

LUNG FUNCTION IN PERINATALLY HIV-INFECTED ADOLESCENTS ON ANTIRETROVIRAL THERAPY IN CAPE TOWN, SOUTH AFRICA

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- b. Githinji LN, Gray DM, Zar HJ: **Lung function in HIV-infected children and adolescents.** *Pneumonia* 2018, **10**.
- c. Githinji LN, Gray DM, Hlengwa S, Machededze T, Zar HJ. **Longitudinal changes in Spirometry in HIV-infected adolescents on antiretroviral therapy.** Submitted to *Clinical Infectious Diseases* journal in October 2018. Revisions from peer-reviewers submitted in January 2019
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I collected data, did data analysis and data interpretation and wrote the manuscript. SH collected data. DG, LM and HZ reviewed the manuscript and added conceptual and intellectual comments. All authors read the manuscript prior to submission.

2. Githinji LN, Gray DM, Zar HJ: **Lung function in HIV-infected children and adolescents.** *Pneumonia* 2018, **10**.

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I collected data, did data analysis and interpretation of results and wrote the manuscript. SH collected data. TM offered expert advice in data analysis. DG and HZ reviewed the manuscript and added conceptual and intellectual comments. All authors read the manuscript prior to submission.

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I collected data, did the data analysis and result interpretation and wrote the manuscript. LZ read and interpreted the echocardiograms. JL provided expert advice on the cardiac aspects of the study. HZ, DG, LM, LZ, JL and HZ reviewed the manuscript and added conceptual and intellectual comments. All authors read the manuscript prior to submission and contributed within their area of expertise.

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Abstract

Background: Lung disease is a common complication of human immunodeficiency virus (HIV) infection in children and adolescents. As antiretroviral programmes have strengthened and HIV diagnosed earlier, survival of perinatally HIV-infected children has improved. Therefore, an increasing number of perinatally HIV-infected children are surviving into adolescence, with development of chronic multisystem disease including chronic lung disease (CLD). However, there is limited information on the determinants, spectrum and progression of lung disease. Lung function testing, an objective, non-invasive, reproducible tool, is useful in characterising CLD and in monitoring disease progression.

Aim: To investigate the spectrum, determinants and progression of lung function in perinatally HIV-infected adolescents on antiretroviral therapy (ART) in Cape Town, South Africa. Specific objectives included describing the spectrum and determinants of lung function; investigating cardiopulmonary dysfunction and investigating progression of lung function over two years.

Methods: The study population was from a prospective cohort, the Cape Town Adolescent Anti-retroviral cohort (CTAAC), that enrolled 515 perinatally HIV-infected adolescents on ART and 110 age-matched HIV-uninfected adolescents followed six-monthly for two years in Cape Town, South Africa. Eligibility criteria were adolescents, aged 9-14 years, with perinatally acquired HIV, who had been on ART for at least six months. Comprehensive lung function testing was done, and clinical and lung function data collected at baseline, 12 and 24 months.

Results: At baseline, HIV-infected adolescents had lower forced expiratory volume in 1 second (FEV_{1}), forced vital capacity (FVC), FEV_{1}/FVC , diffusing capacity for carbon

monoxide, respiratory system compliance and functional residual capacity; and higher airway resistance and lung clearance index compared to HIV-uninfected adolescents, $p < 0.05$ for all. At 24 months, FEV₁ and FVC remained lower in the HIV-infected compared to the uninfected, $p < 0.05$ and had similar growth-related change over time in both groups. Previous pulmonary tuberculosis (PTB) or severe lower respiratory tract infections (LRTI) was associated with reduced FEV₁ and FVC, $p < 0.05$ for both. Impaired cardiopulmonary function was detected in 13% of HIV-infected adolescents and 8% of HIV-uninfected adolescents, $p = 0.136$. Past PTB was significantly associated with a low cardiopulmonary function, OR 2.3, 95%CI 1.2-4.4.

Conclusion: Perinatally HIV-infected adolescents had lower lung function and higher resistance and ventilation inhomogeneity compared to age-matched HIV-uninfected adolescents at baseline. Lung function tracked, remaining lower at two years. Previous PTB or severe LRTI were predictors of lower lung function. Co-existent cardiopulmonary dysfunction occurred in a minority.

These data highlight respiratory disease risk in this vulnerable group and may inform policy to strengthen strategies to prevent and manage HIV-associated lung or cardiopulmonary disease.

Four of the chapters (2–5) of this thesis are presented as published manuscripts.

Chapter 1 encompasses an overview of the burden of HIV disease and the spectrum of HIV-associated chronic lung disease in adolescents and the utility of lung function in the diagnosis of chronic lung disease. Study methodology is also detailed in this chapter.

Chapter 2 (published manuscript) comprises a comprehensive review of published data on lung function (over and above the literature included in the individual papers) in HIV-infected children and adolescents and summarises studies that have been done in Africa, USA, Europe and Asia.

Chapter 3 (published manuscript) describes the spectrum and determinants of comprehensive lung function parameters (flow, volume, compliance, resistance, ventilation inhomogeneity) in perinatally HIV-infected adolescents with a comparator group of age-matched HIV-uninfected adolescents.

Chapter 4 (published manuscript) further explores the prevalence and determinants of co-existent cardiopulmonary dysfunction in perinatally HIV-infected adolescents on ART.

Chapter 5 (published manuscript) describes the progressive changes in spirometry over two years in perinatally HIV-infected adolescents compared to HIV-uninfected age-matched controls. It also addresses the associations of low lung function, factors amenable to public health interventions.

Chapter 6 is a summary of the study findings and recommendations.

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Abbreviations

| | |
|--------|--|
| 6MWT: | Six-minute-walk test |
| ART: | Antiretroviral therapy |
| BDR: | Bronchodilator responsiveness |
| CLD: | Chronic lung disease |
| DLCO: | Single breath diffusion test for carbon monoxide |
| FAC: | Percentage fractional area change |
| FOT: | Forced oscillation technique |
| FRC: | Functional residual capacity |
| IQR: | Interquartile range |
| LCI: | Lung clearance index |
| LLN: | Lower limit of normal |
| MBW: | Nitrogen multiple-breath washout test |
| OIs: | Opportunistic infections |
| PCP: | Pneumocystis jirovecii pneumonia |
| PMTCT: | Prevention of mother-to-child transmission |
| PTB: | Pulmonary tuberculosis |
| TAPSE: | Tricuspid annular plane systolic excursion |
| VA: | Alveolar volume |

Chapter 1:

1. Introduction

1.1 HIV prevalence in children and adolescents

In 2017, there were 36.9 million people living with HIV globally of which 1.8 million were children less than 15 years old. Among these children, 59% had access to antiretroviral therapy (ART). [1]

There were 2.1 million adolescents aged 10-19 years living with HIV globally in 2017. Among 40 countries with available data, only about 43% of adolescents aged 10-19 years living with HIV received ART in 2017. Of the 250 000 adolescents (ages 15-19 years) who were newly infected with HIV in 2017, 66% were female. [2]

In Eastern and Southern Africa, there were 77 000 new HIV infections among children (<15 years) in 2016. There has been a 56% reduction in new infections among children in this region since 2000. Fifty-one per cent of children less than 15 years were accessing ART in Eastern and Southern Africa. [3] Adolescent girls and women aged 15-24 years accounted for 25% of new HIV-infections in sub-Saharan Africa.

In South Africa, 280 000 children, aged 0-14 years, were living with HIV, of which 58% were on ART in 2017. [4] There were 13 000 new infections and 8 600 deaths attributable to acquired immune deficiency syndrome (AIDS) among children aged 0-14 years. There were 1.3 million orphans, aged 0-17 years, as a result of AIDS in 2017. [4]

1.2 Chronic lung disease in HIV-infected children and adolescents

With the more widespread availability of ART, HIV has now become a chronic disease. [5] The adolescent age group is among the most vulnerable to HIV in sub-Saharan Africa. [3] HIV chronic lung disease (CLD) is a common sequela of HIV infection. [6, 7] Diagnosis of CLD requires clinical, radiological, pulmonary function testing or histopathological diagnosis. The syndrome of chronic lung disease reported in HIV-infected adolescents includes irreversible airway obstruction, chronic respiratory symptoms and exercise intolerance.

The spectrum of chronic lung disease is worse in adolescents where there was delayed initiation of ART, [8, 9] emphasising that early ART initiation is beneficial to mitigate the damage that HIV or opportunistic infections (OIs) have on the lung.

1.2.1 Clinical spectrum of chronic lung disease

In 116 adolescents with vertically transmitted HIV in Zimbabwe (69% on ART), Ferrand et al. [10] reported 66% had chronic respiratory symptoms and 55% with non-tuberculous chronic lung disease had chest computed tomography scan findings of bronchiolitis obliterans.

Chronic lung disease was reported to be more prevalent in HIV-infected than HIV-uninfected children (71% vs 27%) in a cohort of 194 children who had respiratory symptoms persisting for more than one month. [11] In a subset of 42 patients who underwent invasive investigation for CLD, Jeena et al. [11] reported lymphocytic interstitial pneumonitis in 57%, tuberculosis in 29% and non-specific interstitial pneumonitis in 14%. Tuberculosis (TB), diagnosed by histology, was common in a cohort of HIV-infected children in South Africa with chronic respiratory symptoms and significant peripheral lymphadenopathy. [12] This was also found

in a Zimbabwe cohort of 32 older children and adolescents with delayed HIV diagnosis, where Ferrand et al. [13] reported 59% had TB.

Increased asthma prevalence in a cohort of HIV-infected children on ART has been reported. [14] The diagnosis of asthma was, however, based on the use of asthma medication which may have led to overdiagnosis, as asthma medications are commonly used for symptom relief in HIV-related chronic lung disease. Shearer [15] reported a higher rate of bronchodilator reversibility in HIV-exposed uninfected youth compared to HIV-infected youth (17% vs 9%, $p=0.05$). Asthma symptoms and use of asthma medications and atopic dermatitis were also reported to be higher in HIV-infected children compared to HIV-exposed but uninfected children. [16] This may be due to the immune reconstitution of CD4 T cells which regulate the type II inflammatory pathway in asthma.

The aetiology of CLD is broad, ranging from chronic infection to non-infective causes. With the advent of ART, the incidence of opportunistic infections has reduced substantially. [7] Further, Kaposi's sarcoma, lymphocytic infectious pneumonia (LIP), *Pneumocystis jirovecii* pneumonia (PCP) infection have a markedly reduced incidence in the ART era. [17] Bronchiolitis obliterans on chest tomography scan (CT scan) has been identified as the commonest radiological manifestation in CLD in HIV-infected adolescents. [10, 18]

1.2.2 Lung function abnormalities in chronic lung disease

The spectrum of lung function abnormalities in HIV-related chronic lung disease may present as an obstructive pattern on spirometry (bronchiectasis, bronchiolitis obliterans), restrictive pattern (chronic PCP, tuberculosis, lymphocytic interstitial pneumonitis (LIP), bronchiectasis) or mixed obstructive-restrictive pattern due to combinations of these. Interstitial pneumonitis,

LIP, PCP are likely to lead to a reduced diffusion factor for carbon monoxide (DLCO), table 1. Diffusion abnormalities though rarely reported in this age group have been reported in younger children [19] who had a faster clearance of radiolabelled aerosol and the faster clearance correlated with chronic respiratory symptoms in older children. Other less reported lung function abnormalities include reduced lung compliance [20] and increased airway resistance in HIV-infected children. [21]

Other clinical entities with non-specific lung function abnormalities that have been encountered in HIV-associated CLD include recurrent lower respiratory tract infections [9] and immune reconstitution inflammatory syndrome (IRIS). [7] Growth impairment in HIV-infected children (stunting or wasting) may also affect lung growth and lung function.

There is scarce longitudinal lung function data in HIV-infected adolescents on ART. Possible reasons for decreased lung function and decline over time as shown in previous HIV-infected adult studies includes immune activation in HIV leading to alveolitis. [22] Opportunistic infections (OIs) like PCP or TB have been reported to cause a decline in FEV₁, FVC and DLCO. [23, 24] HIV per se has been considered responsible for the decline in lung function after controlling for other respiratory infections. [25] Increased HIV viral load accelerates lung function decline, [25] and achievement of viral suppression by ART may preserve lung function at near or equivalent levels to HIV-uninfected individuals.

Table 1: Spectra of chronic lung disease in HIV infected adolescents [7] and associated lung function abnormality

| Infective causes | Lung function abnormality |
|--|---|
| Pulmonary Tuberculosis (PTB) | Mixed obstructive-restrictive pattern on spirometry or purely restrictive (if fibrosis) |
| Persistent and recurrent pneumonia due to recurrent viral and bacterial infections | Restrictive pattern on spirometry or mixed obstructive-restrictive pattern |
| Opportunistic infections like PCP* | Reduced DLCO |
| Post-infectious bronchiolitis obliterans | Obstructive or mixed pattern spirometry |
| Non-infective causes | |
| Bronchiectasis | Obstructive or mixed pattern spirometry |
| Lymphocytic interstitial pneumonitis (LIP) | Reduced DLCO |
| Immune reconstitution inflammatory syndrome (IRIS) following ART or TB therapy | Mixed obstructive-restrictive pattern spirometry or restrictive pattern |
| Malignancy e.g. pulmonary Kaposi's sarcoma | Restrictive pattern spirometry |
| Pulmonary hypertension | Reduced DLCO |
| Non-specific interstitial pneumonitis | Reduced DLCO |

* PCP-Pneumocystis jirovecii pneumonia; DLCO diffusion factor for carbon monoxide; ART antiretroviral therapy

2. Use of lung function to measure chronic lung disease

Different lung function tests measure various aspects of pulmonary function hence comprehensive measures are needed. These tests may aid in diagnosing and monitoring CLD. They include spirometry and bronchodilator response test, carbon monoxide single breath lung diffusion test, multiple breath nitrogen wash-out, forced oscillation and six-minute walk test as detailed in table 2.

Table 2: Lung function parameters

| Test | Measurement | Disease definition |
|--------------------------------|--|--|
| Spirometry | FEV ₁ , FVC, FEF ₂₅₇₅ , FEV ₁ /FVC | Obstructive or restrictive disease |
| Single breath diffusion for CO | DLCO, KCO | Interstitial lung disease, emphysema |
| Multiple breath washout test | FRC, LCI | Obstructive lung disease and ventilation homogeneity |
| Forced oscillation Technique | Resistance and compliance | Respiratory system resistance and compliance |
| Six-minute walk | Distance walked, SaO ₂ , HR, RR, BP before and after test and measure of dyspnoea in six minutes (Borg scale) | Cardiopulmonary functional status |

Forced expiratory volume in 1s (FEV₁), forced vital capacity (FVC), forced expiratory flow between 25-75 sec of vital capacity (FEF₂₅₇₅), transfer factor for carbon monoxide (TLCO), carbon monoxide transfer coefficient (KCO), functional residual capacity (FRC), lung clearance index (LCI), Oxygen saturation (SaO₂), blood pressure (BP), heart rate (HR), respiratory rate (RR)

2.1 Spirometry

Spirometry provides information on dynamic lung volumes and airflow and can identify patterns of lung disease. The commonly used outcomes in spirometry are forced vital capacity (FVC), forced expiratory volume in the first second (FEV₁), FEV₁/FVC ratio, and forced expiratory flow between 25 and 75% of vital capacity (FEF₂₅₋₇₅), which can be seen on the spirogram in figure 2. The test requires that the patient understands what is expected, can follow the instructions and uses maximal effort to comply with the current paediatric spirometry standards. [26, 27] Information on the reversibility of airway obstruction after bronchodilator is useful to differentiate asthma from non-reversible airway obstructive disease. Airflow in restrictive disorders is usually reduced in proportion to the reduction in lung volumes; thus, the ratio of FEV₁/FVC remains unchanged or is increased (>80%). In obstructive disorders, airflow is reduced more than lung volume and thus FEV₁/FVC decreases. [28, 29]

Mixed obstructive-restrictive diseases are suspected when reductions in the magnitude of forced expiratory flow appear disproportionately large in relation to reduction in the FEV₁/FVC ratio. [28] Declines in FVC caused by airway closure and gas trapping are accompanied by increases in the residual volume/total lung capacity (RV/TLC) ratio. Classically, RV/TLC remains unchanged in restrictive disorders. [28] TLC is reduced in restrictive diseases. In obstructive diseases, FRC will be increased. Spirometry does not measure RV, TLC or FRC (figure 1), other lung function tests and radiological tests may be required to confirm true restrictive or obstructive disease. Recent grading of severity of airway obstruction published in 2014 reported values of FEV₁ >-2 z-scores normal as per the reference standards, [30] whereas mild dysfunction is ≤2 z-scores and severe dysfunction ≥3 z-scores. The Global Lung Initiative (GLI) [31] has provided reference equations for use in multi-ethnic groups in ages 3-95 years and introduced reporting references as the lower limit of normal (LLN), which denotes 1.64 SD, z-scores and per cent predicted.

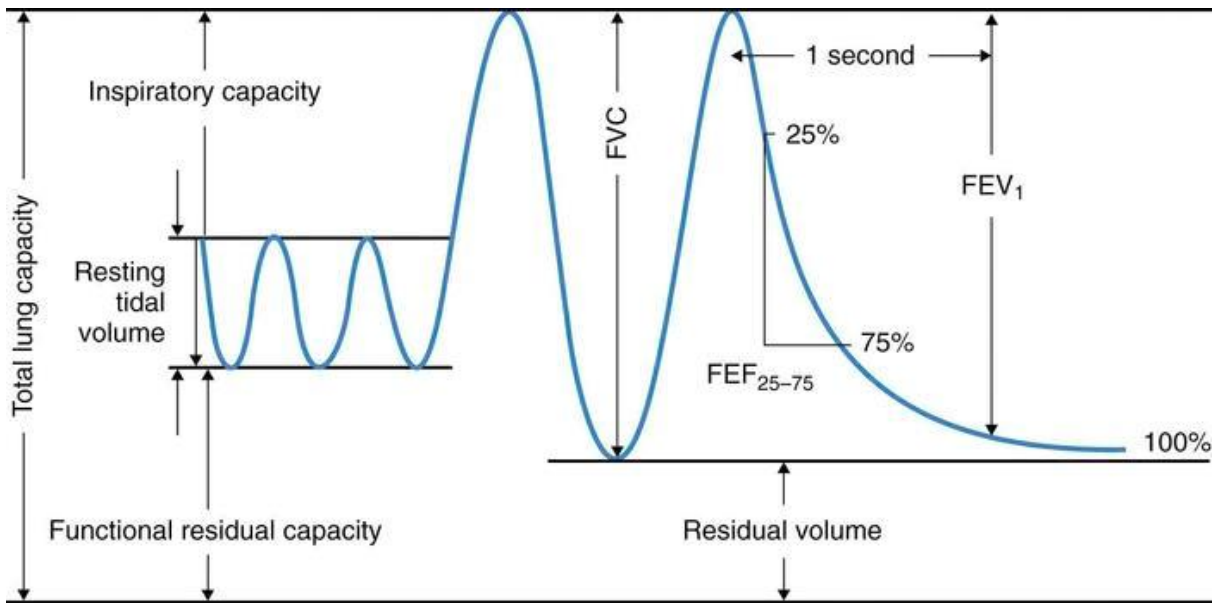


Figure 1: Lung volumes and lung capacities [32]

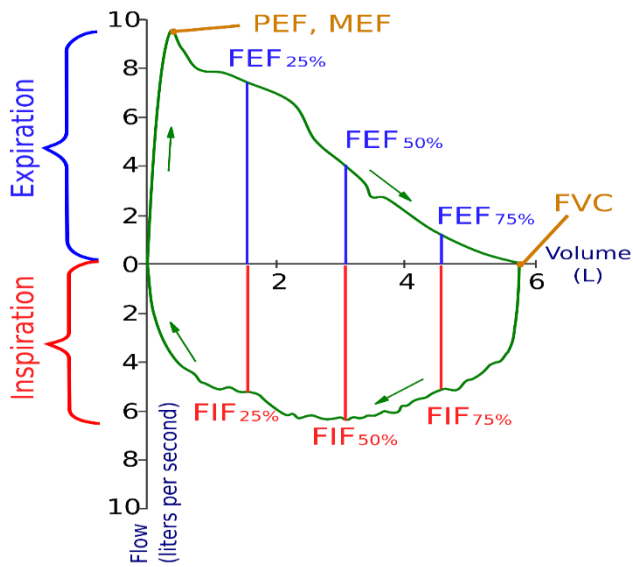


Figure 2: Forced expiratory and inspiratory flow volume loop [33]

2.2 Single breath test for carbon monoxide

Transfer factor for carbon monoxide of the lung (TLCO) or diffusion factor for carbon monoxide (DLCO), measured by single breath test, is a measure of the impedance to gas flow across the alveolar-capillary interface. It measures the rate at which carbon monoxide is transferred from the lung to the pulmonary capillary bed and is a reflection of the efficiency of diffusion across the capillary-alveolar surface. TLCO is the volume of carbon monoxide (CO) transferred from alveolar gas to blood (V_{CO}) in mm/min divided by the difference between mean alveolar-capillary CO pressure (P_{ACO}) and mean pulmonary-capillary CO pressure (P_{aCO}).

$$TLCO = V_{CO} \div (P_{ACO} - P_{aCO})$$

Current standards when performing the test are specified in the ATS/ERS guidelines. [34, 35] TLCO below the lower limit of normal (LLN) can be due to emphysema, interstitial lung diseases, congestive heart failure, alveolar proteinases, bronchiectasis, bronchiolitis obliterans, anaemia among others. TLCO may be elevated in exercise, in patients with right to left shunts, polycythaemia and acute pulmonary haemorrhage. [28, 36] The GLI has provided reference values for ages 3-95 years [37]; 85% of participants were Caucasians. TLCO has been used to diagnose and monitor diffusion impairments in patients with chronic pulmonary diseases.

