 6.4) years, with 161 (80%) taking non-
156 nucleoside reverse transcriptase inhibitor-based (first-line) ART and the remainder taking a protease
157 inhibitor based regimen (

Table 3). The median CD4 count at HIV diagnosis (available for 105 participants) was 353 (IQR 134 – 696) cells/µl and the CD4 count at enrolment was 726 (IQR 476 – 941) cells/µl. The majority of participants (79%) had an HIV viral load <400copies/ml (Table 1), and 194 (96%) were taking co-trimoxazole.

None of the HIV-uninfected participants screened positive using the WHO TB symptom screen. Within the HIV infected group, 153 participants produced samples for mycobacterial culture, 35 could not despite sputum induction, and 16 were too ill or did not return for the procedure. None grew *Mycobacterium tuberculosis*, but 3 grew non-tuberculous mycobacteria (not speciated).

A significantly higher proportion of HIV-infected participants were stunted than HIV-uninfected participants (35.8% vs. 7.3%, $p<0.001$), but prevalence of wasting was similar in the two groups (Table 1). Seventy-six HIV-infected children had been previously treated for tuberculosis, compared with one from the HIV-uninfected group.

The proportion treated for asthma was similar in both groups. Chronic respiratory symptoms (breathlessness, cough or wheeze) were reported by 25.3% of HIV-infected children but only by one (0.7%) HIV-uninfected child. Wheeze was infrequently reported in either group (Table 1).

Incremental Shuttle Walk Test (ISWT) could not be performed in 15 HIV infected participants due to resting hypoxaemia (n=2), resting dyspnoea (n=9), or loss to follow-up (n=4). One HIV-uninfected participant had tachypnoea at rest. The distance attained during ISWT was significantly reduced in the HIV-infected group (mean 771m [SD 216] compared with 889m [SD 227] in the HIV-uninfected group, $p<0.001$). Shortly after completion of the test, mean heart rate was not significantly difference at 62% (SD 12.0) and 67% (SD 10.5) of their predicted maximal heart rates for the HIV infected and non-infected groups respectively.

One hundred and seventy-seven (88%) HIV-infected and 130 (87%) HIV-uninfected participants had high-quality spirometry traces. Quality grading according to ATS standards did not differ between groups either before or after reversibility testing. Of those high quality (interpretable) traces, a quarter of all HIV-infected participants had abnormal lung function on spirometry. This was significant higher than among HIV-uninfected participants (24.3% vs 11.5%, p=0.01). This was reflected in lower $FEV_1$, FVC and FEF25-75 indices in the HIV-infected group ($p<0.05$) (Figure 2). There was no significant difference in the $FEV_1$:FVC ratio (p=0.08) between the two groups, although those with a history of lung infection had a lower $FEV_1$:FVC than those without (p=0.03). For those with spirometric abnormality, post bronchodilator reversibility traces were complete and adequate in 31 HIV-infected and 6 HIV-uninfected participants, demonstrating reversibility in 11 (35.4%) and 2 (33.33%) respectively.

Predictors of abnormal lung function are summarised in Table 4. When subdivided into restrictive and obstructive types, numerators were insufficient to draw robust conclusions: data are therefore presented as "normal" or "abnormal". The presence of any respiratory symptom, except wheeze, was significantly associated with abnormal lung function. Older age at HIV diagnosis was positively associated with abnormal lung function (p=0.025), as was wasting (OR 8.1, 95% CI 2.3-31.7). After fitting a multivariate model, wasting was the only independent predictor: OR 4.7 (95% CI 1.2 – 18.6).

We investigated the potential for prior respiratory infection to impact on lung function by stratified analysis (Figure 2 and Supplemental Table S1). Among those with previous infection (any of: TB; PCP; NTM by sputum culture; hospitalisation for chest infection), those with later presentation and ART initiation were more likely to have abnormal lung function ($p<0.05$). Dyspnoea, tachypnoea, oxygen desaturation and wasting were also disproportionately apparent ($p<0.05$ for each). However,

among those without previous infection, cough and sputum production were more commonly seen in those with abnormal spirometry ($p<0.05$)

## DISCUSSION

A quarter of HIV infected children experienced chronic respiratory symptoms, despite being treated with ART and with good virological control in the majority. In contrast, only one HIV-uninfected participant reported respiratory symptoms. More than one third of the HIV-infected children in our study had been previously treated for TB, although we were unable to determine how the diagnosis of TB had been made due to a lack of robust records. As noted in other cohorts, where background TB rates are high, over-treatment is likely in those with chronic respiratory symptoms due to a lack of diagnostic and alternative therapeutic strategies for chronic respiratory symptoms.[18, 19] Notably, no TB was found in our study on microbiological investigation.