2.3 Multiple breath washout (MBW) test

Functional residual capacity (FRC) and lung clearance index (LCI) can be measured by inert gas washout tests, the most commonly used measure being the nitrogen (N_2) multiple breath washout test. FRC measurement is a marker of normal lung growth but may also be increased in severe obstructive lung disease. [38] The total amount of nitrogen washed from the lungs

provides a measure of the patient's resting FRC. FRC equals the product of the washout volume divided by the nitrogen concentration of air in the lungs. The current standards for lung volume measurement have been specified by Robinson et al. [39]

Lung clearance index is a measure of ventilation homogeneity and has been found to be an early marker of lung function impairment in children with cystic fibrosis. [40] LCI is the ratio of cumulative expired washout volume (CEV) and FRC. The CEV reflects the sum of the tidal volumes required to reduce the concentration to $1/40^{\text{th}}$ of the concentration of the inert gas prior to the washout. In children with the non-homogenous lung disease, washout takes longer and requires more tidal breaths and thus the LCI is elevated compared to healthy children. In healthy people, LCI values do not change through later childhood and adult life. [28] Although reference equations exist for MBW in adolescents, [41] there are no appropriate reference ranges for African youth using N_2 MBW.

2.4 Forced oscillation technique (FOT)

FOT measures the impedance of the respiratory system and is measured during tidal breathing. External pressure oscillations are delivered at the airway opening and flow and pressure changes are measured to obtain the impedance (Z_{rs}), and its component parts resistance (R_{rs}), and reactance (X_{rs}) of the respiratory system. The current standards when performing FOT are spelt out by Oostveen et al. [42] R_{rs} depends on the resistive behaviour of the respiratory system and the reactive component, X_{rs} depends on the elastic and inert behaviour of the respiratory system. FOT has been used to diagnose airway obstruction and assess bronchodilator responses in asthma. [43-45] R_{rs} and X_{rs} are reported to be abnormal in children with chronic lung disease. [46-48] X_{rs} has been used to monitor improvement in cystic

fibrosis after a pulmonary exacerbation. [28] Calogero et al. [49] have provided reference values for respiratory impedance and bronchodilator responsiveness in Caucasian children 2-13 years of age, but currently, no reference ranges exist for African children and adolescents.

2.5 Six-minute walk test

The six minutes' walk test assesses functional status in chronic heart and or lung disease and has been used to monitor treatment response or to predict hospitalisation or mortality. Despite its utility in adults, six-minute walk test is not commonly performed in children due to lack of standardised guidelines and reference values in Paediatrics. Past studies have documented reference values for use in healthy children and adolescents. [50-52]

2.6 Study Methodology

2.6.1 Aim

To investigate the spectrum and determinants of lung function and progression over two years in perinatally HIV-infected adolescents on ART in Cape Town, South Africa.

2.6.2 Specific objectives

1. To describe the spectrum and determinants of lung function in perinatally HIV-infected adolescents on ART in Cape Town, South Africa.
2. To investigate the prevalence and determinants of cardiopulmonary dysfunction in HIV-infected adolescents on ART.
3. To describe the progression of lung function over two years in HIV-infected adolescents on ART and to determine risk factors associated with low lung function over time.

2.6.3 Study design

This was a prospective study of children enrolled in the Cape Town Adolescents Antiretroviral cohort (CTAAC). CTAAC aims to investigate chronic diseases in HIV-infected adolescents and markers of disease severity or progression. Enrolment of the study subjects began in October 2013 and was completed in March 2015.

2.6.4 Setting

The study was carried out at the Red Cross War Memorial Children's Hospital, Research Centre for Adolescent and Child Health (REACH).

2.6.5 Study population

Five hundred and fifteen HIV-infected adolescents and 110 healthy age, sex, ethnicity matched HIV-uninfected adolescents were enrolled and followed for two years. The HIV-infected participants were recruited from seven ART clinics within Cape Town namely Nolungile, Gugulethu, Cross Roads, Mitchell's Plain, Infectious disease clinics at Red Cross War Memorial children's and Tygerberg hospitals and Adolescent HIV clinic at Groote Schuur hospital. The HIV-uninfected adolescents were from Masiphumelele Youth Centre, a voluntary social service institution, located in a township in Cape Town.

2.6.6 Inclusion criteria

All participants enrolled in CTAAC study were eligible for inclusion. Eligibility criteria for CTAAC were HIV-infected adolescents, aged 9-14 years, who had been on ART for at least six months and who are resident in Cape Town area and where informed consent and assent for participation had been provided. The comparator group were healthy HIV-uninfected age-matched adolescents, where informed consent and assent was provided and had no known pre-existing lung disease.

2.6.7 Routine health care and follow up

All adolescents in this cohort were receiving routine health care at primary health care sites and/or clinics according to the Western Cape Department of Health Guidelines. If impaired lung function was detected as part of the study, this information was communicated back to the primary health care provider enabling the primary health care provider to adjust management according to standard care guidelines. Participants were followed up six-monthly from enrolment at REACH.

2.6.8 Data collection

The following measures were taken:

- Clinical parameters-signs and symptoms of respiratory disease, e.g. cough, wheeze, digital clubbing, intercurrent illness, intercurrent hospital visits/admissions, history of pulmonary tuberculosis and lower respiratory tract infections, history of smoking either passive or active, history of doctor-diagnosed asthma and anthropometry data, i.e. weight, height, body mass index (BMI). Clinical data were collected at baseline and annually. Clinical examination was conducted by a qualified medical officer. A standardised respiratory questionnaire (Appendix 1) was administered at enrolment and six-monthly. Data extraction from hospital records for past lower respiratory tract infections requiring hospitalisation or PTB was done at enrolment.
- Laboratory measures of disease, collected annually, included full blood count, HIV viral load (CAP method/Roche COBAS Ampliprep, Germany) and CD4 cell count (Beckman Coulter FC500 MPL analysers, USA and sputum microbiology.
- Lung function at baseline and progression over two years

The lung function testing at enrolment included spirometry, single breath carbon monoxide diffusion test (TLCO), multiple breath nitrogen washout (MBW), forced oscillation technique (FOT), bronchodilator response test and 6MWT. The testing at 24 months which is longitudinally reported was spirometry with bronchodilator responsiveness.

- Echocardiography to assess cardiac function at enrolment.

Cardiac function testing was assessed by echocardiography, performed by a trained research echocardiographer using either a Philips IE33 or CX50 echo machines (Phillips, Netherlands) using standardised techniques. [53, 54]

2.6.9 Lung function testing

Lung function testing included spirometry, FOT, six-minute walk test, N₂ multiple breath washout, single breath diffusion test for carbon monoxide and bronchodilator response testing. All testing was done according to the American Thoracic Society and European Respiratory Society (ATS/ERS) standards. [27, 32, 34, 35, 38, 42, 55] Spirometry, single breath diffusion test for carbon monoxide and multiple breath washout test was done using NDD EasyOne Pro machine (NDD, Switzerland) and for the FOT, a custom-made setup (University of Szeged, Hungary).

2.6.10 Bronchodilator testing

After performing DLCO and MBW tests, bronchodilator (400 µg of salbutamol) was given via MDI-spacer and FOT and spirometry was repeated after 15 minutes.

2.6.11 Evaluation of results

Lung function results were evaluated using z-scores, per cent predicted and lower limit of normal according to the Global Lung Initiative [31, 37]. The association of low lung function with socio-demographic factors, HIV-related factors and other clinical factors was investigated using Directed acyclic graphs. [56]

2.6.12 Ethical considerations

This study was approved by the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town (REC Ref: 051/2013), see Appendix 2. Consent from a parent or legal guardian and assent from the participant were obtained in the participant's first language by a trained study counsellor during enrolment, Appendix 3. The study was on a voluntary basis and participants were aware they could opt out of the study at their own will. Confidentiality was maintained by ensuring study participants were assigned unique study codes and hard copies of data locked in cabinets with restricted access and the computerised records were password restricted.

Potential risks in this sub-study were minimal, e.g. minimal discomfort involved when phlebotomy and lung function procedures were performed. Phlebotomy was done by trained nurses. Standardised phlebotomy kits were made available. We also ensured sterilisation of the sensor on the machine used for lung function was done daily with alcohol swabs. Lung function testing was done by a trained respiratory technologist. A new spirette (mouthpiece) was allocated to each study participant for each visit and was disposed of after use.

Results of lung function tests were available to the participant's primary health care provider. Participants needing specialist review were referred to a pulmonologist. The study potential benefits outweighed the minimal study risks.

Pertaining to the use of vulnerable population, i.e. adolescents, HIV infected, low socioeconomic status, the study was carried out in accordance with standard operating procedures published in January 2013 by Faculty of Health Sciences, Human Research Ethics committee that addresses research in a vulnerable population. The participants were reimbursed R80 for short visits and R200 for long visits predominantly to cover the costs of transportation

2.6.13 Sample size and power calculation

We used Satterthwaite's test to estimate power for a two sample means test, assuming unequal variances. Mean and standard deviation (SD) was obtained from a convenience sample of the whole cohort; mean (SD) of FEV₁ for HIV- uninfected and HIV-infected of 1.85 (0.50) and 1.56 (0.45), respectively and mean (SD) of DLCO of 18.40 (4.20) and 16.50 (3.38) of HIV-uninfected and HIV-infected, respectively. With the 515 HIV-infected adolescents and 110 HIV-uninfected adolescents already enrolled in the CTAAC cohort, the study had a power of 98% to describe the spectrum of lung function of HIV-infected adolescents on ART, with a type 1 error rate of 5%.

2.6.14 Data analysis

Data analysis and monitoring were undertaken continuously over the study period. Data were analysed with *Stata* Version 15.0.

An initial one-way analysis was conducted to assess the distribution of each variable. Continuous variables were summarised using mean and standard deviation for normally distributed variables and median and inter-quartile range (IQR) for non-normally distributed variables. Categorical variables were summarised with percentages in each category.

A two-way analysis including graphs, plots and tables were used to look for an association between the outcome and the exposure of interest, including potential confounders. Group differences for continuous variables were evaluated using T-test for normally distributed variables or Wilcoxon test or Kruskal-Wallis test for non-normally distributed variables. Group differences for categorical variables were evaluated using the Chi-Square. The final analysis utilised multivariate linear regression analysis for continuous outcomes or multivariate logistic regression for binary outcomes. Linear mixed effect modelling was used to explore longitudinal changes over two years in lung function. The impact of potential predictors and confounders was assessed using appropriate regression analysis.

For objective 1:

To describe the spectrum and determinants of lung function in HIV-infected adolescents on ART in Cape Town, South Africa.

Summary statistics included means, standard deviation (SD) or median, interquartile range (IQR) and z-scores. Linear regression was used to investigate the determinants of lung function.

For objective 2:

To investigate cardiopulmonary function in HIV-infected adolescents on ART.

This was summarised using mean (SD) and median (IQR). Logistic regression was used to compute determinants of cardiopulmonary function.

For objective 3:

To investigate the progression of lung function over two years in HIV-infected adolescents on ART.

This was done using cross-sectional time-dependent analysis to assess the patterns in mean lung function over time in the cases and controls. To explore lung growth in this population we controlled for baseline lung function and described lung function longitudinally as a change in z-score expected for age, height and gender. The impact of potential predictors and confounders was assessed using mixed model regression analysis.

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Chapter 2:

Lung function in HIV-infected children and adolescents

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Abstract

Background: The advent of anti-retroviral therapy has led to improved survival of HIV-infected children to adulthood, and to HIV becoming a chronic disease in older children and adolescents. Chronic lung disease is common among adolescents. Lung function measurement may help to delineate the spectrum, pathophysiology and guide therapy for HIV-related chronic lung disease.

Aim: The aim was to review the available data on the spectrum and determinants of lung function abnormalities and the impact of antiretroviral therapy on lung function in perinatally HIV-infected children and adolescents.

Methods: Electronic databases, "PUBMED" and "African wide" and "CINAHL" via EBSCO Host, using the MeSH terms, "Respiratory function" AND "HIV" OR "Acquired Immunodeficiency Syndrome" AND "Children" OR "Adolescents" were searched for relevant articles on lung function in HIV-infected children and adolescents. The search was limited to English language articles published between January 1984 and September 2017.

Results: Eighteen articles were identified which included studies from Africa, USA and Italy, representing 2051 HIV-infected children and adolescents, 68% on antiretroviral therapy, age 50 days to 24 years. Lung function abnormalities showed HIV-infected participants had increased lower airway expiratory obstruction that was irreversible and reduced functional aerobic impairment on exercise, compared to HIV-uninfected participants. Mosaic attenuation, extent of bronchiectasis, history of previous pulmonary tuberculosis or previous lower respiratory tract infection and cough for more than one month were associated with low lung function. Pulmonary function tests in children established on antiretroviral therapy did not show aerobic impairment and had less severe airway obstruction.

Conclusion: There is increasing evidence that HIV-infected children and adolescents have high prevalence of lung function impairment, predominantly irreversible lower airway obstruction and reduced aerobic function.

Background

Improved survival of perinatally HIV-infected children to adolescence has occurred with the scale-up of paediatric antiretroviral therapy (ART) and prevention of mother to child transmission (PMTCT) programmes. This has led to a large cohort of youth living with vertically transmitted HIV in sub-Saharan Africa. [1] Of the 2.3 million children living with HIV globally, 43% are on ART. [2, 3] In 2016, seven million people were reported to be living with HIV in South Africa, of which 350,000 were between 10-19 years old. [2]

HIV-related chronic lung disease (CLD) is a major cause of morbidity and mortality. [4, 5] In the post-ART era, the spectrum of CLD has changed from lymphocytic interstitial pneumonitis (LIP) being most predominant to bronchiolitis obliterans and bronchiectasis being more prevalent patterns. [5, 6] The spectrum of chronic lung disease in HIV infection has broad clinical phenotypes. For example, bronchiolitis obliterans or bronchiectasis may present as an obstructive or mixed pattern on spirometry, [5] while chronic *Pneumocystis jirovecii* pneumonia (PCP), pulmonary tuberculosis (PTB) or LIP have a restrictive or pattern spirometry. Interstitial pneumonitis, LIP and PCP are likely to lead to a reduced diffusion capacity for carbon monoxide (DLCO).

Comprehensive lung function measures are therefore needed to delineate the spectrum of CLD, monitor progression and to guide therapy and treatment response. These include measurements of lung capacities and flow such as spirometry and bronchodilator response testing, measure of lung volumes with plethysmography, measure of resistance and compliance with tests such as the forced oscillation technique (FOT), interrupter technique or single-breath occlusion technique, measure of gas diffusion with single-breath carbon

monoxide lung diffusion test to assess alveolar-capillary membrane function, measurement of ventilation distribution with multiple breath nitrogen wash-out test (MBW), cardiopulmonary functional assessment with six-minute walk test (6MWT) and exercise (treadmill) testing.

The aim of this study was to review the available data on the spectrum and determinants of lung function abnormalities in perinatally HIV-infected children and adolescents.

Methodology

A review of published literature was done by searching “PUBMED”, “African wide” and “CINAHL” via EBSCO Host using the MeSH terms “Respiratory function” AND “HIV” OR “Acquired Immunodeficiency Syndrome” AND “Children” OR “Adolescents”; full search terms are shown in table 1. Search was limited to English language, and publication date January 1984 - September 2017. Articles involving infants, child, adolescents or youth, HIV-infected or exposed and lung function testing were included. Articles on adult studies or healthy populations were excluded. Where full articles could not be retrieved on Endnote, we requested the full article from the corresponding author by email. In addition to database searches, other relevant references from previous original articles were hand-searched through google scholar. Data regarding patient characteristics, lung function test used, and outcome were abstracted and summarised in table format.

Results

The process of the literature search is shown in Figure 1. After combining all the search terms, 146 articles were found; eight additional articles were obtained by hand-searching, figure 1. One hundred and thirty-six studies were excluded because they were unrelated to lung function or were not related to the population of interest or only a conference abstract was

available. Eighteen full articles were found and are included in this review, table 2. Included studies were published between July 1997 and September 2017. Of the 18 included studies, 11 were from Africa, six from USA and one from Italy. Three studies were done in infants (of which two also included HIV-exposed uninfected infants), [7-9] two were done in children <8 years [10, 11] and 13 were done in adolescents and youth (9-24 years), table 2. Eleven studies had a comparator group (control), table 2. All the HIV- infected participants were perinatally infected.

Baseline characteristics of participants differed among studies with median age ranging from 50 days to 24 years. Number of participants in each study ranged from 100 to 600 with a total of 2051 HIV-infected participants pooled from all studies. Severity of disease differed; Ferrand et al. [6] reported 66% had chronic cough, McHugh et al. [1] reported 54% had chronic cough. Githinji et al. [12] reported 3.5% had clubbing while Mwalukomo reported 22% with digital clubbing. [13]

Participants were reported to have been on ART in 75% of the studies, table 2. Duration of ART was reported in five studies and ranged from 2-8 years. [12, 14-16] In three studies, no participant was on ART, table 2; two of these studies were done in sub-Saharan Africa [1, 17] and one in Italy in the pre-ART era. [11]

Lung function measures reported were spirometry with bronchodilator response testing and exercise testing (with treadmill or incremental shuttle walk test or 6MWT. One study included comprehensive lung function testing including forced oscillation technique (FOT) and MBW test. [12]

Spirometry testing was standardised in all studies as per American Thoracic Society/European Respiratory Society criteria. [18] Definition of restrictive pattern spirometry varied across studies with most reporting reduced forced vital capacity (FVC) as a spirometry pattern. Definition of obstructive pattern also varied across studies with some studies using the lower limit of normal of forced expiratory volume in 1sec/ forced vital capacity (FEV_1/FVC), as per the global lung initiative reference [19] and others using FEV_1/FVC less than 80%. Shearer et al. [20] had a broad inclusion criteria of obstructive spirometry pattern including $FEF_{25-75} < 65\%$ or FEV_1/FVC less than 80%.

Assessment and definition of bronchodilator responsiveness varied among the studies. Criteria for bronchodilator responsiveness (BDR) in most studies was change in $FEV_1 > 12\%$. Shearer et al. [20] used albuterol and a change of $\geq 10\%$ in FEV_1 . Three studies used 2.5 mg nebulized salbutamol [1, 13, 16] while the rest used 400 μg inhaled salbutamol.

Of the ten studies reporting spirometry findings, table 2, nine reported obstructive spirometry pattern, of which six studies demonstrated low rates of bronchodilator reversibility; and in five studies with a comparator group, this rate of irreversible obstruction spirometry was higher in the HIV-infected. Rylance et al. [16] reported 11 (35%) out of 31 HIV-infected children with obstructive spirometry had positive bronchodilator responsiveness, while Githinji et al. [12] reported 15% of HIV-infected had bronchodilator responsiveness compared to 8% HIV-uninfected adolescents, $p=0.058$. Mwalukomo et al. [13] reported 31.9% of the HIV-infected participants had bronchodilator responsiveness. Shearer et al. [20] reported similar rates of obstructive pattern spirometry between HIV-infected youth and HIV-exposed uninfected

youth (22% vs 21%), but a lower rate of bronchodilator responsiveness in the HIV-infected youth, 17% vs 9%, $p=0.05$).

Two studies reported diffusion tests, table 2, found to be lower or impaired in the HIV-infected group compared to the uninfected. Airway obstruction and reduced diffusion capacity were consistent findings across age-groups from childhood [10, 11] to adolescence, table 2.

Seven studies reported exercise tests for cardiopulmonary function status (6MWT or treadmill test), table 2, which showed that HIV-infected participants had functional aerobic impairment except for one study where no difference in distance walked or oxygen desaturation was reported after exertion, table 2.

Determinants of lung function were reported in three studies. [5, 6, 12, 13] History of previous lower respiratory tract infection or pulmonary tuberculosis was associated with reduced FEV₁ and DLCO. [12] Cough >one month was 2.9 times more likely to be associated with abnormal spirometry, 95% CI 1.21-7.10. [13] Mosaic attenuation and extent of bronchiectasis were significantly associated with reduced FEV₁, ($r=-0.52$ and $r=-0.50$, $p<0.001$, respectively). [5]

One study reported MBW and FOT besides spirometry, table 2, where HIV-infected adolescents had increased resistance, lower compliance, reduced functional residual capacity and increased lung clearance index compared to HIV-uninfected adolescents.

Two studies involved HIV-exposed uninfected children. [7, 9] and one study had HIV-exposed uninfected youth as a comparator group. [20] Forced expiratory flow was about 20% less in the HIV-exposed group but this difference was not significant. [21]

A summary of all studies included in this review is presented in table 2. Overall, results showed that HIV-infected participants had reduced flow and volume and functional aerobic impairment on exercise, reduced compliance, increased respiratory system resistance and reduced diffusion capacity compared to HIV-uninfected participants. Participants who had longer ART duration had less severe respiratory symptoms, less severe lower airway obstruction and no aerobic impairment.

Discussion

This review provides evidence of impairments in lung function in perinatally HIV-infected children and youth which were predominantly irreversible lower airway obstruction, reduction in exercise tolerance and reduced diffusion capacity. [1, 6, 15, 16] Fixed airflow obstruction was the most commonly reported finding, irrespective of ART status, table 2.

Irreversible airway obstruction is likely to be a response to airway epithelium injury by opportunistic infections (OIs) or from HIV, repair of which can lead to proliferation of granulation tissue, fibrosis of airways and subsequent obliteration of the lumen. [22] Bronchiolitis obliterans, which may result in irreversible lower airway obstruction, has been reported as a predominant pathology, evidenced by radiological manifestation of mosaic attenuation on chest tomography in HIV-infected adolescents with delayed access to ART. [5, 6] Systemic inflammatory markers have also been found to be increased in uncontrolled HIV or following repeated infections. [23] Lung infections like PCP [24] have been associated with increased metalloproteinases and chronic airflow obstruction in adults but none of the studies in this review reported prior PCP in participants.

ART has been reported in HIV-infected adults to be independently associated with irreversible airway obstruction, but the mechanism remains unproven. [25, 26] A direct effect of ART on inflammation in the lung and airways by reduction of peroxisome-proliferator-activated receptor has been reported in adults. [27]

Bronchodilator reversibility was reported to be present in 15-35% of participants. Despite the available evidence that irreversible airway obstruction is common in HIV-infected children, use of inhaled asthma medications has been reported to be widely used in HIV-infected children and adolescents. [28] Although bronchodilator reversibility was more common in HIV-exposed uninfected youths than in the HIV-infected youths, Shearer [20] reported that self-reported asthma diagnosis was higher in HIV-infected youths than uninfected. This may be due to constellation of symptoms of asthma-like respiratory illness, e.g. wheeze and cough in HIV-infected population and physician use of inhalers in the patients who present with such symptoms.

Differences in disease severity across study populations were more likely a result of varying duration of HIV infection and ART use. Those who had a duration of ART therapy of more than seven years reported lower prevalence of respiratory symptoms [12] than those who initiated ART in later childhood. [1, 6] Chronic lung diseases like bronchiectasis and bronchiolitis obliterans are likely to have occurred by the time of ART initiation in most of these studies reporting higher prevalence of chronic cough and wheeze. [1, 5, 6]

Functional aerobic impairment was more common in HIV-infected participants than uninfected. [1, 16, 17, 29, 30] Those on ART were reported to have done better on exertion than those not on ART. [15] The exercise intolerance may have been due to impaired

ventilation-perfusion mechanics with possible heart dysfunction, though no study in this review reported cardiac function. The cardio-pulmonary function status results across studies were inconsistent due to patient selection differences, with Githinji et al. [12] reporting no significant difference in exercise status between the HIV-infected adolescents and the uninfected and Chisati et al. [17] reporting low aerobic endurance in the HIV-infected group. These differences may be explained by differences in the cohorts and ART use; all children in the former cohort were stable on ART for a median duration of 8 years whereas none of the youth in the latter study were on ART.

While adult studies [25, 31] have reported diffusion impairment as predominant lung function abnormality, diffusion impairment in HIV-infected children and adolescents has not been commonly investigated. However, HIV-infected adolescents on ART were reported to have lower diffusion capacity compared to HIV-uninfected adolescents [12] suggesting that HIV or opportunistic infections may impair oxygen diffusion either by thickening of alveolar-capillary membrane due to interstitial inflammation or post-inflammation fibrosis or due to reduced surface area for gas exchange like in HIV-related bronchiectasis or bronchiolitis obliterans. Alveolar-capillary membrane integrity may be damaged by HIV and/or opportunistic infections well before the presence of clinical symptoms as reported by Alderson et al. [10] Emphysema, unlike in adults, was not documented as a common presentation in HIV-infected adolescents in Zimbabwe. [5, 6] Inflammation of the alveolar-capillary membrane by opportunistic infections like PCP and other AIDS related complications have also been documented. [31, 32] Low diffusion capacity has been reported in adult patients who had previous TB in a South

Africa cohort. [33] One study reported that pulmonary tuberculosis was associated with reduced DLCO in HIV-infected adolescents. [12]

The evidence on impact of HIV in-utero on infants born to HIV-infected mothers is evolving with only a few studies to date investigating HIV-exposed uninfected infants. These found no difference in spirometry pattern or forced expiratory flow on thoraco-abdominal compression between HIV-unexposed infants and HIV-exposed uninfected infants. [9, 20] Gray, [7] however, reported increased tidal volumes in HIV-exposed uninfected infants compared to unexposed infants soon after birth.

Although accelerated lung function decline has been shown in HIV-infected adults, [34, 35] published data on longitudinal lung function changes in HIV-infected children and adolescents are lacking. HIV per se has been reported to cause decline in lung function after controlling for other respiratory infections. [31] Bacteria pneumonia in HIV has been associated with permanent declines in FEV₁, FVC, FEV₁/FVC and DLCO. [36] Pneumonia and pulmonary tuberculosis were reported to be more common in HIV-infected adolescents than uninfected in two of the studies [12, 16] A result of pre-natal and postnatal origin of adult chronic obstructive airway disease as reported in several studies, [7, 37, 38] remains to be proven by longitudinal studies where these HIV-infected children and adolescents are followed to adulthood.

Limitations of this review include heterogeneity among studies and lack of reporting by some studies on duration of ART. The studies were also carried out in different eras of PMTCT and ART roll-out, where ART was initiated at varying CD4 counts or clinical stages. [39] Description of obstructive and restrictive spirometry patterns was not uniform across studies with most

studies reporting reduced FVC as a spirometry pattern per se and one study also including FEF₂₅₋₇₅ in the definition of obstructive spirometry. Determinants of lung function were also not widely reported. Almost all studies were cross sectional with very limited data on longitudinal changes in lung function over time.

Conclusion

There is increasing evidence that HIV-infected children and adolescents have high prevalence of lung function impairment, predominantly irreversible lower airway obstruction and reduced aerobic function. Lung function impairment was milder in cohorts of adolescents/children who had had earlier access to ART. Lung function impairment starts early in life in the absence of ART as evidenced by the papers published in the pre-ART era. Achievement of viral suppression through ART may preserve lung function, though at a lower level compared to HIV-uninfected individuals. ([1, 12, 20, 34]

Future directions

There is a need for longitudinal studies on lung function in HIV-infected children and adolescents in the post-ART era into adulthood as there is increasing evidence that chronic obstructive pulmonary disease has its origins in early life. [38] There is also need for more studies comparing lung function among HIV-infected, HIV-exposed uninfected and HIV-uninfected children and adolescents, to provide evidence on how exposure to maternal virus in-utero may affect lung function and how early intervention with ART in HIV-pregnant mothers may help to preserve lung function in infants and children.

Table 1: Search strategy for review of lung function in HIV-infected children and adolescents

| Database | MeSH | Key words |
|----------|--|---|
| PUBMED | Respiratory Function Tests HIV OR Acquired Immuno-deficiency Syndrome | Lung function test OR pulmonary function test OR Respiratory Function Tests HIV OR human immunodeficiency virus OR AIDS OR Acquired immunodeficiency syndrome OR Acquired Immuno-deficiency Syndrome or Acquired Immunodeficiency Syndrome Children OR pediatric OR paediatric OR neonates OR Adolescents OR teenagers OR youth OR young people OR infants Search, Query, Items found, Time #35, "Search ("Respiratory Function Tests"[Mesh]) OR (Respiratory Function Tests) OR pulmonary function test) OR lung function test)) AND ("Acquired Immunodeficiency Syndrome"[Mesh]) OR "HIV"[Mesh])) OR (HIV) OR human immunodeficiency virus) OR AIDS) OR Acquired immunodeficiency syndrome) OR Acquired Immuno-deficiency Syndrome) OR Acquired Immunodeficiency Syndrome) AND (Children) OR pediatric) OR paediatric) OR Adolescents) OR youth) OR young people) OR infants) OR teenagers) OR neonates) Sort by: [relevance]", 146,08:58:16 #34, "Search ("Respiratory Function Tests"[Mesh]) OR (Respiratory Function Tests) OR pulmonary function test) OR lung function test) AND ("Acquired Immunodeficiency Syndrome"[Mesh]) OR "HIV"[Mesh]) OR ((HIV) OR human immunodeficiency virus) OR AIDS) OR Acquired immunodeficiency syndrome) OR Acquired Immuno-deficiency Syndrome) OR Acquired Immunodeficiency Syndrome) Sort by: [relevance]", 659,08:54:18 #33, "Search (Children) OR pediatric) OR paediatric) OR Adolescents) OR youth) OR young people) OR infants) OR teenagers) OR neonates Sort by: [relevance]", 4074285,08:53:22 #32, "Search infants Sort by: [relevance]", 1118057,08:51:23 #31, "Search young people Sort by: [relevance]", 805914,08:51:04 #30, "Search youth Sort by: [relevance]", 1839127, 08:50:35 #29, "Search teenagers Sort by: [relevance]", 1822104, 08:50:16 #28, "Search Adolescents Sort by: [relevance]", 1843588, 08:49:54 |

#27, "Search neonates Sort by: [relevance]", 572698, 08:49:40
#26, "Search paediatric Sort by: [relevance]", 413775, 08:49:16
#25, "Search pediatric Sort by: [relevance]", 612753, 08:48:58
#24, "Search Children Sort by: [relevance]", 2174198,08:48:33
#23, "Search ("Acquired Immunodeficiency Syndrome"[Mesh]) OR "HIV"[Mesh]) OR ((HIV OR human immunodeficiency virus) OR AIDS) OR Acquired immunodeficiency syndrome) OR Acquired Immuno-deficiency Syndrome) OR Acquired Immunodeficiency Syndrome) Sort by: [relevance]", 424852, 08:47:05
#22, "Search (HIV) OR human immunodeficiency virus) OR AIDS) OR Acquired immunodeficiency syndrome) OR Acquired Immuno-deficiency Syndrome) OR Acquired Immunodeficiency Syndrome Sort by: [relevance]", 424852, 08:46:36
#21, "Search Acquired Immunodeficiency Syndrome Sort by: [relevance]", 88073, 08:44:20
#20, "Search Acquired Immuno-Deficiency Syndrome Sort by: [pubsolr12]", 88141, 08:44:00
#19, "Search Acquired immunodeficiency syndrome Sort by: [relevance]", 88073, 08:43:37
#18, "Search AIDS Sort by: [relevance]", 250617, 08:43:14
#17, "Search human immunodeficiency virus Sort by: [relevance]", 328144, 08:42:43
#16, "Search HIV Sort by: [relevance]", 316415, 08:42:22
#15, "Search ("Acquired Immunodeficiency Syndrome"[Mesh]) OR "HIV"[Mesh] Sort by: [relevance]", 152381, 08:41:48
#14, "Search ("Respiratory Function Tests"[Mesh]) OR (Respiratory Function Tests) OR pulmonary function test) OR lung function test) Sort by: [relevance]", 224085, 08:40:33
#13, "Search "Respiratory Function Tests"[Mesh] Sort by: [relevance]", 213706, 08:40:04
#12, "Search (Respiratory Function Tests) OR pulmonary function test) OR lung function test Sort by: [relevance]", 224085, 08:38:54
#11, "Search Respiratory Function Tests Sort by: [relevance]", 218117, 08:38:08
#10, "Search pulmonary function test Sort by: [relevance]", 222124, 08:37:37
#9, "Search lung function test Sort by: [relevance]", 221928, 08:37:11
#8, "Search ""Acquired Immunodeficiency Syndrome"[Mesh] Sort by: [relevance]", 74419, 08:36:14
#4, "Search "HIV"[Mesh] Sort by: [relevance]", 88572, 08:29:34

African wide
and CINAHL
via EBSCO
Host

lung function OR lung function test* OR pulmonary function OR pulmonary function test*
OR Respiratory Function OR Respiratory Function Test*

HIV OR human immunodeficiency virus OR AIDS OR Acquired immunodeficiency syndrome
OR Acquired Immuno-deficiency Syndrome or Acquired Immunodeficiency Syndrome
Child* OR pediatric OR paediatric OR neonat* OR Adolescent* OR teenage* OR youth OR
young people OR infant*OR young adult*

Table 2: Summary of studies on lung function in HIV-infected children and adolescents

| Author, Journal | Symptoms | Study design & country | Participant characteristics | Lung function test | Summary of results |
|--|--|----------------------------|--|--|---|
| Desai et al., [5] 2017 Clin Infec Dis | -25% chronic cough -5% wheeze -18% resting hypoxia | Cross-sectional, Zimbabwe | HIV-infected adolescents, median age 11 years, n=193, ART duration 5 years | spirometry with BDR* | -Mosaic attenuation and bronchiectasis on HRCT strongly correlated with FEV ₁ , r=-0.52, and r=-0.50, p<0.001 respectively |
| Shearer et al., [20] 2017 J Allergy Clin & Immuno | -34% had history of physician-diagnosed asthma | Cohort, USA | 218 HIV-infected, all on ART; 152 HIV-uninfected exposed; median age 17 years | Spirometry with BDR | -Obstructive spirometry pattern similar in both groups (22% vs 21%). -17% HIV-exposed uninfected youth had positive BDR vs 9% in HIV-infected youth, p=0.052 |
| Githinji et al. [12] 2017 Annals of ATS | -10% had history of asthma -4% had clubbing -15% anytime cough | Cohort study, South Africa | 515 HIV-infected adolescents, median age 12 years; mean ART duration 8 years, and 110 HIV-uninfected | Spirometry with BDR, FOT, N ₂ MBW, Single breath CO 6MWT | -Flow, volume, compliance, diffusion capacity lower in HIV-infected than uninfected; Higher resistance and LCI in HIV-infected compared to uninfected, p<0.05 -No cardiorespiratory function impairment on exercise testing in both groups |
| Gray D. et al. [7] 2017 Thorax | - | Birth cohort | 129 infants HIV-exposed uninfected; 546 infants born to HIV-uninfected | Tidal breathing and flow volume loops | -HIV-exposed infants had higher tidal volumes compared to infants born to |

| | | | | | |
|--|---|------------------------------|--|--|--|
| | | | mothers; median age 50 days | | HIV-uninfected mothers, p=0.04 |
| McHugh et al. [1] 2016 AIDS | -54% chronic cough -16% reported dyspnoea | Cross-sectional, Zimbabwe | 385 HIV-infected children, median age 11 years, none on ART | Spirometry with BDR, shuttle walk test | -10% obstructive spirometry; 1.3% BDR -18% reduced FVC -10% desaturated to <88% on exercise |
| Rylance et al. [15] 2016 Arch dis child (poster abstract) | -Those receiving ART, 15% had dyspnoea -15% had daily cough | Cross-sectional | 385 HIV-infected ART-naïve; 202 on ART; median age 11 years | Spirometry 6MWT | -Proportion of abnormal spirometry similar in ART-exposed and ART-naïve group (25.6% vs 24.3%) -Less distance in 6MWT in ART-naïve group, p<0.001 |
| Mwalukomo et al. [13] 2016 Peds Inf Dis | -8% had history of wheeze -22% had finger clubbing -20% had resting hypoxia | Cross-sectional, Malawi | 160 HIV-infected; median age 11 years 71% on ART median duration 3.5 years | Spirometry with BDR | -18% obstructive spirometry, 20% reduced FVC; 32% had + BDR |
| Rylance et al. [16] 2016 AIDS | -15% had chronic cough -15% had dyspnoea -5% had wheeze | Cross-sectional, Zimbabwe | 150 HIV-uninfected; 202 HIV-infected; median age 11 years ART mean duration 5 years | Spirometry with BDR, Shuttle walk test | -Lower FEV ₁ , FVC, and FEF ₅₀ in HIV-infected, p<0.05. 11 (35%) out of 31 with obstructive spirometry had + BDR -Less distance walked in HIV-infected, p<0.001 |
| Chisati et al. [17] 2015 Malawi Med. Journal | - | Cross-sectional, Malawi | 55 HIV-infected youth, not on ART and 78 uninfected youth, mean age 24 years | Treadmill exercise test | -Lower VO ₂ max (aerobic endurance) in HIV-infected compared to uninfected, p=0.01 |

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|--|---|----------------------------------|--|--|---|
| Masekela et al. [14] 2012 Int J Tuberc Lung Dis | - | Cross-sectional, South Africa | 35, 6-18 years with HIV-related bronchiectasis, all on ART | Spirometry with BDR | -Median FEV ₁ was 53% |
| Ferrand et al. [6] 2012 Clin Inf Dis | -35% resting hypoxia -66% recurrent cough -10% clubbing | Cross sectional, Zimbabwe | 116 adolescents mean age 14 years, vertically HIV-infected, 69% ART mean duration 20 months | Spirometry with BDR, 200 m brisk walk | -45% had FEV ₁ <80%; 47% had CXR abnormalities, 55% had mosaic attenuation on HRCT |
| Samadi et al. 2012 (unpublished data) | - | Cross-sectional, South Africa | 56 HIV-infected on INH prophylaxis, 7-10y, none on ART | Spirometry with BDR | -21% had abnormal spirometry; 18% had positive BDR |
| Cade et al. [29] 2002 Ped Rehab | | Cross-sectional, USA | 15 HIV-infected adolescents, 14 on ART & 15 matched HIV-uninfected, median age 18 years | Treadmill exercise test | -Peak oxygen consumption, treadmill duration and oxygen pulse were lower in HIV infected adolescents compared to uninfected, p<0.05 for all |
| Colin A et al. [9] 2001 AJRCCM | - | Cohort, USA | 285 HIV-exposed uninfected infants born to HIV-infected mothers, 92 HIV-unexposed uninfected infants | Vmax FRC by rapid thoracic compression | -Forced expiratory flow was ≈20% less in the HIV-exposed group but this difference was non-significant |
| Keyser et al. [30] 2000 Arch Phys Med Rehabil | - | Cross-sectional, USA | 17 HIV-infected mean age 18 years; all on ART | treadmill exercise test | -Peak oxygen consumption was lower than expected (functional aerobic impairment) |
| Platzker et al. [8] 2000 AJRCCM | - | Cohort, USA | 41 infants born to HIV-infected mothers (34% of HIV-infected infants), mean age 24 months | Thoraco-abdominal compression | -Respiratory system compliance reduced and declined more after TAC in HIV-infected, p=0.003 |

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|---|---|--|--|--|--|
| | | | | | -Higher resistance in HIV-infected infants compared to uninfected, p=0.03 |
| Alderson et al. [10] 1999 Radiology | - | Cohort, USA | 132 HIV-infected children, mean age 47 months and 160 HIV-exposed uninfected infants; mean age 10 months | Lung diffusion capacity using ^{99m} Tc DTPA | -HIV-infected children had faster clearance of ^{99m} Tc DTPA compared to HIV-exposed uninfected children, p<0.05, in the absence of clinical symptoms |
| De Martino et al. [11] 1997 Paeds Pulm | - | Prospective longitudinal cohort, Italy | 54 children, median age 64 months, with perinatal HIV infection, none on ART and 315 healthy controls | Interrupter technique | -Airway resistance greater in HIV-infected than uninfected, p<0.001 |

+BDR: positive bronchodilator responsiveness in FEV₁>12%; FEV₁: forced expiratory volume in 1s; FVC: forced vital capacity; FEF₂₅₋₇₅: forced expiratory flow between 25-75 sec of vital capacity; ^{99m}Tc DTPA: diethylene triamine pentaacetic acid; 6MWT: six-minute walk test; FOT: forced oscillation technique; N₂MBW: nitrogen multiple breath wash-out test; CO: carbon monoxide; HRCT: high resolution chest tomography

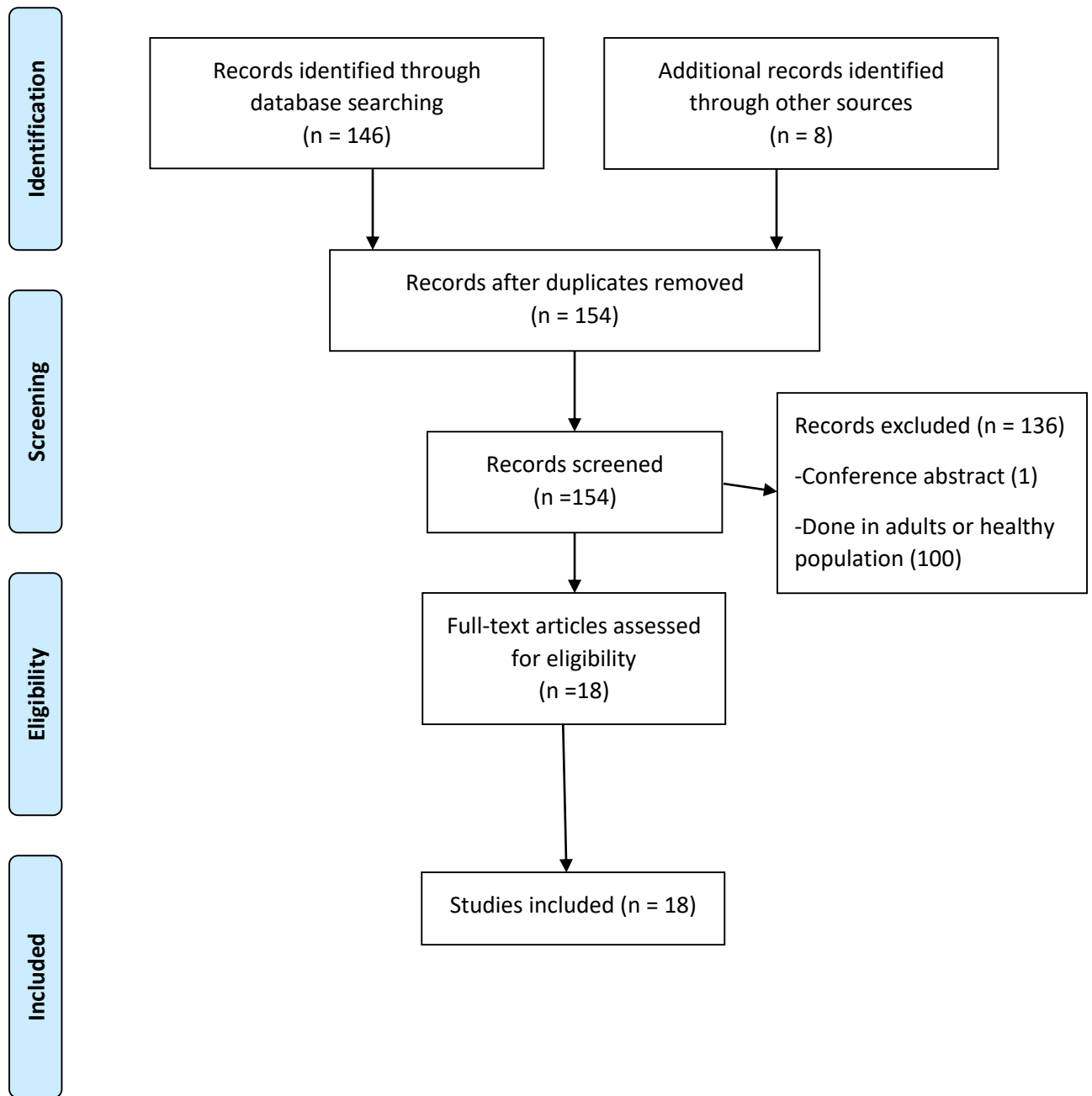


Figure 1: PRISMA Flow Diagram

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Chapter 3:

Lung Function in South African Adolescents Infected Perinatally with HIV and Treated Long-term with Antiretroviral Therapy

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Abstract

Rationale: Lung disease is a common cause of mortality and morbidity in HIV-infected adolescents, but there is limited information on the spectrum of lung function impairment in adolescents on antiretroviral therapy.