We found much higher rates of abnormal lung function among HIV-infected compared to HIV-uninfected children. Development of airways disease in early life has been described in the context of HIV infection: airway resistance measurements indicate that abnormalities of airway calibre are established in those as young as one year old.[20] However, in resource limited settings, no longitudinal studies have been conducted to define how such abnormalities change over time, or how they respond to ART. Interestingly, in our current study, obstructive lung disease was less common than in a study conducted in Malawi (4% vs 26%).[21] In the Malawi study, however, 31% of individuals were ART-naïve or had been taking ART for a relatively shorter duration (median 20 months).[2]

In the pre-ART era, the most common cause of CLD was lymphocytic interstitial pneumonitis (LIP) found in 30-40% of HIV-infected children.[7, 22] LIP responds well to ART and the prevalence has significantly declined with increased availability of ART.[23] We found low rates of wheeze and bronchodilator response which would suggest that the chronic respiratory symptoms are not likely

to be due to asthma. Similarly, although household air pollution is detrimental to lung function and can cause these symptoms, rates of biomass as the main fuel for cooking or lighting are lower in our cohort (less than 1 in 5) than other reported peri-urban series in sub-Saharan Africa, due to access to mains electricity.[24]

In the ART era, multiple aetiological factors may contribute to lung disease, including long-term sequelae of repeated bacterial and viral respiratory tract infections and possibly HIV-induced chronic inflammation and immune senescence caused by dysregulated immune activation.[25, 26] Such processes account for the high prevalence of bronchiectasis in this population, and may also contribute to obliterative bronchiolitis (OB).[18, 22] The latter has been described in a previous study from Zimbabwe. It seems to represent, as in non-HIV related OB, a final common pathway of lung injury typified radiologically by air trapping and mosaic attenuation of the lung parenchyma.[2, 9] The features on plain chest radiography are subtle and non-specific, and high resolution CT is the definitive imaging modality. The lack of HRCT facilities may explain why OB is not recognised in Africa, despite the high prevalence of chronic respiratory symptoms.[27-30] Clinically, OB usually presents as an obstructive lung disease with minimal response to inhaled beta agonists. However, the pattern of spirometry abnormalities observed in our study was mainly restrictive. This might be explained by chronic immune activation causing scarring and fibrosis, resulting in a restrictive lung function abnormality.[31-33] We found a reduced $FEV_1$/FVC ratio - a more obstructive pattern - in those with a previous history of infection. This would be consistent with unrecognised OB which has been treated as TB or other infection. However, TB in adults is itself known to leave evidence of airway obstruction, even in those cured of disease.

. our finding that later diagnosis is associated with abnormal spirometry suggests that early diagnosis and treatment of HIV may prevent development or progression of chronic lung disease. Established disease appears to persist despite otherwise effective ART. This was apparent children

with a history of infection, where later initiation of ART, and signs of reduced gas-exchange capacity (oxygen desaturation and tachypnoea) predicted lung function abnormality. Wasting, also a predictor of lung function abnormality, could represent by association many risk factors for lung disease, including nutrition and chronic inflammatory disease.

In our cohort, there was no significant correlation between lung function abnormality and SWT distance. The Incremental Shuttle Walk Test (ISWT) is a valid and reproducible predictor of aerobic exercise capacity ($VO_2$ peak). As a maximal test, it is less variable than self-paced protocols such as the six-minute walk test.[13] $VO_2$ peak is determined by both respiratory and cardiovascular fitness. It is possible that the reduced exercise capacity in HIV infected children might be partly due to cardiac disease. Previously noted echocardiographic abnormalities in Zimbabwean children include LV hypertrophy and diastolic dysfunction, seen in 74/110 (67%) and 27/110 (24%) respectively.[34] We are further investigating this possibility using echocardiography in our cohort.