Objectives: To investigate lung function in HIV-infected adolescents on antiretroviral therapy in the Cape Town Adolescent Antiretroviral Cohort.

Methods: A total of 515 South African adolescents aged 9 to 14 years, stable on antiretroviral therapy for at least six months, underwent baseline lung function testing. Measures included spirometry, nitrogen multiple breath washout, forced oscillation technique, six-minute walk test, single breath carbon monoxide diffusion testing, and bronchodilator response testing. A comparator group of 110 age and ethnically matched HIV-uninfected adolescents was also tested.

Results: For the HIV-infected adolescents (mean (SD) age 12 (1.6) years, 52% male), the median (IQR) duration of antiretroviral therapy was 7.6 (4.6-9.2) years. The median (IQR) nadir CD4 was 510.5 (274-903) cells/mm³. HIV-infected adolescents had significantly lower forced expiratory volume in 1 sec (FEV₁), forced vital capacity (FVC), FEV₁/FVC, diffusing capacity for carbon monoxide (DLCO), respiratory system compliance, and functional residual capacity (FRC) than HIV-uninfected adolescents ($p < 0.05$ for all associations). HIV-infected adolescents had higher airway resistance and lung clearance index than HIV-uninfected adolescents ($p < 0.05$ for all associations). Although generally small in magnitude, these differences remained significant after adjusting for age, sex and height. In addition, age, sex, height, and history of past lower respiratory tract infection or pulmonary tuberculosis were associated with reduced lung function.

Conclusions: Perinatally infected South African HIV-infected adolescents on antiretroviral therapy have lower lung function than uninfected adolescents. History of severe lower respiratory tract infection or pulmonary tuberculosis is associated with lower lung function.

Background

Respiratory disease is the major cause of morbidity and mortality in HIV-infected children and adolescents. This burden of disease occurs predominantly in sub-Saharan Africa. [1] South Africa has the world's greatest burden of HIV infected people with an estimated 6.1 million persons infected, including 410 000 children under 15 years. [2] Since 2004, access to paediatric antiretroviral therapy has expanded globally, resulting in improved survival of HIV-infected children. [3] In South Africa, the number of children less than 15 years of age receiving antiretroviral therapy increased from 4200 in 2004 to 152 000 in 2011. [4] In response to treatment, the incidence of opportunistic infections has declined dramatically [5] Accordingly, HIV is evolving into a chronic illness among adolescents. [3]

HIV-associated chronic lung disease is common [5-7] but there is limited information on the determinants, spectrum or progression of chronic lung disease in perinatally infected adolescents. Studies of lung function in HIV-infected children in pre-antiretroviral therapy era have reported increased airway resistance, [8] while a few studies in children on antiretroviral therapy found airway obstruction, [6, 9-12] and increased bronchodilator responsiveness. [13, 14] Studies of adults on antiretroviral therapy [15-17] have documented irreversible airway obstruction, reduced diffusion capacity and increased bronchodilator response with the most prominent being diffusion impairment. [17, 18] Studies of comprehensive lung function testing in HIV-infected adolescents on antiretroviral therapy are limited, despite a high burden of respiratory disease.

The aetiology of HIV-associated chronic lung disease is broad, ranging from chronic infection to non-infective causes. [5] Diagnosis of chronic lung disease is made in South Africa by clinical criteria, imaging and lung function tests. Measurement of lung function offers an objective, reproducible, non-invasive measure of lung disease. Comprehensive lung function measures strengthen the ability to detect and diagnose the spectrum of lung function impairment.

The aim of this study was to investigate the spectrum and determinants of lung function in HIV-infected adolescents on antiretroviral therapy in Cape Town, South Africa. Some of the results of these studies have been previously reported in the form of an abstract. [19]

Methods

Study subjects

Patients at the Research Centre for Adolescent and Children Health at Red Cross Children's Hospital, South Africa Study subjects were recruited between August 2013 to April 2015 into a prospective study, the Cape Town Adolescent Anti-retroviral cohort (CTAAC), a longitudinal cohort study of HIV-infected adolescents on antiretroviral therapy. Eligibility was limited to adolescents, aged 9-14 years, with vertically transmitted HIV, who had been on antiretroviral therapy for at least 6 months, knew their HIV status, and where informed parental consent and participant assent was obtained. After enrolment, patients followed up monthly.

Lung function measures

Lung function measurements (Appendix 4) were generally performed on eligible subjects at the time of enrolment in CTAAC. Lung function testing was deferred if the participant had an acute respiratory illness. Testing, therefore, represented baseline lung function measured at a time when the participant was well.

Age, sex and ethnicity matched HIV-uninfected adolescents without known pre-existing lung disease were enrolled from the Masiphumelele Youth Centre, a community based vocational centre in Cape Town to serve as controls. Perinatal HIV-exposure of the HIV-uninfected participants was not known.

Lung function testing included spirometry [forced expiratory volume in one sec (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio]; single breath carbon monoxide diffusion test (DLCO); multiple breath nitrogen washout (multiple breath washout) measuring functional residual capacity (FRC) and ventilation homogeneity with the lung clearance index; the forced oscillation technique (forced oscillation technique testing) measuring respiratory system resistance and compliance; and the six-minute walk test. Spirometry was repeated 15 minutes after administering 400 µg salbutamol via a metered dose inhaler equipped with a spacer.

Lung function testing was performed using the NDD Easyone Pro LAB (NDD, Switzerland) for spirometry, single breath carbon monoxide diffusion test, and multiple breath washout. Forced oscillation technique testing was completed using custom-made equipment (University of Szeged, Hungary). [20] All testing adhered

to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. [21-27]

The highest FVC and FEV₁ from any of the acceptable manoeuvres was reported. Z-standardization for spirometry outcome variables was performed with Global Lung Initiative (GLI) software using African-American reference population. [28] Bronchodilator response was considered positive if there was an increase in FEV₁ of $\geq 12\%$ after bronchodilator administration. [22] The lower limit of normal (LLN) was calculated using the African-American reference cohort in GLI software, -1.64 standard deviations (SD) below the mean. [28]

Forced oscillation technique testing was performed first with the participant seated, nose clip on, cheeks firmly supported and breathing quietly through a mouthpiece and filter, as per consensus guidelines for testing. [25] Three 20-sec epochs were collected. Tests were considered acceptable if breathing was regular and free from leak or glottic closure. We collected a minimum of three repeatable tests, defined as visually overlapping spectral recordings within 10% of each other. The analysis model used was frequency range 10-20 Hz for respiratory system resistance (R) and 4-26 Hz for respiratory system compliance (C). In the absence of appropriate reference ranges, we used our HIV-uninfected adolescents as the reference population.

We followed ATS/ERS recommendations for acceptability of single breath CO testing. [24] We performed a minimum of two trials which met quality control standards and were within 3 ml/min/mmHg or 10%. Up to four attempts could be

done per session. Diffusion factor for carbon monoxide (DLCO) was adjusted for the patient's haemoglobin done on the day of lung function testing. The average of two best manoeuvres was taken for analysis. LLN was estimated using the Kim and Hall Equations. [29]

Multiple-breath washout testing adhered to international recommendations. [23, 27] Tests were considered acceptable if there was regular breathing pattern, especially for five tidal breaths before washout and first four breaths of washout, devoid of sighs, apnoea or leaks, and complete washout. We ensured adequate time between tests (at least 4 minutes) for gas concentration to equilibrate. Multiple-breath washout repeatability was defined by three technically acceptable tests with FRC within 10% of highest value and only excluded test if FRC differed by >25% of the median. For lung clearance index repeatability, we excluded test if lung clearance index differed by >1 FRC turnover (TO) from the median. Where there were two technically acceptable tests, we confirmed FRC repeatability if FRC is within 10% of the highest value, and lung clearance index was within one turnover. In the absence of appropriate reference ranges for African adolescents for multiple breath washout, we used the HIV-uninfected adolescents as the reference population.

For the six-minute walk test, participants were instructed to walk to and fro for six minutes. This was done in the research centre where two marked cones had been placed 30 m apart, as per standardised recommendations. [26] Heart rate, respiratory rate, blood pressure, Borg scale for dyspnoea and oxygen saturation was

recorded before and at the end of testing. Distance covered in meters was recorded at the end of the test, with published reference data used as normative values. [30]

Demographic data

Demographic and exposure data were measured at enrolment. Smoking history was self-reported by questionnaire completed by the adolescent and by the parent or guardian. Passive smoke exposure was regarded if either respondent reported a smoker in the household; active smoking was attributed if the study participant self-reported smoking. Antiretroviral therapy adherence data was collected via questionnaires to both participant and caregiver and graded according to the respondent's subjective rating of adherence as either good or poor. Other covariates were extracted from medical records or measured at the study visit. Participants were screened for respiratory symptoms using a validated respiratory questionnaire derived from the International Study of Asthma and Allergies in Childhood study. [31]

Statistical analysis

Data were analysed using STATA 12.0, (StataCorp, College Station, Texas, USA). Lung function measures were summarised with mean (with SD) or median (with interquartile ranges, IQR). We used our HIV-uninfected controls as the reference population for normative values for lung clearance index and forced oscillation technique testing outcome variables in the absence of reference data in African adolescent populations. Z-scores for height and weight were calculated using LMS (lambda-mu-sigma) growth charts. [32]

Comparison of lung function between HIV-infected and uninfected subjects was computed using independent two sample t-test with equal variances, where data were normally distributed and Wilcoxon sum rank test for data that was not normally distributed. Z-test was used to compute differences between two proportions. All statistical tests were 2-sided at $\alpha=0.05$. Univariable and multivariable linear regression was done to explore associations of lung function outcomes using the following covariates: HIV status, age, sex, height z-score, smoking status, CD4 count and viral load at the time of first study visit, antiretroviral therapy adherence, antiretroviral therapy duration, past lower respiratory tract infections, pulmonary tuberculosis (pulmonary TB) and self-reported respiratory symptoms.

Ethical approval was obtained from the Faculty of Health Sciences, University of Cape Town Human Research and Ethics Committee. Informed consent and assent were obtained from study participants and a parent or guardian.

Results

A total of 567 HIV- infected adolescents and 114 demographically matched HIV-uninfected control subjects were enrolled from August 2013 to April 2015. Fifty-six (9.9%) participants did not perform lung function testing and were excluded from this analysis, providing 515 HIV-infected adolescents and 110 HIV-uninfected adolescents with lung function results (figure 1).

The success rate for spirometry testing in HIV-infected adolescents was 97% and 88% for single breath test for CO, but 38% for multiple breath washout (figure 1).

The mean (SD) age of participants was 12 (1.7) years; 52% were male (table 1). Median (IQR) duration of antiretroviral therapy was 7.6 (4.6-9.2) years. Median (IQR) nadir CD4 count was 510 (274-903) cells /mm³.

Past pulmonary TB was reported in 37% of HIV-infected adolescents, compared to none of the HIV-uninfected adolescents (table 1). Prior *Pneumocystis jirovecii* pneumonia (PCP) was only reported in 1.4% of cases. Prior pneumonia was more commonly reported in HIV-infected than uninfected adolescents. Passive smoke exposure occurred in 25% of HIV-infected children, with a similar prevalence in the HIV-uninfected group (table 1). Seventy-seven (15%) HIV-infected adolescents had a history of a self-reported cough as compared to 7.3% HIV-uninfected adolescents (p=0.033). HIV-infected adolescents had more reported wheeze than HIV-uninfected adolescents, although the difference was not statistically significant (10.9% vs 5.5%, p=0.084), table 1. Eighteen (3.5%) HIV-infected participants had digital clubbing compared to only one uninfected adolescent. There was no difference between respiratory rate or oxygen saturation between the two groups.

HIV-infected adolescents had lower z-FVC compared to HIV-uninfected adolescents, (table 2). Overall, 75 (15%) HIV-infected adolescents and nine (8%) HIV-uninfected had a positive bronchodilator response, p=0.077, (table 2). There was no difference in the distance walked or oxygen saturation after six-minute walk test between the two groups, (table 3).

HIV infection was associated with lower z-FEV₁ (coefficient -0.52; 95%CI -0.79 to -0.26), lower z-DLCO (coefficient -0.23; 95%CI -0.35 to -0.11), lower z-FRC (coefficient -0.62; 95%CI -0.87 to -0.36), lower z-compliance (coefficient -0.21; 95%CI -1.61 to -0.80) and increased z-lung clearance index (coefficient 0.84; 95%CI 0.34 to 1.34 and increased z-resistance at 8Hz (coefficient 0.42, 95%CI 0.22 to 0.62), (table 4).

The associations of lung function outcomes in HIV-infected adolescents are shown in tables S1-7. History of LRTI and PTB were associated with lower z-FEV₁, after adjusting for smoking, ART duration and HIV status, coefficient -0.30; 95%CI -0.57 to -0.04 and -0.25; 95%CI -0.50 to -0.00 for previous LRTI and PTB respectively, (table S1). Longer ART duration was associated with a decrease in z-Reactance at 8Hz (coefficient -0.06; 95%CI -0.11 to -0.02), (table S6). History of LRTI was associated with increased z-LCI (coefficient 0.55; 95%CI 0.05 to 1.04; adjusting for HIV, smoking, ART duration and history of PTB). History of PTB was associated with lower z-DLCO (coefficient -0.13; 95% CI -0.23 to -0.02, adjusting for HIV, smoking, ART duration and history of PTB), (table S2). Smoke exposure was not associated with lung function, (tables S1-S7).

On average those who had digital clubbing had lower z-FEV₁ compared to those who had no clubbing while controlling for smoking, HIV infection, history of past LRTI and pulmonary TB, table 5 (coefficient -1.17, 95% CI -1.78 to -0.56). History of self-reported wheeze and asthma was significantly associated with lower z-FEV₁, (coefficient 0.92, 95%CI -1.27 to -0.56 and coefficient -1.00, 95%CI -1.34 to -0.65), respectively, (table 5).

Discussion

This study provides comprehensive lung function data on a large cohort of unselected perinatally HIV-infected adolescents established on antiretroviral therapy, showing that lung function outcomes are impaired compared to HIV-uninfected adolescents including expiratory flow volumes, ventilation homogeneity, resistance and compliance. Although the mean z-FEV₁ and z-FVC in the HIV-infected adolescents were within normal limits, they were significantly lower than in the HIV-uninfected. Further, the lung clearance index was increased in HIV-infected children reflecting ventilation impairment. The higher resistance and lower compliance found in HIV-infected adolescents suggests smaller airways or chronic inflammation.

Overall lung function impairment was mild in the HIV-infected cohort compared to the uninfected patient cohort. The HIV-infected cohort represented a relatively healthy group of perinatally infected adolescents with minimal symptoms, which may partly explain the relatively mild lung function impairment.

Other African studies in Malawian and Zimbabwean HIV-infected adolescents [6, 11, 33] have reported more severe symptoms and lung function impairment. In a study [6] of 116 Zimbabwean HIV-infected adolescents (69% on antiretroviral therapy), 52 (45%) were reported to have an FEV₁<80%. A cross-sectional study of 145 Malawian HIV-infected adolescents of which 72% were on antiretroviral therapy, reported 18% to have obstructive disease. [11] Patient selection, with more severely symptomatic children being selected for lung function, may have influenced measurements in these studies.

The relatively preserved lung function in our cohort may also be a result of long-term antiretroviral therapy use, good adherence to antiretroviral treatment, and access to health care enabling timely diagnosis and treatment of intercurrent infections. Although our HIV-infected adolescents had been on antiretroviral therapy for approximately 8 years, the median age of initiation of antiretroviral therapy occurred only at around 5 years of age, suggesting that use of antiretroviral therapy, even when initiated late in childhood, is an important strategy to preserve lung health. The reasons for documented low lung function in HIV-infected children may be related to prior illness, or HIV infection specifically. Opportunistic infections (OIs) have been reported to increase cytokines in serum and sputum, so promoting HIV mediated chronic airway inflammation. [15]

Only a minority of adolescents had bronchodilator responsiveness, similar to that reported in a Malawian study. [11] Several mechanisms may predispose to irreversible airway damage in this group including recurrent infection, fibrosis and increased oxidative stress mediated by downregulation of glutathione pathways by the HIV virus. [34] Irreversible airway damage may also reflect bronchiolitis obliterans which has been commonly reported in a Zimbabwean cohort. [6]

In our cohort, previous respiratory tract infection was common and associated with lower lung function. Previous lower respiratory tract infection or pulmonary TB was strongly associated with reduced lung function, both of which were more common in HIV-infected participants. This is consistent with data from HIV-uninfected children

showing lower respiratory tract infection in childhood to be associated with lower lung function in later life. [35, 36]

Bacterial pneumonia in HIV-infected adults has been associated with permanent declines in FEV₁, FVC, FEV₁/FVC and DLCO. [37] Opportunistic infections like *Pneumocystis jirovecii* pneumonia have also been associated with increased metalloproteinases and chronic airflow obstruction in HIV infected adults. [38] Very few HIV-infected adolescents reported prior PCP in this cohort. Further HIV-infected adolescents had higher airway resistance possibly due to smaller airways from impaired growth, repeated lower respiratory tract infections, OIs or increased airway inflammation. Higher airway resistance has been demonstrated in HIV-infected children before widespread use of antiretroviral therapy. [8]

Although low lung function on spirometry was common in HIV-infected adolescents, diffusion abnormalities were uncommon. Very few children were active smokers and only approximately a quarter reported passive smoke exposure; in contrast, HIV-infected adult studies have reported diffusion impairment in the context of high smoke exposure. [17] The absence of an association between passive smoking and lung function outcomes may possibly reflect underreporting of smoke exposure which was self-reported. The lack of association could also reflect a shorter term, lower dose exposure among adolescents compared to that reported in the adult emphysema literature. Diffusion abnormalities reported in both smoking and non-smoking HIV-infected adults have been explained by the presence of radiological emphysema due to loss of capillary blood volume. [39]

Emphysema may not be important in the aetiology of chronic lung disease in HIV-infected adolescents given the imaging findings that have been previously published that show predominantly bronchiolitis obliterans. [6] Other mechanisms postulated include alveolar-capillary membrane inflammation due to opportunistic infections like PCP or pulmonary TB. [18, 39] The lower DLCO in HIV-infected adolescents in our study cohort may represent a loss of alveolar volume, because of previous respiratory infection or ongoing inflammation.

The six-minute walk test is an insensitive measure of cardiopulmonary compromise which only becomes impaired in moderate to severe disease. Therefore, the finding that the distance walked and arterial oxygen saturation on the six-minute walk test was similar in HIV-infected children and controls probably reflects the lack of severe cardiorespiratory compromise and relatively mild lung disease.

Limitations

One limitation of the study is that childhood history of prior lower respiratory infection or pulmonary TB were based on recall. Despite this, there was a strong association between prior lower respiratory tract infection or pulmonary TB and impaired lung function.

A second limitation is a lack of appropriate normative reference values in African population for lung function, but the study is strengthened by the inclusion of an age-matched control group. Further, the use of the GLI African-American data for spirometry normal values provided an appropriate reference standard.

We did not obtain official birth records on the HIV-uninfected participants to establish HIV exposure perinatally; however, given the very long time from exposure to enrolment in this cohort, any possible residual effect of HIV exposure on lung health is unclear and likely to be minimal. As the cohort was enrolled at 9-14 years of age, it is impossible to obtain data on HIV exposure in-utero in HIV-negative individuals during pregnancy. A decade ago, it was not routine to test pregnant woman and the enrolled adolescents will not know if they were HIV exposed. Even if we tested their mother's now, there would be no way of knowing whether they were HIV-infected at the time of pregnancy. A further limitation is that few children met the strict criteria for multiple breath washout quality analysis as any who had sighs, glottis closure or hyperventilation during testing were excluded. This occurred in both HIV-infected adolescents and controls. However, success rates in performing spirometry were more than 95% and there were also high rates of success for DLCO, forced oscillation technique testing and six-minute walk test.

Another limitation is the inability to correlate lung function with precise radiological abnormalities as we did not obtain radiological imaging due to concerns about exposure to radiation and cost. These findings may not be generalizable to other settings in sub-Saharan Africa where adolescents may be more severely symptomatic and have more delayed access to antiretroviral therapy and to health care. Nevertheless, the results suggest that antiretroviral therapy may be effective for preserving lung health even when started late in childhood.

Conclusions

South African HIV-infected adolescents on antiretroviral therapy have significantly lower lung function than HIV-uninfected adolescents. Despite being well controlled on antiretroviral therapy, the study showed mild lung function impairment in HIV-infected adolescents, though significantly lower compared to age-matched HIV-uninfected. Previous lower respiratory infection and pulmonary TB were associated with lower lung function. With the growing number of perinatally HIV-infected adolescents in Africa facing improved survival and wellbeing on antiretroviral therapy, investigating the implications of these findings on long term respiratory health in this population is a priority which we are currently undertaking.

Table 1: Characteristics of the study population

| | HIV-infected n=515 | HIV-uninfected n=110 | P-value |
|---|-----------------------|-------------------------|---------|
| Age, years | 12.0 (1.6) | 11.8 (1.8) | 0.355 |
| Height for age z-score | -1.3 (1.0) | -0.5 (1.1) | <0.001 |
| Weight for age z-score | -0.8 (1.1) | 0.1(1.2) | <0.001 |
| Male, n (%) | 266 (52.1) | 48 (45.3) | 0.127 |
| Smoke exposure, n (%) none | 379 (74.3) | 83 (78.3) | 0.388 |
| passive | 127 (24.9) | 20 (18.9) | 0.185 |
| active | 2 (0.4) | 1 (0.9) | 0.458 |
| Past lower respiratory tract infection, n (%) | 147 (28.8) | 1 (0.9) | <0.001 |
| Past pulmonary TB, n (%) | 301 (37.1) | 0 (0) | <0.001 |
| PCP, n (%) | 7 (1.4%) | 0(0) | 0.220 |
| History of cough, n (%) | 77 (15.0) | 8 (7.3) | 0.033 |
| History of wheeze, n (%) | 56 (10.9) | 6 (5.5) | 0.084 |
| History of asthma, n (%) | 51 (9.9) | 7 (6.4) | 0.246 |
| Shortness of breath, n (%) | 17 (3.3) | 2 (1.8) | 0.411 |
| Clubbing, n (%) | 18 (3.5) | 1 (0.9) | 0.152 |
| Respiratory rate, breaths/min | 21.5 (3.4) | 20.8 (5.1) | 0.189 |
| Oxygen saturation (%) | 98.6 (0.9) | 98.8 (0.5) | 0.063 |
| CD4 nadir count, (cells/mm ³), n=442 | 510.5 (274-903) | | |
| CD4 current, (cells/mm ³), n=510 | 713 (561-957.5) | | |
| Viral load zenith, (copies/mL), n=195 | 226 (40-3927) | | |
| Antiretroviral therapy duration, (years), n=497 | 7.6 (4.6-9.2) | | |
| Age at antiretroviral therapy initiation, (years) | 5.0 (3.5) | | |
| Poor antiretroviral therapy adherence, n (%) | 51 (10.8) | | |

Values are mean (SD) or median (IQR); P value computed by two-sample test for proportions or independent two-sample T-test. lower respiratory tract infection, lower respiratory tract infections; pulmonary TB, pulmonary tuberculosis; antiretroviral therapy, antiretroviral therapy; PCP, *Pneumocystis jirovecii* pneumonia

Table 2: Baseline lung function tests

| Spirometry | HIV-infected n=499 | HIV-uninfected n=106) | P-value |
|---|-------------------------------|----------------------------------|----------------|
| FEV ₁ z-score | -1.0 (1.3) | -0.5 (1.0) | <0.001 |
| FVC z-score | -1.1 (1.3) | -0.8 (1.04) | 0.064 |
| FEV ₁ /FVC z-score | 0.2 (1.4) | 0.9 (1.2) | <0.001 |
| +BDR, n (%) | 75 (15) | 9 (8) | 0.006 |
| Single breath CO diffusion test | n=454 | n=98 | |
| DLCO z-score | -0.6 (0.5) | -0.3 (0.7) | 0.002 |
| DLCO /VA z-score | 0.7 (1.1) | 0.5 (1.2) | 0.078 |
| Multiple breath washout (med/IQR) | n=193 | n=41 | |
| FRC z-score | -0.6 (-1.1 to -0.2) | -0.2 (-0.8- 0.7) | 0.001 |
| LCI z-score | 0.6 (-0.2-1.6) | -0.2 (-0.8- 0.9) | 0.002 |
| Forced oscillation technique testing (med/IQR) | n=397 | n=104 | P-value |
| z- score Resistance at 8Hz | 0.4 (-0.2 to 0.9) | -0.1 (-0.7 to 0.7) | <0.0001 |
| z- score Reactance at 8 Hz | -0.2(-1.0 to 0.3) | 0.2 (-0.5 to 0.7) | 0.0001 |
| z- score mean respiratory system resistance | 0.3 (-0.2 to 0.8) | -0.1 (-0.8 to 0.7) | 0.0001 |
| z- score compliance | -0.2 (-0.5 to 0.3) | 0.2 (-0.3 to 1.3) | <0.0001 |

Values are mean (SD) unless otherwise indicated. P values computed by T-test or Wilcoxon sum rank test depending on data normality. forced oscillation technique testing and multiple breath washout compared to control data; Spirometry reference equation: GLI 2012 [28] DLCO referenced to Kim et al., 2012 [29]

FEV₁, forced expiratory volume in 1s; FVC, forced vital capacity; LCI, lung clearance index; FRC, functional residual capacity; DLCO, transfer factor for carbon monoxide; VA, alveolar volume

Table 3: Six-minute walk test

| Six-min walk test | HIV-infected n=339 | HIV-uninfected n=99 | P-value |
|----------------------------------|-------------------------------|--------------------------------|----------------|
| Distance walked, metres | 433.7 (57.0) | 443.5 (60.5) | 0.138 |
| Pulse before test, beats per min | 78.1 (12.2) | 83.0 (13.5) | <0.001 |
| Pulse after test, beats per min | 82.3 (13.9) | 87.2 (14.4) | 0.002 |
| Oxygen saturation after test (%) | 98.7 (0.8) | 98.8 (0.6) | 0.473 |
| MAP before test (mmHg) | 77.6 (7.9) | 81.4 (7.9) | <0.001 |

P-value computed using independent two-sample T-test. MAP, mean arterial pressure

Table 4: Effect of HIV-infection on lung function

| variable | coefficient | P value | 95%CI |
|--|--------------------|----------------|----------------|
| zFEV₁ (n=605) | | | |
| HIV status | -0.52 | <0.001 | -0.79 to -0.26 |
| z-DLCO (n=552) | | | |
| HIV infection | -0.23 | <0.001 | -0.35 to -0.11 |
| z-score FRC (n=234) | | | |
| HIV infection | -0.62 | <0.001 | -0.87 to -0.36 |
| z-score LCI (n=234) | | | |
| HIV infection | 0.84 | 0.001 | 0.34 to 1.34 |
| z-score Resistance at 8Hz (n=501) | | | |
| HIV infection | 0.42 | <0.001 | 0.22 to 0.62 |
| z-score Reactance at 8Hz (n=501) | | | |
| HIV infection | -0.51 | <0.001 | -0.77 to -0.25 |
| z-score Compliance (n=501) | | | |
| HIV infection | -1.21 | <0.001 | -1.61 to -0.80 |

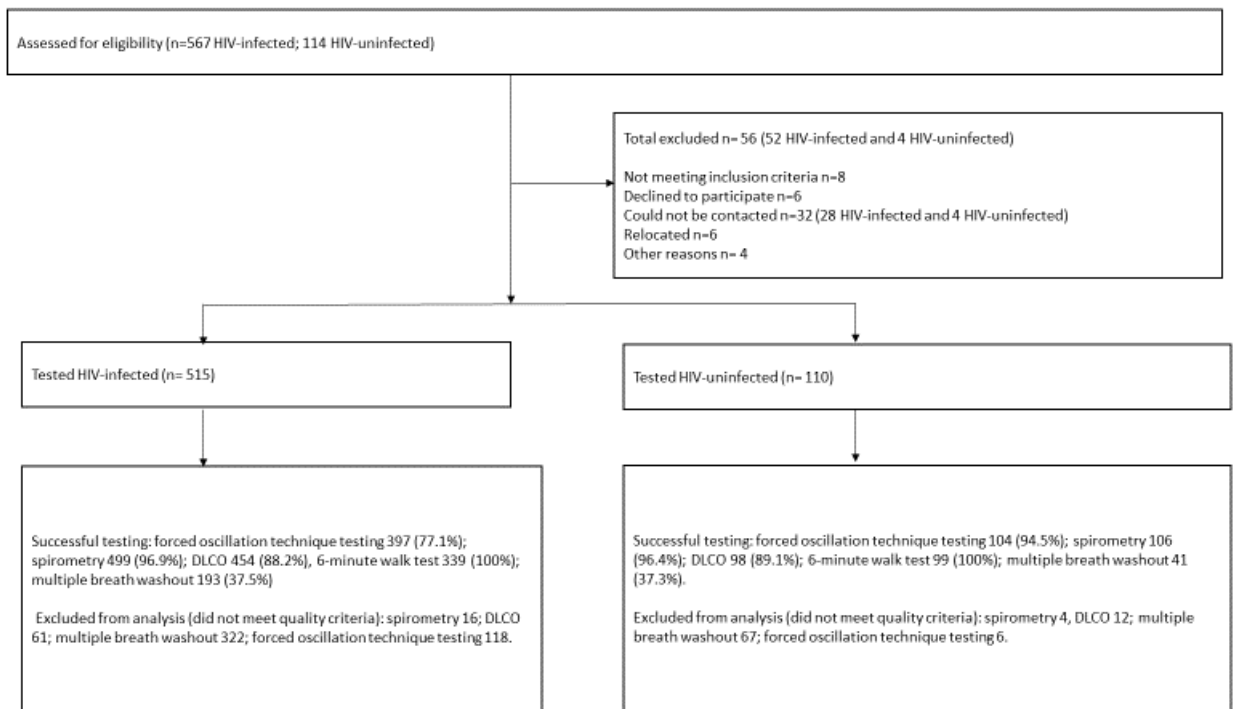
P value adjusted for HIV infection: linear regression

FEV₁, forced expiratory volume in 1s; lung clearance index, lung clearance index; FRC, functional residual capacity; DLCO, transfer factor for carbon monoxide.

Table 5: Effect of self-reported respiratory symptoms on lung function in HIV-infected adolescents

| Symptoms | z-FEV ₁ | | z-DLCO | | z-LCI | |
|-------------------|--------------------|----------------|--------|----------------|--------|---------------|
| | Coeff* | 95% CI | Coeff* | 95% CI | Coeff* | 95% CI |
| History of asthma | -1.00 | -1.34 to -0.65 | -0.01 | -0.16 to 0.15 | 0.48 | -0.18 to 1.14 |
| History of wheeze | -0.92 | -1.27 to -0.56 | -0.01 | -0.17 to 0.15 | -0.11 | -0.78 to 0.55 |
| History of cough | -0.30 | -0.61 to 0.01 | 0.10 | -0.04 to 0.25 | 0.54 | -0.07 to 1.15 |
| Digital clubbing | -1.17 | -1.78 to -0.56 | -0.36 | -0.64 to -0.08 | 0.01 | -1.32 to 1.34 |

* Adjusted for HIV status, smoking, history of lower respiratory tract infections, history of pulmonary TB and antiretroviral therapy duration
 Coeff, coefficient; CI confidence interval, LCI, Lung clearance index; FEV₁, forced expiratory volume in 1s; DLCO, transfer factor for carbon monoxide



DLCO, transfer factor for carbon monoxide

Figure 1: Cohort description

Supplementary Material

Table S1: Associations of z-FEV₁ in HIV-infected adolescents

| | | Univariable analysis | | | Multivariable analysis n=465 | |
|---|---------|----------------------|-------|----------------|------------------------------|----------------|
| | | N | Coeff | 95%CI | Coeff | 95%CI |
| Smoking | None | 457 | ref | - | - | - |
| | Passive | 148 | 0.09 | -0.15 to 0.33 | 0.11 | -0.17 to 0.39 |
| | Active | 3 | -0.95 | -2.42 to 0.52 | -1.65 | -3.49 to 0.20 |
| HIV status | Yes | 503 | -0.52 | -0.79 to -0.25 | Omitted* | |
| CD4+ T-cell count (cells/mm ³) | | 501 | 0.00 | -0.00 to 0.00 | - | - |
| HIV viral load (copies/mL) <50 | | 502 | | | | |
| | | 383 | ref | | | |
| | | 55 | -0.45 | -0.82 to -0.07 | | |
| | | 37 | -0.45 | -0.89 to -0.01 | - | - |
| | | 27 | -0.67 | -1.18 to -0.15 | | |
| ART duration (Y) | | 486 | -0.02 | -0.03 to -0.00 | -0.03 | -0.07 to 0.02 |
| Age at ART initiation (Y) | | 494 | -0.01 | -0.04 to 0.03 | | |
| ART adherence | Good | 382 | ref | - | - | - |
| | Poor | 116 | 0.16 | -0.11 to 0.44 | - | - |
| History of LRTI | No | 395 | ref | - | - | - |
| | Yes | 146 | -0.45 | -0.70 to -0.21 | -0.30 | -0.57 to -0.04 |
| History of PTB | No | 290 | ref | - | - | - |
| | Yes | 305 | -0.39 | -0.59 to -0.18 | -0.25 | -0.50 to -0.00 |

Y: years; FEV₁: forced expiratory volume in 1 second; PTB: pulmonary tuberculosis; ART: antiretroviral therapy; LRTI: lower respiratory tract infection; *HIV status omitted because of collinearity

Table S2: Associations of z-DLCO in HIV-infected adolescents

| | | Univariable analysis | | | Multivariable analysis n=415 | |
|---|------------|----------------------|-------|----------------|------------------------------|----------------|
| | | N | Coeff | 95%CI | Coeff | 95%CI |
| Smoking | None | 409 | ref | - | - | - |
| | Passive | 137 | 0.04 | -0.07 to 0.15 | -0.01 | -0.13 to 0.10 |
| | Active | 2 | -0.16 | -0.94 to 0.63 | -0.02 | -0.75 to 0.71 |
| HIV status | Yes | 549 | -0.23 | -0.35 to -0.11 | Omitted* | |
| CD4+ T-cell count (cells/mm ³) | | 449 | -0.00 | -0.00 to 0.00 | - | - |
| HIV viral load (copies/mL) <50 | | 450 | | | - | - |
| | | 344 | ref | | | |
| | 50-1000 | 50 | -0.03 | -0.18 to 0.13 | | |
| | 1001-10000 | 33 | -0.03 | -0.22 to 0.16 | - | - |
| | >10000 | 23 | -0.07 | -0.29 to 0.15 | | |
| ART duration (Y) | | 443 | -0.01 | -0.02 to 0.01 | -0.00 | -0.02 to 0.01 |
| Age at ART initiation (Y) | | 443 | 0.04 | 0.02 to 0.05 | - | - |
| ART adherence | Good | 339 | ref | - | - | - |
| | Poor | 107 | 0.03 | -0.09 to 0.15 | - | - |
| History of LRTI | No | 359 | ref | - | - | - |
| | Yes | 129 | -0.11 | -0.22 to -0.00 | -0.08 | -0.19 to 0.03 |
| History of PTB | No | 266 | ref | - | - | - |
| | Yes | 270 | -0.18 | -0.27 to -0.08 | -0.13 | -0.23 to -0.02 |

Y: years, DLCO: diffusion factor for carbon monoxide; PTB: pulmonary tuberculosis; ART: antiretroviral therapy; LRTI: lower respiratory tract infection; *HIV status omitted because of collinearity

Table S3: Associations of z-FRC in HIV-infected adolescents

| | | Univariable analysis | | | Multivariable analysis n=183 | |
|--|---------|----------------------|-------|----------------|------------------------------|---------------|
| | | N | Coeff | 95% CI | Coeff | 95% CI |
| Smoking | None | 164 | - | - | - | - |
| | Passive | 68 | 0.03 | -0.20 to 0.26 | -0.07 | -0.30 to 0.16 |
| | Active | 1 | 0.29 | -1.29 to 1.87 | 0.58 | -0.94 to 1.90 |
| HIV status | | 233 | -0.61 | -0.87 to -0.35 | Omitted* | |
| CD4+ T-cell count (cells/mm ³) | | 191 | -0.00 | -0.00 to 0.00 | - | - |
| HIV viral load (copies/mL) | | 147 | - | - | - | - |
| <50 | | | | | | |
| 50-1000 | | 20 | -0.07 | -0.40 to 0.26 | | |
| 1001-10000 | | 9 | 0.18 | -0.29 to 0.66 | | |
| >10000 | | 15 | -0.17 | -0.55 to 0.21 | | |
| ART duration (Y) | | 188 | -0.01 | -0.04 to 0.02 | -0.01 | -0.04 to 0.03 |
| Age at ART initiation (Y) | | 188 | 0.04 | 0.01 to 0.07 | | |
| ART adherence | Good | 140 | - | - | - | - |
| | Poor | 50 | 0.14 | -0.09 to 0.37 | - | - |
| History of LRTI | No | 150 | - | - | - | - |
| | Yes | 57 | -0.15 | -0.37 to 0.07 | -0.10 | -0.34 to 0.13 |
| History of PTB | No | 112 | - | - | - | - |
| | Yes | 115 | -0.26 | -0.46 to -0.05 | -0.02 | -0.24 to 0.19 |

Y: years; FRC: functional residual capacity; PTB: pulmonary tuberculosis; ART: antiretroviral therapy; LRTI: lower respiratory tract infection; *HIV status omitted because of collinearity

Table S4: Associations of z-LCI in HIV-infected adolescents

| | | Univariable analysis | | | Multivariable analysis n=183 | |
|--|---------|----------------------|-------|----------------|------------------------------|---------------|
| | | N | Coeff | 95% CI | Coeff | 95% CI |
| Smoking | None | 164 | - | - | - | - |
| | Passive | 68 | 0.06 | -0.36 to 0.48 | 0.16 | -0.32 to 0.65 |
| | Active | 1 | 4.49 | 1.56 to 7.42 | 3.5 | 0.54 to 6.54 |
| HIV status | | 233 | 0.85 | 0.35 to 1.35 | Omitted* | |
| CD4+ T-cell count (cells/mm ³) | | 191 | -0.00 | -0.00 to 0.00 | - | - |
| HIV viral load (copies/mL) | | 147 | - | - | - | - |
| <50 | | | | | | |
| 50-1000 | | 20 | 0.57 | -0.16 to 1.31 | | |
| 1001-10000 | | 9 | 0.07 | -0.99 to 1.12 | | |
| >10000 | | 15 | 0.13 | -0.71 to 0.96 | | |
| ART duration (Y) | | 188 | 0.12 | 0.05 to 0.19 | 0.10 | 0.03 to 0.18 |
| Age at ART initiation (Y) | | 188 | -0.08 | -0.14 to -0.02 | | |
| ART adherence | Good | 140 | - | - | - | - |
| | Poor | 50 | -0.23 | -0.74 to 0.28 | - | - |
| History of LRTI | No | 150 | - | - | - | - |
| | Yes | 57 | 0.82 | 0.36 to 1.27 | 0.55 | 0.05 to 1.04 |
| History of PTB | No | 112 | - | - | - | - |
| | Yes | 115 | 0.46 | 0.07 to 0.85 | 0.08 | -0.38 to 0.54 |

Y: years; LCI: lung clearance index; PTB: pulmonary tuberculosis; ART: antiretroviral therapy; LRTI: lower respiratory tract infection; *HIV status omitted because of collinearity