The strengths of this study was that it was prospectively conducted, recruitment was unselective (i.e. not based on presence of respiratory symptoms) and from a general HIV clinic. It included an HIV-uninfected comparison group recruited from the same geographical area. Study limitations include self-report of illness which may result in recall bias. Spirometry was not available for all participants due to exclusion of poor quality traces and ISWT was not universally performed in HIV-uninfected participants due to staff shortages. Furthermore, we were unable to measure lung volumes. Our HIV-uninfected group were not matched by sex, resulting in females being slightly more frequently represented compared to in the HIV-infected group. However, this should not affect interpretation for spirometry data as lung function indices use sex-specific normal ranges. For exercise capacity, male children tend to outperform females, but sub-analysis of our data stratified by sex revealed no difference in findings (data not shown).[35]

Our study demonstrates that there is a large burden of chronic respiratory morbidity among HIV-infected children established on ART. Despite efforts to eliminate mother to child transmission, 300,000 infants were newly infected with HIV in 2011, and a record number (630,000) were receiving ART in low income countries.[36, 37] Thus, HIV will continue to place a heavy burden on paediatric clinical services in sub-Saharan Africa, where 90% of the world's HIV-infected children live.[38] Delay in diagnosis of HIV infection and initiation of ART may increase the risk of developing chronic lung disease. The WHO until 2015 had recommended that children aged over five years should be treated with ART once CD4 count drops to 500cells/µl or Stage 3 or 4 disease. The recent START and TEMPRANO trials in adults demonstrated that treating HIV infection irrespective of immunological status reduces the risk of AIDS and non-AIDS events.[39, 40] In children, earlier HIV recognition, may reduce the risk of developing chronic lung disease by preventing recurrent respiratory tract infection and by reducing chronic pulmonary inflammation. Adoption of universal treatment of all HIV-infected individuals regardless of disease stage may therefore be advantageous, and some additional benefit might be expected from co-trimoxazole prophylaxis by lowering the rates of lower respiratory tract infection. However, once established, chronic lung disease appears to persist despite ART. Hitherto the main focus of HIV programs has been on meeting the need for massive scale-up of paediatric antiretroviral therapy (ART) and on improving infant survival. There is now a pressing need to address the long term complications of HIV infection among the increasing numbers of children growing up with HIV, and novel diagnostic and therapeutic strategies for chronic lung disease are urgently required.

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## CONTRIBUTIONS

Study conception: RAF, JR, KK, JM; Study design: RAF, JR, KK, JM; Protocol development: RAF, JR, KK, JM; Training, quality control: JR; data collection: GM, RAF, EM, SW; Data analysis: JR, RAF, KK; Drafting and revision of manuscript: all authors.

## COMPETING INTERESTS

The authors confirm that they have no competing interests.

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412 **Table 1: Summary characteristics of study participants by HIV status**