Table S5: Associations of z-Resistance at 8Hz in HIV-infected adolescents

| | | Univariable analysis | | | Multivariable analysis n=366 | |
|--|---------|----------------------|-------|----------------|------------------------------|---------------|
| | | N | Coeff | 95% CI | Coeff | 95% CI |
| Smoking | None | 378 | - | - | - | - |
| | Passive | 115 | -0.06 | -0.26 to 0.13 | -0.04 | -0.26 to 0.18 |
| | Active | 3 | 0.17 | -0.89 to 1.23 | 0.72 | -0.53 to 1.98 |
| HIV status | | 497 | 0.43 | 0.23 to 0.62 | Omitted* | |
| CD4+ T-cell count (cells/mm ³) | | 391 | 0.00 | -0.00 to 0.00 | - | - |
| HIV viral load (copies/mL) <50 | | 292 | - | - | - | - |
| 50-1000 | | 46 | 0.18 | -0.10 to 0.45 | | |
| 1001-10000 | | 32 | -0.27 | -0.60 to 0.05 | | |
| >10000 | | 22 | 0.15 | -0.23 to 0.53 | | |
| ART duration (Y) | | 386 | 0.02 | -0.01 to 0.05 | 0.02 | -0.02 to 0.05 |
| Age at ART initiation (Y) | | 386 | -0.05 | -0.08 to -0.03 | - | - |
| ART adherence | Good | 298 | - | - | - | - |
| | Poor | 90 | -0.03 | -0.24 to 0.18 | - | - |
| History of LRTI | No | 327 | - | - | - | - |
| | Yes | 112 | -0.01 | -0.21 to 0.19 | -0.11 | -0.32 to 0.09 |
| History of PTB | No | 247 | - | - | - | - |
| | Yes | 238 | 0.17 | 0.01 to 0.34 | 0.02 | -0.17 to 0.21 |

Y: years; PTB: pulmonary tuberculosis; ART: antiretroviral therapy; LRTI: lower respiratory tract infection;

*HIV status omitted because of collinearity

Table S6: Associations of z-Reactance at 8Hz in HIV-infected adolescents

| | | Univariable analysis | | | Multivariable analysis n=366 | |
|--|---------|----------------------|-------|----------------|------------------------------|----------------|
| | | N | Coeff | 95% CI | Coeff | 95% CI |
| Smoking | None | 378 | - | - | - | - |
| | Passive | 115 | 0.16 | -0.09 to 0.42 | 0.14 | -0.16 to 0.44 |
| | Active | 3 | -1.79 | -3.19 to -0.40 | -2.85 | -4.61 to -1.08 |
| HIV status | | 497 | -0.52 | -0.78 to -0.26 | Omitted* | |
| CD4+ T-cell count (cells/mm ³) | | 391 | -0.00 | -0.00 to 0.00 | - | - |
| HIV viral load (copies/mL) <50 | | 292 | - | - | - | - |
| 50-1000 | | 46 | -0.40 | -0.79 to -0.00 | | |
| 1001-10000 | | 32 | 0.08 | -0.39 to 0.54 | | |
| >10000 | | 22 | -0.17 | -0.72 to 0.38 | | |
| ART duration (Y) | | 386 | -0.07 | -0.12 to -0.03 | -0.06 | -0.11 to -0.02 |
| Age at ART initiation (Y) | | 386 | 0.09 | 0.06 to 0.13 | - | - |
| ART adherence | Good | 298 | - | - | - | - |
| | Poor | 90 | 0.17 | -0.13 to 0.47 | - | - |
| History of LRTI | No | 327 | - | - | - | - |
| | Yes | 112 | -0.32 | -0.60 to -0.05 | -0.10 | -0.39 to 0.19 |
| History of PTB | No | 247 | - | - | - | - |
| | Yes | 238 | -0.33 | -0.54 to -0.11 | -0.12 | -0.39 to 0.39 |

Y: years; PTB: pulmonary tuberculosis; ART: antiretroviral therapy; LRTI: lower respiratory tract infection;

*HIV status omitted because of collinearity

Table S7: Associations of z-Compliance in HIV-infected adolescents

| | | Univariable analysis | | | Multivariable analysis n=366 | |
|--|---------|----------------------|-------|----------------|------------------------------|---------------|
| | | N | Coeff | 95% CI | Coeff | 95% CI |
| Smoking | None | 378 | - | - | - | - |
| | Passive | 115 | 0.22 | -0.19 to 0.62 | 0.12 | -0.13 to 0.37 |
| | Active | 3 | -0.81 | -3.01 to 1.40 | -0.97 | -2.42 to 0.47 |
| HIV status | | 497 | -1.21 | -1.62 to -0.80 | Omitted* | |
| CD4+ T-cell count (cells/mm ³) | | 391 | -0.00 | -0.00 to 0.00 | - | - |
| HIV viral load (copies/mL) <50 | | 292 | - | - | - | - |
| 50-1000 | | 46 | -0.28 | -0.59 to 0.03 | | |
| 1001-10000 | | 32 | 0.08 | -0.29 to 0.44 | | |
| >10000 | | 22 | -0.36 | -0.80 to 0.07 | | |
| ART duration (Y) | | 386 | -0.02 | -0.05 to 0.02 | -0.01 | -0.05 to 0.02 |
| Age at ART initiation (Y) | | 386 | 0.04 | 0.02 to 0.07 | - | - |
| ART adherence | Good | 298 | - | | - | - |
| | Poor | 90 | 0.00 | -0.24 to 0.24 | - | - |
| History of LRTI | No | 327 | - | - | - | - |
| | Yes | 112 | -0.32 | -0.75 to 0.12 | 0.05 | -0.18 to 0.29 |
| History of PTB | No | 247 | - | - | - | - |
| | Yes | 238 | -0.59 | -0.94 to -0.25 | -0.12 | -0.34 to 0.10 |

Y: years; PTB: pulmonary tuberculosis; ART: antiretroviral therapy; LRTI: lower respiratory tract infection;

*HIV status omitted because of collinearity

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Chapter 4:

Cardiopulmonary dysfunction in perinatally HIV-infected South African adolescents on antiretroviral therapy: Baseline findings from the Cape Town Adolescent Antiretroviral Cohort

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Abstract

Introduction

Antiretroviral therapy (ART) has reduced morbidity and mortality in sub-Saharan Africa, but the burden of coexistent cardiopulmonary disease in perinatally HIV-infected adolescents on ART has not been well described. The aim of this study was to investigate the prevalence and associations of cardiopulmonary dysfunction in adolescents with perinatally acquired HIV on antiretroviral therapy.

Methods

For this cross-sectional analysis, 515 perinatally HIV-infected adolescents ages 9-14 years on ART for at least six months, and a comparator group of 110 age-matched HIV-uninfected adolescents were tested between August 2013-April 2015 using echocardiography, six-minute walk test (6MWT) and spirometry. Those with either abnormal spirometry or abnormal 6MWT and any right or left systolic or diastolic dysfunction or abnormal mean pulmonary arterial pressure were considered as having impaired cardiopulmonary function. Logistic regression was used to investigate determinants of impaired cardiopulmonary function.

Results

Overall, 474 adolescents with perinatally acquired HIV (mean [SD] age, 12 [1.6] years; median [IQR] ART duration, 7 [4.6-9.3] years; median [IQR] CD4 count, 712 [571-959] cell/mm³) and 109 HIV-uninfected adolescents (mean (SD) age 11.8 (1.8) years, had successful cardiac and lung function testing. Impaired cardiopulmonary function was detected in 13% of adolescents with perinatally acquired HIV and 8% of HIV-uninfected adolescents, $p=0.136$. Among adolescents with perinatally acquired HIV those with low tricuspid annular plane systolic excursion (TAPSE) had lower mean z -FEV₁, 1.9 (1.4). versus -1.6 (1.3), $p = 0.07$, but this difference was not statistically significant. Height (OR 0.7, 95%CI 0.5-0.9), body mass index (OR 0.7, 95%CI 0.5-0.9) and past pulmonary tuberculosis (OR 2.3, 95%CI 1.2-4.4) were significantly associated with a low cardiopulmonary function.

Conclusion

Despite being on ART, cardiopulmonary dysfunction occurs in an appreciable proportion of perinatally HIV-infected adolescents but no significant difference to uninfected controls. This finding requires further exploration. Factors associated with dysfunction may be amenable to public health interventions to reduce cardiopulmonary disease in this population.

Introduction

With improved survival of adolescents with perinatally acquired HIV on antiretroviral therapy (ART), HIV has become a chronic disease, with perinatally infected children surviving into adolescence and adulthood. [1] HIV-related cardiac disease [2-4] and/or chronic lung disease has been reported in sub-Saharan Africa. [5, 6] Symptoms which can be attributed to either cardiac or lung disease like tachypnoea or dyspnoea can overlap posing a diagnostic challenge.

In the pre-ART era, a high prevalence of cardiac abnormalities especially left ventricular dysfunction and cardiomyopathy were reported, [7] but a sharp decline has been found with the use of ART. [8, 9] Although ART has reduced the incidence and severity of acute pulmonary infections, lung function abnormalities are still highly prevalent. [10, 11] Right heart dysfunction may be secondary to chronic lung disease. Miller et al. [4] reported a 29% prevalence of right ventricle dilatation in a Zimbabwe cohort of perinatally HIV-infected adolescents (71% on ART, median duration of ART 20 months). Nearly 50% of this population had chronic lung disease.

ART also has an impact on exercise tolerance, which is a marker of cardiopulmonary function status. HIV-infected children on ART in Malawi had better exercise tolerance compared with HIV-infected ART-naive children. [12] ART-naive HIV-infected children had worse symptoms of cough, dyspnoea, hypoxaemia and low exercise tolerance compared with those on ART. [13] Although ART is reported to improve cardiac function, exercise tolerance and lung function, children on ART still have lower lung function, [11] lower exercise tolerance [12] and lower cardiac function [14] compared to HIV-uninfected children. Most of these published studies were from the era when ART access was not

universal, was initiated based on clinical and immunological severity; hence the need for studies from well-established ART cohorts.

Risk factors for cardiopulmonary dysfunction may include HIV immunosuppression, opportunistic infections, poor ART adherence, late age at initiation of ART, malnutrition or smoking. Other risk factors may be specific to cardiac dysfunction like dyslipidaemia. Further, transplacental exposure to drugs like zidovudine has been reported to impact fetal cardiac development. [15] Lung disease may lead to right heart strain and pulmonary hypertension progressing to cor-pulmonale. [16] Conversely, cardiac dysfunction as measured by a reduced left ventricular ejection fraction may result in fluid overload causing pulmonary oedema and subsequent poor lung compliance. HIV has also been documented to cause primary pulmonary hypertension in adults [17] and adolescents. [18]

The aim of this study was to investigate the prevalence and determinants of cardiopulmonary dysfunction in perinatally HIV-infected adolescents on antiretroviral therapy.

Methods

A prospective study, the Cape Town Adolescent Anti-retroviral cohort (CTAAC), previously described [11], enrolled 515 perinatally HIV-infected adolescents on ART and 110 age-matched HIV-uninfected adolescents. Patients were enrolled from August 2013 to April 2015 and followed six-monthly at the Research Centre for Adolescent and Child Health at Red Cross War Memorial Children's Hospital, South Africa.

Participants were eligible for the study if they were adolescents aged 9-14 years, with perinatal HIV infection, had been on ART for at least six months and knew their HIV status. Informed parental consent and participant assent were obtained. Age-matched HIV-

uninfected adolescents without known pre-existing lung or cardiac disease were enrolled from Masiphumelele Youth Centre in Cape Town, South Africa. Perinatal HIV exposure of the HIV-uninfected participants was unknown, but all tested negative for HIV prior to enrolment in the study. Ethical approval was obtained from the Human Research Ethics Committee of the University of Cape Town.

Data presented here are from the enrolment visit. Data on demography, self-reported smoking history and cardiorespiratory symptoms, ART duration and adherence, previous pulmonary tuberculosis (PTB) and other lower respiratory tract infections (LRTI) were collected by validated questionnaires. A history of previous PTB or LRTI was extracted from hospital records and supplemented by participant or caregiver report. Respiratory symptoms of wheeze, shortness of breath were self-reported. Blood was taken for CD4 count (Beckman Coulter®, USA) and HIV viral load (Roche COBAS Ampliprep, Germany). Adherence to ART was self-reported and measured using any missed doses in the last 30 days.

Lung function testing

Lung function testing [11] included spirometry measuring forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and FEV₁/FVC as measures of dynamic lung volumes and airflow obstruction; and the six-minute walk test measuring effort tolerance. Lung function testing was deferred if the participant had an acute respiratory illness. Spirometry was done using the NDD Easyone Pro LAB (NDD, Switzerland). All testing adhered to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. [19-21] We reported the highest FVC or FEV₁ from any of 3 acceptable spirometric attempts. The lower limit of normal (LLN) for spirometry outcome variables

was calculated using the African-American reference cohort in Global Lung Initiative (GLI) software, -1.64 standard deviations (SD) below the mean. [22]

For the six-minute walk test, the participant was instructed to walk for six minutes, between two marked cones placed 30 metres apart, as per standardized recommendations [21] Heart rate, respiratory rate, blood pressure, Borg scale for dyspnoea and oxygen saturation were recorded before and at the end of testing. Distance covered in meters was recorded at the end of the test, with published reference data used as normative values. [23]

Spirometry patterns were used to define abnormal lung function. Abnormal lung function was defined as either abnormal 6MWT or abnormal spirometry with obstructive (FEV_1/FVC less than the lower limit of normal (LLN)), restrictive ($FVC < LLN$ with normal FEV_1/FVC) or mixed pattern spirometry ($FEV_1 < LLN$, $FEV_1/FVC < LLN$ and $FVC < LLN$). Abnormal 6MWT was defined as desaturation $> 5\%$ post exercise or oxygen saturation $< 94\%$ at rest after exercise.

Cardiac function testing

Cardiac function testing [24] was assessed by echocardiography, performed by a trained research echocardiographer using either a Philips IE33 or CX50 echo machines (Phillips, Netherlands) using standardized techniques. [25, 26] All echocardiographs were interpreted by a single cardiologist. A random subset of 10% was also read by a second blinded cardiologist. Both cardiologists were blinded to the HIV status of the participant. Inter-reader disagreements were resolved by consensus.

Right ventricular (RV) systolic function was determined by calculating the percentage fractional area change (FAC) and the volumetric RV ejection fraction i.e. tricuspid annular plane systolic excursion (TAPSE). TAPSE was measured using M Mode echo [27] and FAC

was measured by a 2-D technique for tracing the area during systole and diastole by using the formula = (RV end-diastolic area - RV end-systolic area)/RV end-diastolic area X 100. [28] Pulmonary artery pressures (systolic and diastolic) were estimated using standard continuous and pulse wave Doppler methods. Cardiac dimensions were assessed in the standard manner either using direct measurement of 2-D images or M Mode recordings. TAPSE is a global parameter for right ventricular function which describes apex-to-base shortening. [29, 30] TAPSE has been found to be highly specific and easy method to estimate the right ventricular ejection fraction. [31, 32] FAC has better correlation with cardiac MRI derived RV systolic dysfunction. [33]

Left ventricular (LV) systolic function was determined by measuring shortening fraction (M-mode) and deriving ejection fraction using the Teichholz method [34] and the modified Simpson's method. [35] Left ventricular diastolic function was measured using Doppler assessment of mitral inflow. Tissue Doppler techniques were used to measure mitral annular velocity.

The following definitions were used to define abnormal findings:

1. Right ventricular systolic dysfunction was defined as low TAPSE or low FAC. TAPSE z-score <2 (z-score was calculated based on published normal values). [36] Z-scores were normalised to body surface area. [37] A fractional area change measurement of the RV (FAC) $\leq 34\%$ was considered abnormal. [38]
2. Pulmonary hypertension: Mean pulmonary arterial pressure (mPAP) was calculated using the Chemla equation. [2, 39] ($mPAP = (0.61 \times PAPs) + 2 \text{ mmHg}$) was normal if less than 25 mmHg. [2]
3. LV systolic dysfunction: Left ventricular shortening fraction (LVSF) $\leq 25\%$. [27]

4. LV diastolic dysfunction: E-wave/A-wave normal range was calculated according to age as per Eidem et al. [40]
5. Cardiopulmonary dysfunction was defined as any right ventricle or left ventricle systolic or diastolic dysfunction or abnormal mean pulmonary arterial pressure and abnormal spirometry or abnormal 6MWT.

Data analysis

Descriptive statistics were used to describe characteristics of the study population and to summarise cardiopulmonary outcomes by HIV status. Comparison of cardiopulmonary and clinical outcomes by HIV status was compiled using the two-sample test of proportions. Independent two-sample t-test was used to compare lung function in the adolescents with perinatally acquired HIV between those with low TAPSE and normal TAPSE. A new variable, cardiopulmonary status was generated; those with either obstructive or restrictive or mixed spirometry or abnormal 6MWT and any right or left systolic or diastolic dysfunction or abnormal mean pulmonary arterial pressure were considered as an impaired cardiopulmonary function. Univariate and multivariate logistic regression was done using the cardiopulmonary function as the outcome variable.

Results

Five hundred and fifteen adolescents with perinatally acquired HIV and 110 HIV-uninfected controls had lung function testing. Four hundred and seventy-four adolescents with perinatally acquired HIV and 109 HIV-uninfected completed echocardiogram testing; 478 APH, 104 uninfected adolescents completed six-minute walk test, figure 1. Mean (SD) age was 12 (1.6) years and 50% were male. Median (IQR) duration of ART was 7.6 (4.6-9.3) years. Median (IQR) CD4 count was 712 (571-959) cells/mm³, 77.9% had viral load <50 copies/ml, table 1. Sixty per cent of the participants were on two nucleoside-reverse

transcriptase inhibitors (NRTI), 75% on abacavir and one non-nucleoside-reverse transcriptase inhibitors (NNRTI), 98% were on efavirenz. Median age at ART initiation was 4.4 (2.0-7.0) years, with 29.7% initiating ART at <2 years of age. Cough and digital clubbing were more common in the adolescents with perinatally acquired HIV, $p=0.05$ for both, table 1. History of shortness of breath occurred rarely in less than 5% of participants, table 1.

Adolescents with perinatally acquired HIV with mixed pattern spirometry had a higher rate of RV dysfunction, figure 2. None of the uninfected adolescents had mixed pattern spirometry. Obstructive and mixed pattern spirometry was reported in five per cent of the HIV-infected adolescents, table 2. Right ventricle dysfunction (32%) was more common than left ventricular dysfunction (7%) in the adolescents with perinatally acquired HIV but not significant different to uninfected adolescents (26.6% of whom had RV dysfunction and 5.5% LV dysfunction), table 2. Thirteen per cent of perinatally HIV-infected and 8.3% of uninfected adolescents had impaired cardiopulmonary function, table 2. Sixteen per cent of HIV-infected and 5% of uninfected adolescents had FEF_{25-75} less than the lower limit of normal for age, sex and height, table 2. A pictorial diagram that shows the proportions of the various cardiac and lung function abnormalities in the HIV-infected cohort is presented, figure 3. Only 2 HIV-infected children had pulmonary hypertension, table 2. Among adolescents with perinatally acquired HIV those with low TAPSE had lower mean $z\text{-FEV}_1$, 1.9 (1.4) versus -1.6 (1.3), $p = 0.07$, Table 3, but this difference was not statistically significant.

The cardiopulmonary functional status as measured by distance walked in six-minutes, Borg scale and oxygen saturation were not different between the two groups, table 2. Mean Borg scale was 1.3 in both groups, table 2. Mean pulse rate and mean arterial

pressures were lower in the adolescents with perinatally acquired HIV compared to the HIV-uninfected, $p < 0.05$ for all, table 2.

Height (OR 0.7, 95% CI 0.5-0.9), body mass index (OR 0.7, 95%CI 0.5-0.9) and past pulmonary tuberculosis (OR 2.3, 95%CI 1.2-4.4) were significantly associated with low cardiopulmonary function, table 4. Those with digital clubbing had significantly higher odds for impaired cardiopulmonary function, OR 4.5, 95% CI 1.6-12.3, adjusted for age, sex, height and HIV status, table 5.

Discussion

This study provides comprehensive lung function and cardiac function data on a large cohort of perinatally HIV-infected South African adolescents on ART showing that a proportion of HIV-infected adolescents had cardiopulmonary dysfunction despite being on long-term ART and having well-controlled HIV disease. However, the prevalence of cardiopulmonary dysfunction was similar to the uninfected adolescents. This may be due to lack of validated local African reference values for cardiac function parameters or measurement error in the echocardiography measurements.

Low FEF₂₅₋₇₅, a marker of small airways disease, was significantly higher in the HIV-infected group, consistent with studies of HIV-infected adolescents in sub-Saharan Africa that have reported a predominance of small airways lung disease. [6, 41] However, we did not include low FEF₂₅₋₇₅ in the definition of impaired cardiopulmonary function but used FEV₁ or FVC as large airways disease is regarded as the standard measurement of clinically relevant lung disease. [42]

Height, Body Mass Index and previous history of pulmonary tuberculosis were associated with impaired cardiopulmonary function; and digital clubbing had higher odds of low cardiopulmonary function. Those with low TAPSE had lower lung function.