| | **HIV uninfected (n=150)** | **HIV infected (n=202)** | **p-value** |
|---|---|---|---|
| Age, median years (IQR) | 11.1 (9.0 – 12.8) | 11.1 (9.0 – 12.9) | 0.77 |
| Sex, female, n (%) | 63 (42.0) | 111 (55.0) | 0.018 |
| Orphan, n (%) | 20 (13.5) | 102 (51.0) | < 0.001 |
| Mother known to be HIV infected | 13 (8.7) | 202 (100) | < 0.001 |
| Active smoker, n (%) | 0 (0) | 0 (0) | - |
| Passive smoke exposure at home, n (%) | 27 (18.0) | 42 (20.9) | 0.18 |
| Any respiratory complaint*, n (%) | 1 (0.7) | 51 (25.3) | < 0.001 |
| Dyspnoea (MRC grade>1), n (%) | 0 (0) | 30 (14.9) | < 0.001 |
| Daily cough for >1 month, n (%) | 1 (0.7) | 30 (14.9) | < 0.001 |
| Sputum production, n (%) | 1 (0.7) | 20 (10.0) | < 0.001 |
| Wheeze, n (%) | 0 (0) | 9 (4.5) | 0.007 |
| Resting tachypnoea: rate >25, n (%) | 9 (6.0) | 28 (14.1) | 0.016 |
| Hospital admission for RTI in last year, n (%) | 3 (2.0) | 4 (2.0) | 0.96 |
| Antibiotics for RTI in last year, n (%) | 3 (2.0) | 45 (22.3) | < 0.001 |
| Previously diagnosed or treated | | | |
| Asthma, n (%) | 3 (2.0) | 7 (3.5) | 0.37 |
| PCP, n (%) | 0 (0) | 6 (3.0) | 0.029 |
| Tuberculosis ever, n (%) | 1 (0.7) | 76 (37.8) | < 0.001 |
| Tuberculosis more than once, n (%) | 0 (0) | 5 (2.5) | 0.10 |
| Stunted (HFA z score <2), n (%) | 12 (7.9) | 72 (35.8) | < 0.001 |
| Wasting (BFA z score <2), n (%) | 10 (6.7) | 18 (9.0) | 0.42 |
| Incremental Shuttle Walk Test (ISWT) | **(n=82)** | **(n=187)** | |
| Desaturates during test, n (%) | 5 (6.1) | 22 (11.1) | 0.16 |
| ISWT distance, metres mean (SD) | 889 (227) | 771 (216) | < 0.001 |
| Spirometry interpretation | **(n=130)** | **(n=177)** | |
| Normal, n (%) | 115 (88.5) | 134 (75.7) | 0.003 |
| Obstruction, n (%) | 1 (0.8) | 7 (4.0) | 0.052 |
| Reduced FVC, n (%) | 14 (10.8) | 36 (20.3) | 0.012 |
| Bronchodilator response | **(n=6)** | **(n=31)** | |

| | | | |
|---|---|---|---|
| Reversibility demonstrated, n (%) | 2 (33.3) | 11 (35.5) | 0.92 |

* wheeze, chronic cough or dyspnoea; IQR interquartile range; SD standard deviation; HFA height for age; BFA body mass for age; RTI respiratory tract infection; missing data on n=2.

**Table 2 HIV-specific summary characteristics**

| | **HIV infected (n=202)** |
|---|---|
| Age at diagnosis, median (IQR) | 5.5 (2.8 – 7.5) |
| Age at ART initiation, median (IQR) | 6.1 (3.6 – 8.4) |
| Mode of HIV transmission, n (%) | |
| Mother to child | 201 (99.5) |
| Sexual | 1 (0.5) |
| Reason for HIV testing | |
| Chronic cough | 113 (55.9) |
| Hospital admission | 41 (20.3) |
| Repeated illness | 32 (15.8) |
| Other ‡ | 16 (7.9) |
| CD4 at diagnosis, median (IQR) † | 353 (134 – 696) |
| CD4, median (IQR) at recruitment | 726 (476 – 941) |
| HIV VL <400 copies/ml, n (%) | 155 (78.7) |

† Data available for 105 participants from health records; ‡ Other reasons were: testing in elective male circumcision (n=6); spontaneous healthcare worker initiated (n=2); TB diagnosed (n=1); sexual debut prompted testing (n=1); don't recall (n=6).

**Table 3 HIV treatment regimes**

| | NNRTI based | | PI based | |
|---|---|---|---|---|
| | *NVP* | *EFV* | *ATAZ/r* | *LPV/r* |
| AZT/3TC* | 72 | 23 | 7 | 2 |
| TDF/3TC | 32 | 32 | 25 | - |
| ABC/3TC | - | 1 | 2 | - |
| ABC/DDI | - | 1 | 2 | 1 |
| Total † | 161 | | 39 | |

AZT = zidovudine; 3TC = stavudine; TDF = tenofovir; ABC = abacavir; DDI = didanosine; NVP = nevirapine; EFV = efavirenz; ATAZ/r = ritonavir boosted atazanavir; LPV/r = ritonavir boosted lopinavir.