Contrary to the few published studies [43-45] that showed reduced exercise capacity as measured by treadmill or six-minute walk test in HIV-infected adolescents, this study did not show any differences in distance walked nor oxygen saturation post-exercise between perinatally HIV-infected and uninfected adolescents. This may reflect differences in study populations, as in the current study, the perinatally HIV-infected adolescents were relatively well, established on ART and had good control of HIV disease. It may also be due to differences in the exercise test modalities or outcome measured; the current study reported distance walked and oxygen saturation before and after the test while other studies [44, 45] outcome measure was peak oxygen consumption (VO₂max). [43] Further, the six-minute walk test is an insensitive measure of mild cardiopulmonary compromise. It is a submaximal test and less predictive of VO₂ max compared to the shuttle-walk test. [46] The lower mean heart rate and arterial pressure observed in adolescents with perinatally acquired HIV requires further study.

Right ventricular dysfunction was more common than left ventricular dysfunction in these data. However, this finding must be interpreted cautiously as the normal reference ranges used were from North America and symptoms of RV dysfunction in this cohort were minimal. Miller et al [4] in a Zimbabwe cohort reported 29% of right ventricular dysfunction in a cohort that had a high rate of abnormal lung function. [6] The prevalence of restrictive spirometry pattern in this cohort, table 2, was unexpected and requires further exploration in an ongoing longitudinal study.

The finding that body mass index, height and past pulmonary tuberculosis were associated with impaired cardio-pulmonary dysfunction may reflect the fact that stunting and opportunistic infections affect lung function and may lead to subsequent cardiac dysfunction. Pulmonary TB has also been associated with chronic obstructive pulmonary disease in adults with HIV. [47] Previous data [11] reported that PTB and previous pneumonia were more prevalent in HIV-infected adolescents and were both associated with low lung function. In addition, the higher likelihood of impaired cardiopulmonary dysfunction in those with digital clubbing may reflect lung disease leading to consequent heart dysfunction. Digital clubbing is well known to be associated with chronic cardiac or lung disease. [48] Similarly, nail clubbing was common in a Malawi cohort of HIV-infected adolescents with a high prevalence of chronic lung disease. [49]

The strengths of this study include a large sample size of perinatally HIV-infected adolescents on ART with a comparative group of HIV-uninfected adolescents and the comprehensive lung and cardiac function tests. Our study was limited by the independent assessment of lung and cardiac function rather than a combined measure of cardiopulmonary function such as formal cardiopulmonary exercise testing. This would measure oxygen consumption and carbon dioxide excretion by sampling inspired and expired gas during exercise. [50] Such technology is expensive and inaccessible in most paediatric centres in South Africa. However, we used a broader definition of impaired cardiopulmonary function to include both heart and lung function parameters used in our study. The cardiac function measures, TAPSE and FAC, are limited by the absence of African reference parameters; normative values were derived from a Caucasian population. Nevertheless, the uninfected control group served as a comparator.

Conclusion

This study indicates that cardiopulmonary dysfunction occurs in an appreciable proportion of African adolescents with perinatally acquired HIV despite being on antiretroviral therapy and having well-controlled HIV disease. However, the prevalence of cardiopulmonary dysfunction was similar in HIV-uninfected adolescents, which may reflect lack of validated local African reference values for echocardiography measurements. In turn, this study highlights the need for development of more sensitive markers of lung or heart disease in adolescents with perinatally acquired HIV, given the minimal symptoms that participants reported. The study identified risk factors for cardiopulmonary dysfunction such as prior PTB and impaired nutrition highlighting areas that may be amenable to public health interventions to optimise health.

Table 1: Baseline characteristics of study population

| Variable | HIV-infected n=474 | HIV-uninfected n=109 | *P-value |
|---|--------------------|-------------------------|----------|
| Age, years | 12.0 (1.6) | 11.8 (1.8) | 0.257 |
| Male, n (%) | 247 (51.7) | 47 (45.2) | 0.231 |
| Height z-score | -1.3 (1.1) | -0.5 (1.0) | <0.001 |
| Respiratory rate, breaths/min | 21.5 (3.5) | 20.8 (5.1) | 0.082 |
| Viral load category, copies/ml | | - | |
| <50, n (%) | 369 (77.9) | | |
| 50-1000, n (%) | 46 (9.7) | | |
| 1001-10000, n (%) | 32 (6.8) | | |
| >10000, n (%) | 26 (5.5) | | |
| CD4 count, cells/mm ³ | 712 (571-959) | - | |
| WHO HIV staging at HIV diagnosis, n (%) | | | |
| I | 34 (7.2) | | |
| II | 47 (9.9) | | |
| III | 266 (56.1) | | |
| IV | 105 (22.2) | | |
| ART used | | | |
| NNRTI+2NRTI | 282 (59.5) | | |
| PI+2NRTI | 175 (36.9) | | |
| Others | 9 (1.9) | | |
| Previous PTB, n (%) | 287 (58.5) | 2 (0) | <0.001 |
| Previous severe LRTI, n (%) | 134 (28.3) | 1 (0.9) | <0.001 |
| Tobacco smoke exposure, n (%) | 119 (25.0) | 22 (20.9) | 0.279 |
| Poor ART adherence, n (%) | 111 (23.4) | - | |
| ART duration, years, n (%) | 7.0 (3.0) | - | |
| Age at ART initiation, years | 4.4 (2.0-7.0) | | |
| Shortness of breath, n (%) | 16 (3.4) | 2 (1.8) | 0.402 |
| History of wheeze, n (%) | 51 (10.8) | 6 (5.5) | 0.096 |

| | | | |
|---|-----------|---------|-------|
| History of cough, n (%) | 69 (14.6) | 8 (7.3) | 0.045 |
| History of doctor-diagnosed asthma, n (%) | 57 (12.0) | 6 (5.5) | 0.048 |
| Finger clubbing, n (%) | 16 (3.5) | 0 | 0.052 |

Values are mean/SD except for age at ART initiation, viral load and CD4 which are median (IQR) *P-values derived from chi-square or two sample t-test

ART antiretroviral therapy, PTB pulmonary tuberculosis, NNRTI non-nucleoside reverse transcriptase inhibitor, NRTI nucleoside reverse transcriptase inhibitor, PI protease inhibitor, LRTI lower respiratory tract infections, WHO World Health Organization

Table 2: Cardiopulmonary measurements by HIV status

| Variable, n (%) | n | HIV-infected | n | HIV-uninfected | *P-value |
|--|-----|--------------|-----|----------------|----------|
| Restrictive spirometry | 474 | 110 (23.2) | 109 | 23 (21.1) | 0.637 |
| Obstructive spirometry | 474 | 23 (4.9) | 109 | 4 (3.4) | 0.596 |
| Mixed pattern spirometry | 474 | 22 (4.6) | 109 | 0 | 0.022 |
| FEF ₂₅₋₇₅ below LLN | 474 | 76 (16.0) | 109 | 6 (5.5%) | 0.004 |
| *Right ventricular dysfunction (low TAPSE and low FAC) | 474 | 154 (32.5) | 109 | 29 (26.6) | 0.232 |
| Pulmonary hypertension | 474 | 2 (0.46) | 109 | 0 | 0.476 |
| Left ventricular diastolic dysfunction | 474 | 36 (7.6) | 109 | 6 (5.5) | 0.447 |
| Left ventricle systolic dysfunction | 474 | 1 (0.2) | 109 | 0 | 0.631 |
| *Cardiopulmonary function (impaired) | 474 | 64 (13.5) | 109 | 9 (8.3) | 0.136 |
| Pulse before walk (mean/SD) | 478 | 78.6 (12.5) | 104 | 82.9 (14.2) | 0.002 |
| Pulse after walk (mean/SD) | 478 | 82.9 (13.8) | 104 | 87.1 (15.0) | 0.004 |
| Oxygen saturation before walk (mean/SD) | 478 | 98.4 (2.0) | 104 | 98.6 (0.8)) | 0.315 |
| Oxygen saturation after walk (mean/SD) | 478 | 98.3 (2.7) | 104 | 98.4 (1.4) | 0.675 |
| MAP before 6MWT (mean/SD) | 478 | 79.2 (7.7) | 104 | 82.6 (8.1) | <0.001 |

| | | | | | |
|---------------------------------------|-----|--------------|-----|--------------|-------|
| MAP after 6MWT | 478 | 83.0 (8.7) | 104 | 86.6 (8.5) | 0.001 |
| Borg scale before 6MWT (mean/SD) | 478 | 0.1 (0.2) | 104 | 0.04 (0.2) | 0.577 |
| Borg scale after 6MWT (mean/SD) | 478 | 1.3 (0.6) | 104 | 1.3 (0.7) | 0.737 |
| Distance walked in 6 min (mean/SD) | 478 | 437.8 (60.4) | 104 | 443.8 (60.7) | 0.380 |

*P-value derived from chi-square or two sample t-test

TAPSE tricuspid annular plane systolic excursion, 6MWT six-minute walk test, MAP: mean arterial pressure (calculated from blood pressure), FAC fractional area change, Borg scale (perceived exertion scale) FEF₂₅₋₇₅ forced expiratory flow at 25% and 75% of forced vital capacity, LLN lower limit of normal calculated from African-American reference values. [22]

Table 3: Lung function by Tricuspid Annular Plane Systolic Excursion (TAPSE) index in HIV-infected adolescents

| Lung function | n | Low TAPSE Mean/SD | n | Normal TAPSE Mean/SD | P-value |
|-------------------------|-----|----------------------|-----|-------------------------|---------|
| z-FEV ₁ | 114 | -1.9 (1.4) | 350 | -1.6 (1.3) | 0.07 |
| z-FVC | 114 | -2.0 (1.4) | 350 | -1.8 (1.3) | 0.13 |
| z-FEV ₁ /FVC | 114 | -0.0 (1.6) | 350 | 0.2 (1.4) | 0.16 |
| z-FEF ₂₅₋₇₅ | 114 | -1.0 (1.8) | 350 | -0.8 (1.5) | 0.09 |

P-value from two sample t-test

FEV₁ forced expiratory volume in 1 sec; FVC forced vital capacity; FEF₂₅₋₇₅ forced expiratory flow at 25-75% of vital capacity; TAPSE Tricuspid Annular Plane Systolic Excursion

Table 4: Associations of impaired cardiopulmonary function (n=569) in perinatally HIV-infected and uninfected adolescents

| Variable | Univariate | | | Multivariate | | |
|-------------------------|-------------|----------|------------|--------------|---------|------------|
| | Odd's ratio | *P-value | 95% CI | Odd's ratio | P-value | 95% CI |
| Age | 1.1 | 0.365 | 0.9 to 1.2 | - | | |
| Z-Height | 0.8 | 0.017 | 0.6 to 0.9 | 0.7 | 0.010 | 0.5 to 0.9 |
| Z-BMI | 0.7 | <0.001 | 0.5 to 0.8 | 0.7 | 0.009 | 0.5 to 0.9 |
| ETS exposure | 0.8 | 0.545 | 0.5 to 1.5 | - | | |
| Sex | 0.8 | 0.419 | 0.5 to 1.3 | - | | |
| Past LRTI | 1.6 | 0.124 | 0.9 to 2.7 | 1.4 | 0.321 | 0.7 to 2.5 |
| Past PTB | 2.1 | 0.013 | 1.2 to 3.9 | 2.3 | 0.017 | 1.2 to 4.4 |
| Viral load copies/ml | | | | - | | |
| 50-1000 | 0.8 | 0.677 | 0.3 to 2.2 | | | |
| 1001-10000 | 1.9 | 0.171 | 0.8 to 4.6 | | | |
| >10000 | 1.7 | 0.331 | 0.6 to 4.6 | | | |
| ART duration | 1.1 | 0.062 | 1.0 to 1.2 | 1.1 | 0.228 | 0.9 to 1.2 |

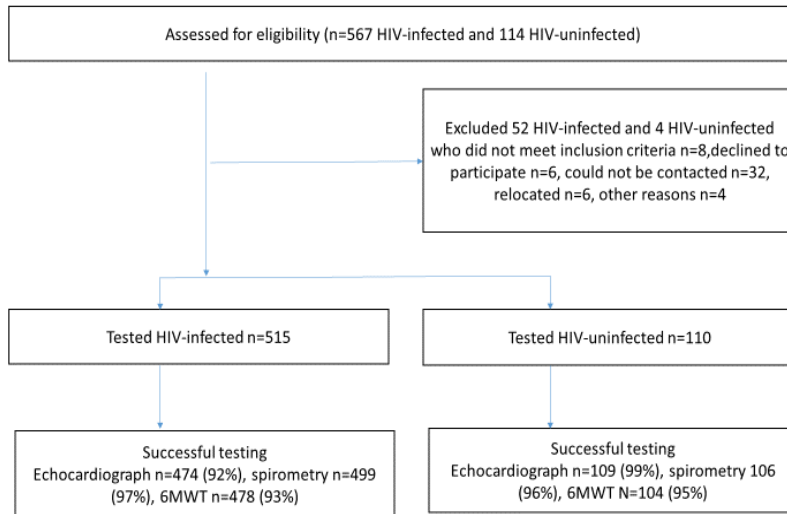
*Adjusted for HIV status. Logistic regression

BMI body mass index, LRTI lower respiratory tract infection, PTB pulmonary tuberculosis, ART antiretroviral therapy, ETS environmental tobacco smoke

Table 5: Association of respiratory symptoms/signs with impaired cardio-pulmonary function (n=569) in perinatally HIV-infected and uninfected adolescents

| Symptom | Univariate | | | ^Multivariate | | |
|---------------------|------------|-------------|---------|---------------|-------------|---------|
| | OR | 95% CI | P-value | OR | 95% CI | P-value |
| Wheeze | 1.5 | 0.7 to 3.2 | 0.249 | 1.4 | 0.7 to 3.0 | 0.369 |
| Shortness of breath | 0.9 | 0.2 to 3.8 | 0.831 | 0.8 | 0.2 to 3.7 | 0.808 |
| Cough | 1.6 | 0.8 to 3.0 | 0.180 | 1.5 | 0.8 to 2.9 | 0.253 |
| Digital clubbing | 5.2 | 1.9 to 14.0 | 0.001 | 4.5 | 1.6 to 12.3 | 0.004 |
| *Asthma | 1.5 | 0.7 to 3.2 | 0.249 | 1.3 | 0.6 to 2.7 | 0.445 |

Symptoms are self-reported except digital clubbing *physician-diagnosed asthma ^adjusted for HIV status, age, sex, height. Logistic regression



6MWT six-minute walk test

Figure 1: Flow diagram for the study population

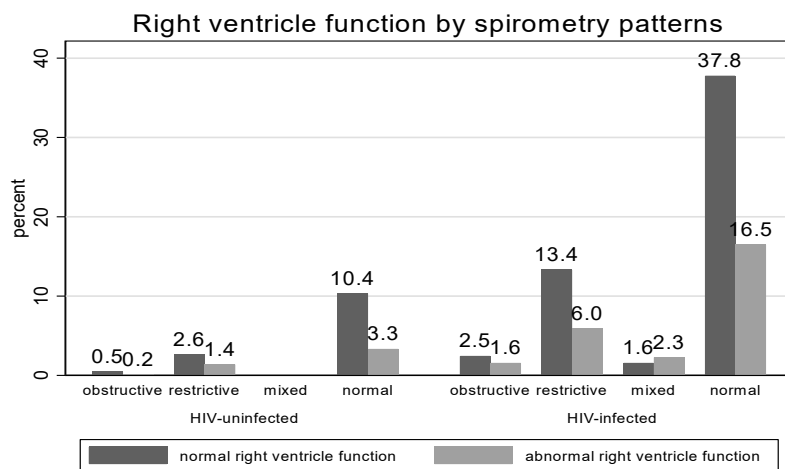


Figure 2: Spirometry pattern and right ventricle function

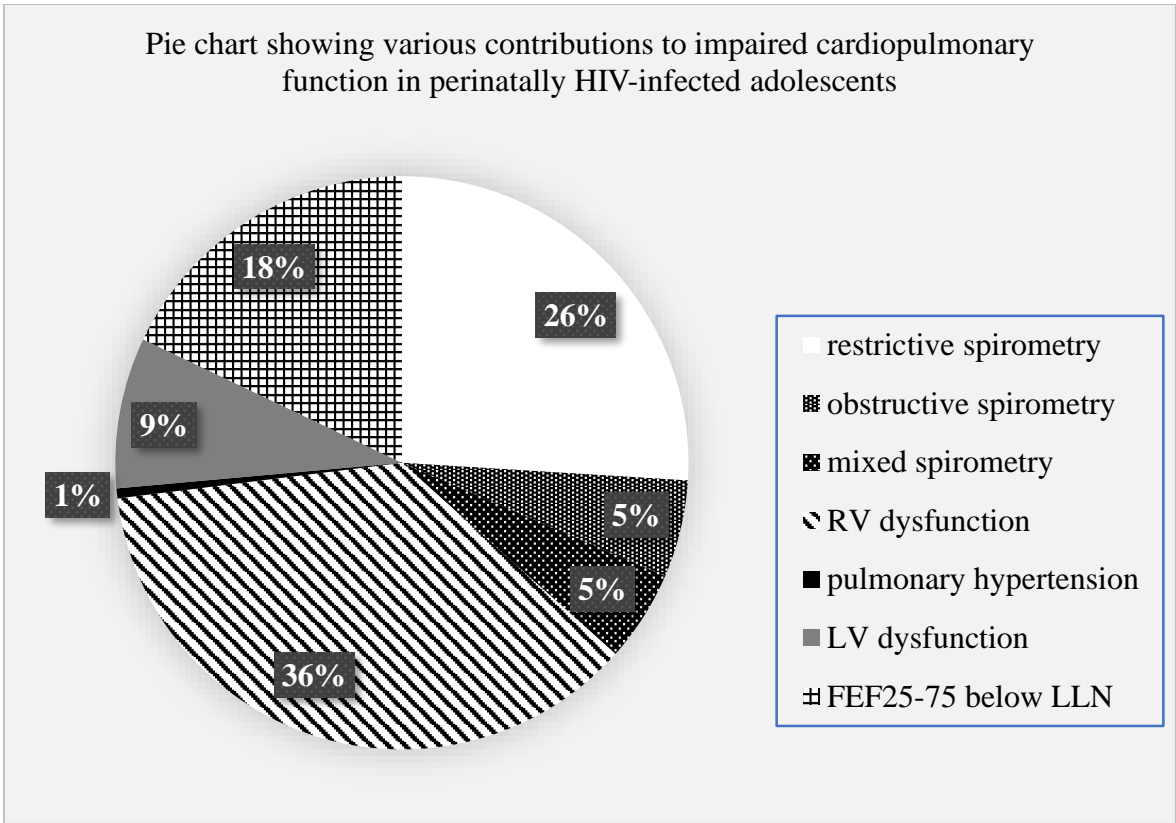


Figure 3: Lung and cardiac functional abnormalities in perinatally HIV-infected adolescents
 RV right ventricle; LV left ventricle, FEF₂₅₋₇₅ forced expiratory flow at 25% and 75% of forced vital capacity. LLN lower limit of normal calculated from African-American reference values. [22]

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Conflict of interest: None

Authors contributions

LG: data collection, statistical analysis and wrote manuscript

SM: analysed cardiac function data and wrote section on cardiac function testing

LZ: read all the echocardiography, critically reviewed the manuscript and provided comments which were incorporated into this manuscript

JL: advised on data analysis, critically reviewed the manuscript and provided comments which were incorporated into this manuscript

DG: data collection, critically reviewed the manuscript and provided comments which were incorporated into this manuscript

LM: conception and designing of study, obtained funding, advised on data analysis, critically reviewed the manuscript and provided comments which were incorporated into this manuscript

HZ: conception and designing of study, obtained funding, advised on data analysis and wrote manuscript.

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Chapter 5:

Longitudinal changes in Spirometry in perinatally HIV-infected South African adolescents on antiretroviral therapy

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Running Title: Changes in lung function of HIV-infected adolescents: a 2-year follow-up study

Summary

Perinatally HIV-infected adolescents had lower lung function over two years and more obstructive and mixed pattern spirometry compared to HIV-uninfected adolescents. Previous pulmonary tuberculosis or lower respiratory tract infection requiring hospitalisation were predictors of low lung function.

Key words: HIV, adolescents, chronic lung disease, lung function

Author contributions: LG: acquisition of data, analysis and interpretation of data and wrote manuscript; DG: conception and design of study and wrote manuscript; SH: acquisition of data; TM: statistical analysis; HZ: conception and design of the study, obtained funding for CTAAC study and wrote manuscript.

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Abstract

Background: Despite increased access to antiretroviral therapy (ART), lung disease remains common in HIV-infected adolescents. There is limited information on changes in lung function over time in perinatally HIV-infected adolescents on ART.

Objective: To investigate the progression of spirometry findings over two years in HIV-infected adolescents on ART in a prospective cohort, the Cape Town Adolescent Anti-retroviral cohort (CTAAC).

Methods: HIV-infected adolescents aged 9-14 years, with at least six months of ART, and a comparator group of healthy HIV-uninfected, age-matched controls were enrolled in CTAAC. Spirometry and bronchodilator testing was done at baseline, 12 and 24 months. Mixed-effect models were used to compute longitudinal changes in lung function.

Results: Five hundred and fifteen HIV-infected adolescents mean (SD) age, 12 (1.6) years, 50.4% male; and 110 HIV-uninfected adolescents, mean (SD) age 11.8 (1.8), 45.6% male, were tested at baseline, 477 (93%) HIV-infected and 102 (93%) HIV-uninfected at 12 months and 473 (92%) HIV-infected and 97 (88%) uninfected adolescents at 24 months. Only 5.4% of the HIV-infected adolescents had HIV viral load >10000 copies/ml at baseline. FEV₁ and FVC were lower in the HIV-infected compared to the uninfected adolescents and tracked with no deterioration or catch up over two years. Previous pulmonary tuberculosis (PTB) or lower respiratory tract infection (LRTI) was significantly associated with reduced FEV₁ and FVC, p<0.05 for both.