*1 individual unknown NNRTI or PI. 1 individual DDI/ATAZ/r with 2$^{nd}$ NRTI unrecorded

**Table 4: Association of factors with abnormal lung function in HIV-infected children**

| | **Normal n=134** | **Abnormal n=43** | **OR (95% CI)** |
|---|---|---|---|
| Age at diagnosis†, n (IQR) | 4.6 (2.7 – 6.7) | 5.8 (3.8 – 8.8) | 1.15 (1.02 – 1.29)* |
| Age at ART initiation†, n (IQR) | 5.8 (3.5 – 8.3) | 6.3 (4.3 – 8.8) | 1.05 (0.95 – 1.17) |
| Years on ART, n (IQR) | 4.6 (2.3 – 6.4) | 5.1 (3.4 – 6.4) | 1.08 (0.95 – 1.23) |
| Any symptom, n (%) | 25 (18.7) | 20 (46.5) | 3.8 (1.7 – 8.5)* |
| Dyspnoea, n (%) | 13 (9.7) | 14 (32.6) | 4.5 (1.7 – 11.5)* |
| Daily cough, n (%) | 12 (9.0) | 13 (30.2) | 4.4 (1.7 – 11.7)* |
| Sputum production, n (%) | 7 (5.2) | 9 (20.9) | 4.8 (1.5 – 16.2)* |
| Wheeze, n (%) | 6 (4.5) | 3 (7.0) | 1.6 (0.2 – 7.9) |
| Passive smoker | 33 (24.6) | 9 (20.9) | 0.8 (0.3 – 2.0) |
| Biomass fuel used for cooking | 21 (15.7) | 8 (18.6) | 1.2 (0.4 – 3.2) |
| Biomass fuel or candles used for lighting | 18 (13.4) | 6 (14.0) | 1.0 (0.3 – 3.0) |
| Previous TB treatment, n (%) | 49 (36.6) | 18 (41.9) | 1.2 (0.6 – 2.7) |
| Stunting (HFA<-2), n (%) | 45 (33.6) | 19 (44.2) | 1.6 (0.7 – 3.3) |
| Wasting (BFA <-2), n (%) | 5 (3.7) | 10 (3.8) | 8.1 (2.3 – 31.7)* |
| Abnormal $SpO_2$ at rest or exercise, n (%) | 11 (8.5) | 9 (23.1) | 3.2 (1.1 – 9.4)* |
| Resting tachypnoea (rate >25), n (%) | 16 (12.1) | 8 (19.1) | 1.7 (0.6 – 4.7) |
| Viral load suppressed <400 copies/ml[a] | 105 (80.2) | 32 (78.1) | 0.9 (0.4 – 2.4) |
| ISWT distance, metres (SD) [b] | 765 (212) | 771 (241) | 1.00 (1.00 – 1.00) |
| CD4 at recruitment[c] | 774 (342) | 688 (344) | 1.00 (1.00 – 1.00) |

[*] Significant at p<0.05

[a] n=131 and n=41 respectively; [b] n=131 and n=38 respectively; [c] n=134 and n=42 respectively

**Figure 1 Flowchart of participant recruitment and testing**

Diagram showing recruitment of HIV-infected participants.

n=4 did not attend for follow-up appointment, and therefore did not undergo SWT
NTM: non-tuberculous mycobacteria

**Figure 2 Spirometric abnormalities in HIV infected and uninfected participants**

Dot plots of all participants with spirometry results, presented as z-scores compared with GLI 2012 reference ranges. Bars illustrate the mean and standard deviations of each group. Dotted horizontal bars show the limits of normality. P values derive from t-tests. HIV infected individuals are further shown by stratification by "Prior lung infection". This is a compound definition, including individuals with any of: TB; PCP; NTM by sputum culture; hospitalisation for chest infection.

Supplementary Table S1

Stratified analysis of the associations of pre-selected clinical and physiological characteristics with abnormal lung function.

| | **No prior infection (n=105)** | | | **Prior infection (n=72)** | | |
|---|---|---|---|---|---|---|
| | **Normal n=82** | **Abnormal n=23** | **OR (95% CI)** | **Normal n=52** | **Abnormal n=20** | **OR (95% CI)** |
| Age at diagnosis†, n (IQR) | 4.6 (2.2 – 6.7) | 4.4 (3.7 – 7.5) | 1.05 (0.90 – 1.23) | 4.6 (3.3 – 6.7) | 7.9 (5.0 – 9.0) | 1.30 (1.07 – 1.59)* |
| Age at ART initiation†, n (IQR) | 6.1 (3.2 – 8.4) | 5.4 (3.7 – 7.9) | 0.96 (0.83 – 1.10) | 5.3 (3.6 – 8.3) | 8.0 (5.7 – 9.9) | 1.19 (1.00 – 1.41)* |
| Years on ART, n (IQR) | 4.3 (2.1 – 6.4) | 5.4 (3.7 – 6.7) | 1.05 (1.00 – 1.42)* | 4.6 (3.0 – 6.5) | 4.5 (2.7 – 6.4) | 0.94 (0.76 – 1.15) |
| Any symptom, n (%) | 14 (17) | 7 (30) | 2.1 (0.7 – 6.1) | 11 (21) | 13 (65) | 6.9 (2.2 – 21.5)* |
| Dyspnoea, n (%) | 9 (11) | 5 (22) | 2.3 (0.7 – 7.5) | 4 (8) | 9 (45) | 9.8 (2.6 – 37.8)* |
| Daily cough, n (%) | 4 (5) | 6 (26) | 6.9 (1.7 – 27.1)* | 8 (15) | 7 (35) | 3.0 (0.9 – 9.7) |
| Sputum production, n (%) | 2 (2) | 4 (17) | 8.4 (1.4 – 49.3)* | 5 (10) | 5 (25) | 3.1 (0.8 – 12.3) |
| Wheeze, n (%) | 4 (5) | 1 (4) | 0.9 (0.1 – 8.3) | 2 (4) | 2 (10) | 2.8 (.04 – 21.2) |
| Passive smoker, n (%) | 22 (27) | 6 (26) | 1.0 (0.3 – 2.8) | 11 (21) | 3 (15) | 0.7 (0.2 – 2.6) |
| Biomass fuel used for cooking, n (%) | 12 (15) | 5 (22) | 1.6 (0.5 – 5.2) | 9 (17) | 3 (15) | 0.8 (0.2 – 3.5) |
| Biomass fuel or candles used for lighting, n (%) | 11 (13) | 3 (13) | 1.0 (0.2 – 3.9) | 7 (13) | 3 (15) | 1.1 (0.3 – 4.9) |
| Stunting (HFA<-2), n (%) | 22 (27) | 7 (30) | 1.2 (0.4 – 3.3) | 23 (44) | 12 (60) | 1.9 (0.7 – 5.4) |
| Wasting (BFA <-2), n (%) | 3 (4) | 3 (13) | 4.0 (0.7 – 21.2) | 2 (4) | 7 (37) | 14.6 (2.7 – 79.3)* |
| Abnormal $SpO_2$ at rest or exercise, n (%) | 7 (9) | 4 (19) | 2.5 (0.6 – 9.3) | 4 (8) | 5 (28) | 4.4 (1.0 – 18.9)* |
| Resting tachypnoea (rate >25), n (%) | 13 (16) | 3 (14) | 0.8 (0.2 – 3.2) | 3 (6) | 5 (25) | 5.3 (1.1 – 25.0)* |

| | | | | | | |
|---|---|---|---|---|---|---|
| Viral load suppressed <400 copies/ml[a] | 60 (75) | 18 (86) | 2.0 (0.5 – 7.5) | 45 (88) | 14 (70) | 0.3 (0.1 – 1.1) |
| ISWT distance, metres (SD)[b] | 803 (223) | 804 (254) | 1.00 (1.00 – 1.00) | 704 (180) | 730 (223) | 1.00 (1.00 – 1.00) |
| CD4 at recruitment[c], cells/µl (SD) | 761 (343) | 658 (259) | 1.00 (1.00 – 1.00) | 794 (342) | 721 (423) | 1.00 (1.00 – 1.00) |

457 "Prior infection" uses a compound definition of any of the following: previous TB treatment; previous PCP treatment; admitted to hospital with

458 infection in the preceding 12 months; mycobacteria isolated in sputum culture

459 a: No prior infection - normal n=80, abnormal n=21; Prior infection - normal n=51, abnormal n=20

460 b: No prior infection - normal n=80, abnormal n=21; Prior infection - normal n=51, abnormal n=17

461 c: No prior infection - normal n=82, abnormal n=22; Prior infection - normal n=52, abnormal n=20

462 * significant at $p<0.05$

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