Conclusion: HIV-infected adolescents had lower lung function over two years than HIV-uninfected. This study highlights the need for lung function surveillance and prevention of LRTIs and PTB in HIV-infected adolescents.

Background

Perinatally acquired HIV has evolved into a chronic disease due to early diagnosis and improved access to antiretroviral therapy (ART) [1, 2] However, of the 2.1 million children under 15 years living with HIV only about 43% were on ART in 2017. [3] Over 90% of HIV-infected adolescents live in sub-Saharan Africa (SSA) with only about 49% accessing ART. Chronic lung disease remains one of the most common concerns in HIV-infected adolescents in the ART era in SSA. [4]

Normal lung growth begins in-utero and continues throughout childhood and adolescence till it reaches a plateau in the early twenties and begins to decline. [5, 6] Lung function tracks through life, with those starting at low lung function continuing along the lower centile into adulthood and also being associated with increased respiratory symptoms and illness. [7] However, most of the evidence on lung function in HIV-infected adolescents is from cross-sectional studies, with limited information on longitudinal lung function changes especially in the context of ART. Prior studies have shown that HIV-infected adolescents on ART have lower lung function compared to HIV-uninfected adolescents. [8, 9] Longitudinal studies are needed to understand the lung function trajectory in HIV-infected adolescents and the impact of low lung function and the risk of later respiratory disease. The aim of this study was to investigate the progression and determinants of lung function over two years in HIV-infected adolescents on ART in a South African perinatally infected adolescent cohort.

Methods

Children were enrolled from October 2013 to March 2015 in a prospective cohort, Cape Town Adolescent Antiretroviral Cohort, CTAAC as previously described. [8] HIV-infected adolescents, aged 9-14 years on ART for more than 6 months, in whom informed parental

consent was obtained and assent provided, were enrolled and followed up 6-monthly. Informed consent and assent was renewed annually.

Children were recruited from primary care clinics and hospital-based ART clinics in Cape Town, South Africa. HIV-uninfected age, sex, ethnicity matched comparator group, with no known pre-existing lung disease was also enrolled. The study was approved by the Faculty of Health Sciences, University of Cape Town Human Research Ethics Committee.

Spirometry and bronchodilator testing was done at baseline, 12 and 24 months. Testing was done using NDD EasyOne Pro LAB (Switzerland). Testing adhered to ERS/ATS guidelines. [10, 11] A minimum of 3 trials to a maximum of 8 attempts were done per patient and at least 3 acceptable, reproducible best quality curves were selected by the respiratory technologist. The highest forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁) from any of the acceptable manoeuvres was reported. 400 µg salbutamol was given via spacer and testing repeated after 15 minutes.

Global Lung Initiative (GLI) [6] African-American reference population was used to calculate the lower limit of normal for the spirometry outcome measures. Obstructive spirometry pattern was defined as FEV₁ over FVC ratio less than the lower limit of normal (FEV₁/FVC <LLN); restrictive spirometry pattern as FVC<LLN and normal FEV₁/FVC ratio and a mixed pattern was defined as FEV₁/FVC<LLN, FVC<LLN and FEV₁<LLN. Bronchodilator response was defined as a change in FEV₁ ≥12% after 400µcg salbutamol administered via MDI-spacer.

Clinical data including age, sex, height, weight, CD4 and viral load were collected annually. Questionnaires were administered semi-annually to study participants to collect data on ART adherence, smoking, isoniazid prophylaxis, respiratory symptoms and other

intercurrent events. ART adherence was calculated by assessing 30-day recall of any missed doses. Data on previous pulmonary tuberculosis (PTB) and past lower respiratory tract infection (LRTI), date of ART initiation, doctor-diagnosed asthma were extracted from hospital records and validated study questionnaires. Data on medications use was extracted from health records.

Data analysis

Two sample t-test was used to compute the comparison of lung function outcome between HIV-infected and uninfected at baseline and 24 months. Demographic and clinical factors were included to determine associations of lung function. The minimum covariates, age, height, sex, HIV, time, smoke exposure, previous PTB and previous LRTIs, figure 1S, were determined using Directed Acyclic Graphs. [12] Mixed-effects modelling was used to compute longitudinal changes over two years and determinants of lung function outcomes.

Results

Five hundred and fifteen HIV-infected adolescents, mean (SD) age, 12 (1.6) years and 110 HIV-uninfected adolescents, mean age 11.8 (1.8) years, were tested at baseline, 477 (93%) HIV-infected and 102 (93%) HIV-uninfected at 12 months and 473 (92%) HIV-infected and 97 (88%) uninfected adolescents at 24 months, with over 90% successfully tested, figure 1. Four hundred and eighty-three (96%) HIV-infected and 88 (83%) HIV-uninfected adolescents had successful bronchodilator testing at baseline, 437 (92.6%) HIV-infected and 91 (94.8%) HIV-uninfected had bronchodilator testing at 12 months and 402 (92.4%) HIV-infected and 88 (93.6%) HIV-uninfected adolescents had a successful bronchodilator test at 24 months, table 2.

At two years of study, the median (IQR) duration of ART was 9.8 (6.8 to 11.3) years, table 1a. The median (IQR) age at ART initiation was 4.3 (1.9 to 7.6) years. HIV-infected

adolescents were shorter and had lower BMI than HIV-uninfected controls at enrolment and at two years, $p < 0.001$, table 1b. Only 5-6% of adolescents had HIV viral load > 10000 copies/ml at both baseline and 24 months, table 1a. History of hospitalisation for LRTI or past pulmonary tuberculosis (PTB) prior to enrolment visit was more common in HIV-infected adolescents compared to uninfected, $P < 0.001$, table 1a. However, intercurrent hospitalisation for non-TB LRTI, ambulatory LRTI not requiring hospitalisation during the 24-month follow-up period was uncommon in both groups, table 1a. However, HIV-infected adolescents had a high incidence rate of intercurrent culture-positive PTB of 3.2% compared to none in the HIV-uninfected adolescents in the two-year study period. Those with culture-positive Mycobacterium tuberculosis over the two years had on average 0.23 lower FEV₁ z-score (95% CI -0.57 to 0.10, $p = 0.174$, adjusted for time and HIV status, data not shown) compared to those without intercurrent tuberculosis disease.

More adolescents reported symptoms of cough or wheeze at enrolment than at the 2-year follow-up period, table 1b. Less than 7% of HIV-infected adolescents used asthma medications at baseline and over two years, table 1a. There was higher use of isoniazid prophylaxis in the HIV-infected adolescents at baseline than at two years, 115 (22.9%) and 33 (7.6%) respectively, table 1a. Cotrimoxazole prophylaxis use was 10% at baseline and 7.6% at two years in the HIV-infected, table 1a. Less than 20% of HIV-infected children reported chronic or recurrent respiratory symptoms at baseline and at two years, table 1b. Mean z-FEV₁/FVC tracked lower in the HIV-infected over time, narrowing at 12 months, mean/SD -0.2 (1.3) in the HIV-infected and 0.0 (1.1) in the HIV-uninfected, $p = 0.231$ (table 2) but the means of the two groups were statistically different at 2 years, mean/SD -0.3 (1.2) in the HIV-infected, 0.0 (1.0) in the HIV-uninfected, $p = 0.008$, figure 2S.

Further, HIV-infected adolescents had lower z-FEV₁ compared to the uninfected adolescents at all time points, table 2, with HIV-infected adolescents having on average 0.52 z-FEV₁ lower than the HIV-uninfected adolescents over two years, (95% CI -0.76 to -0.27, p<0.001), table 3. Although HIV infection was associated with lower FEV₁ or FVC, the change in either FEV₁ or FVC at two years was similar between HIV-infected and uninfected adolescents as depicted by a near parallel slope, figure 2, and a non-significant interaction between HIV and time at two years for both, coefficient -0.03, 95% CI -0.17 to 0.23, p=0.764 for FEV₁ and coefficient -0.14, 95% CI -0.34 to 0.05, p=0.154 for FVC, table 1S.

A history of pulmonary tuberculosis or severe LRTI prior to enrolment were significantly associated with impaired FEV₁ z-score at two years (coefficient -0.27, 95% CI -0.50 to -0.03 p=0.024 for PTB and -0.37, 95% CI -0.62 to -0.13, p=0.003 for LRTI) and FVC z-score (coefficient -0.27, 95% CI -0.49 to -0.05, p=0.015 for PTB and -0.30, 95% CI -0.53 to -0.06, p=0.013 for LRTI), adjusting for smoke exposure, time and HIV infection, tables 4 ,4Sa,4Sb. HIV-infected adolescents with past LRTI requiring hospitalisation had lower lung function compared to those with no prior severe LRTI, figure 3.

FEV₁/FVC z-score tracked lower at all time points in those who had history of severe LRTI compared to those without history of severe LRTI, figure 3S. Those who began ART before 3 years of age had significantly lower rates of history of severe LRTI compared to those who began ART after 3 years, (47% vs 53% at baseline, p=0.006; 49% vs 51% at 12 months, p=0.002; 46% vs 54% at 24 months, p=0.019).

HIV-infected adolescents who had ART initiated before 3 years of age had higher z-FVC, Z-FEV₁ and z-FEV₁/FVC compared to those who had ART initiated above 3 years, as shown

on the lung growth curves, figure 4S, but this difference was not statistically significant, $p > 0.05$ for all, table 5S.

Over 65% of HIV-infected adolescents had a normal spirometry pattern at baseline, 12 and 24 months, table 2S. Mixed spirometry pattern was more common in the HIV-infected compared to HIV-uninfected adolescents at each time point, table 2S and figure 4. Obstructive spirometry pattern increased from a prevalence of 4.8% at baseline to 8.1% at 24 months, table 2S. Bronchodilator responsiveness at baseline was 16.1% and 11.4% in HIV-infected and HIV-uninfected adolescents respectively, table 2. This decreased to 6.2% and 5.7%, respectively, in the HIV-infected and uninfected at 24 months indicating an increase in fixed airway obstruction over the 24 months. On average, those with abnormal spirometry pattern had a higher odds of having either wheeze, shortness of breath, cough on most days, history of doctor diagnosed asthma, adjusted for HIV status, $p < 0.05$ for all, table 5. History of doctor diagnosed asthma (OR 1.96, 95% CI 1.02 to 3.77) or past severe LRTI requiring hospitalisation (OR 1.72, 95% CI 1.02 to 2.91), adjusted for time and HIV status, were significantly associated with bronchodilator responsiveness, table 3S.

Discussion

This study provides novel 2-year follow-up data showing that spirometry in perinatally HIV-infected adolescents established on ART tracks lower compared to uninfected adolescents. Prior LRTI requiring hospitalisation or PTB were associated with low FEV₁ or FVC. Reassuringly this cohort had minimal chronic respiratory symptoms and few intercurrent ambulatory or hospitalised LRTIs during the two-year follow-up. The cohort was well established on ART with a mean duration of ART of nine years

The stable trajectory of spirometry outcomes over two years, suggests that damage to the lung reflected as lower lung function in the HIV-infected group may have occurred early in life due to uncontrolled HIV infection as ART was only initiated on average at 4 years of age in this cohort. In addition, it suggests that in HIV-infected adolescents well established on ART lung growth continues to occur and tracks similar to HIV uninfected youth. Early damage may have been caused by HIV-exposure in-utero in the developing lung or in the early childhood years prior to initiation of ART. This damage may be attributed to early childhood infection, by HIV associated inflammation and immune dysregulation or opportunistic infections in the HIV-infected group who had high rates of prior PTB or LRTI compared to HIV-uninfected participants, as has been reported in other studies. [9, 13] This is supported by the fact that previous LRTI or PTB were associated with lower lung function. The effect of HIV on lung function reduced after adjusting for LRTI and PTB suggesting that pulmonary infection may be a major mechanism by which HIV reduces lung function. Reduction of LRTI by early initiation of ART is possible as evidenced by low rates of severe LRTI in adolescents who had ART initiated before 3 years of age in this cohort.

The incidence of culture confirmed tuberculosis in the HIV-infected adolescents was high over the study period. This is in keeping with published evidence [14] that while long-term ART is associated with a reduction in TB risk, the incidence remains high in HIV-infected populations. South Africa has a high HIV and TB burden [15] and previous reports have shown that adolescent age group has a higher rate of tuberculosis disease. [16] Of concern, was the very low use of cotrimoxazole or isoniazid prophylaxis as effective preventive interventions in this cohort. The low use of cotrimoxazole may reflect current program guidelines as prophylaxis is stopped after immune reconstitution, which had occurred in most participants, as reflected by the median CD4 count of around 700 cells/mm³.

The higher occurrence of obstructive and mixed pattern spirometry in the HIV-infected adolescents may be due to higher rates of bronchiectasis or post-infective bronchiolitis obliterans (BO) in the HIV-infected group. High rates of bronchiectasis in HIV-infected children have been reported. [4, 17] A study on chest computed tomography (CT) in HIV-infected adolescents reported predominantly mosaic attenuation due to possible bronchiolitis obliterans that was inversely correlated with FEV₁. [4] BO is a non-reversible condition due to fibrosis. In our study cohort, bronchodilator responsiveness at two years was less than 10%, suggesting irreversible airway pathology, possibly related to BO. Others have reported irreversible airway pathology in HIV-infected youth compared to HIV-exposed uninfected youth. [18] The high rate of restrictive spirometry pattern in both the HIV-infected and HIV-uninfected adolescents at baseline may be for technical reasons as most participants had never done a lung function test before enrolment but became familiar with the technique of performing spirometry and were able to produce better quality tests in subsequent visits.

The improvement of respiratory symptoms over the two-year period in our cohort may have been due to close follow up of study participants, good clinical management and appropriate treatment or referral when the participant was sick or symptomatic. This cohort also had good adherence to ART, reflected by most adolescents having low HIV viral load.

Birth cohort studies from high-income countries in HIV-uninfected populations have shown that lung function tracks from childhood to adulthood. [7, 19-21] Therefore the lung function of HIV-infected adolescents, which was lower than the HIV-uninfected, is likely to continue to track along the lower centile over time, provided there are no further insults.

Longitudinal studies in HIV-infected children and adolescents are however lacking. With the expected physiological growth and development of the lung from in-utero to early adulthood, [5] it is unclear what effect HIV or opportunistic infections may have on normal physiological growth. In our study, there was no catch-up or deterioration over two years between the HIV-infected and the uninfected adolescents, but a longer follow-up period is needed. The lack of deterioration in lung function over the two years may reflect that this is a relatively healthy HIV-infected cohort (as evidenced by minimal respiratory morbidity in the 2-year period) who were, stable on ART for a mean duration of nine years.

Strengths of the study include a prospective collection of objective measures of lung function, high cohort retention with close follow-up over two years, and a well-controlled HIV cohort. Limitations of the study are that data included spirometry only, but this is the most widely used measure of lung function; further bronchodilator responsiveness was also assessed in this study. In the absence of local South African adolescents' spirometry reference equation, we used the GLI 2012 [6] African-American reference values, which have been shown to be acceptable for sub-Saharan Africa. [22] Further limitation was the lack of chest CT imaging to define lung abnormalities and correlate with lung function. These findings may not be generalizable to HIV-infected adolescent populations that are not well controlled on ART, where more severe lung function abnormalities have been found. [23, 24] However, as ART programmes are strengthened, increasing numbers of perinatally HIV-infected adolescents can be expected to be on therapy and to develop this spectrum of lung disease.

Conclusions

HIV-infected adolescents had lower spirometry at all time points compared to HIV-uninfected adolescents over two years. Lung growth was, however, preserved in the HIV

infected group and similar in both groups. Previous pulmonary tuberculosis or previous LRTI were associated with reduced FEV₁ and FVC. Those with ART initiated before 3 years of age had lower rates of past severe LRTI. There was a high incidence rate of culture confirmed tuberculosis in the HIV-infected adolescents. Interventions to prevent childhood LRTI or TB must be strengthened to optimise lung health. Further longitudinal studies on lung function in HIV-infected children and adolescents on ART are needed to show the long-term effect of HIV or opportunistic infections on subsequent respiratory health and to enable timely treatment of lung disease. Surveillance of lung function in HIV-infected adolescents from early adulthood and throughout adulthood is necessary to detect disease early.

Table 1a: Characteristics of participants tested at both baseline and 24 months by HIV status

| | HIV+ (n=503) | HIV- (n=106) | P-value | HIV+ (n=435) | HIV- (n=94) | P-value [^] |
|---|-----------------|-----------------|---------|---------------------|----------------|----------------------|
| | Baseline | | | 24 months | | |
| Age, years (mean/SD) | 12.0 (1.6) | 11.9 (1.8) | 0.533 | 14.1 (1.6) | 13.8 (1.8) | 0.171 |
| Male, n (%) | 261 (51.9) | 48 (45.2) | 0.216 | 217 (49.9) | 42 (44.7) | 0.360 |
| Age (years) at ART initiation (median/IQR) | 4.3 (1.9-7.6) | | | 4.3 (2.0-7.6) | | |
| ART duration, years median (IQR) | 7.7 (4.6-9.3) | | | 9.8 (6.8-11.3) | | |
| CD4 count, cells/mm³ median (IQR) | 712 (556-959) | | | 695.5 (529.5-887.5) | | |
| Viral load CAT, n (%) | | | | | | |
| <50 copies/ml | 383 (76.1) | | | 260 (59.8) | | |
| 50-1000 copies/ml | 55 (10.9) | | | 108 (24.8) | | |
| 1001-10000 copies/ml | 37 (7.4) | | | 24 (5.5) | | |
| >10000 copies/ml | 27 (5.4) | | | 27 (6.2) | | |
| Missing | 1 (0.2) | | | 16 (3.7) | | |
| Poor ART adherence, n (%) | 116 (23.0) | | | 41 (9.4) | | |
| ART regimen | | | | | | |
| 2xNRTI + NNRTI | 307 (61.2) | | | 249 (57.2) | | |
| 2xNRTI + PI | 166 (33.1) | | | 151 (34.7) | | |
| Other | 29 (5.8) | | | 35 (8.1) | | |
| Tobacco smoke exposure, n (%)[*] | | | | | | |
| passive | 127 (25.3) | 21 (19.8) | 0.236 | 86 (19.8) | 14 (14.9) | 0.274 |
| active | 2 (0.4) | | | 5 (1.2) | | |

| | | | | | | |
|---|------------|-----------|--------|----------|---------|--------|
| History of LRTI requiring hospitalisation before baseline visit, n (%) | 145 (28.8) | 1(0.9) | <0.001 | - | - | |
| Past history of PTB prior to baseline visit, n (%) | 303 (60.2) | 2 (1.9) | <0.001 | - | - | |
| History of asthma, n (%) (ever) (self-reported)* | 62 (12.3) | 6 (5.7) | 0.048 | 32 (7.4) | 7 (7.5) | 0.976 |
| Family history of asthma | 78 (15.5) | 13 (12.3) | 0.395 | | | |
| Isoniazid prophylaxis | 115 (22.9) | 4 (3.8) | <0.001 | 33 (7.6) | 0 | 0.006 |
| Inhaled corticosteroids | 13 (2.6) | 2 (1.9) | 0.674 | 11 (2.5) | 4 (4.3) | 0.360 |
| Short course steroids | 13 (2.6) | - | - | 6 (1.4) | - | - |
| Inhaled Salbutamol | 33 (6.6) | 2 (1.9) | 0.060 | 28 (6.4) | 2 (2.1) | 0.104 |
| Oral salbutamol | 12 (2.4) | - | - | 6 (1.4) | - | - |
| Cotrimoxazole prophylaxis | 50 (9.9) | 0 | - | 33 (7.6) | 0 | - |
| Intercurrent events, n (%) during the cumulative two years (n=609) | | | | | | |
| Hospitalisation | | | | | | |
| Non-TB LRTI, n (%) | - | - | | 5 (0.8) | 1 (0.2) | <0.001 |
| Unconfirmed TB, n (%) | - | - | | 13 (2.1) | 0 | - |
| Asthma | - | - | | 1 (0.2) | 1 (0.2) | 1.000 |
| Ambulatory LRTI | - | - | | 13 (2.1) | 4 (0.7) | <0.001 |
| Culture positive M.tb | | | | 19 (3.1) | 0 | - |

^ P-value t-test or chi-square; * self-reported smoking history;

ART: antiretroviral therapy; LRTI: lower respiratory tract infection; PTB: pulmonary tuberculosis; M.tb: mycobacteria tuberculosis; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor; CAT: category

Table 1b: Clinical characteristics of participants tested at both baseline and 24 months

| | HIV+ (n=503) | HIV- (n=106) | P-value | HIV+ (n=435) | HIV- (n=94) | P-value [^] |
|---|-----------------|-----------------|---------|-----------------|----------------|----------------------|
| | Baseline | | | 24 months | | |
| Height, cm (mean/SD) | 140.0 (10.3) | 144.6 (11.9) | 0.001 | 150.6 (9.8) | 153.0 (9.7) | 0.030 |
| BMI, kg/m ² (mean/SD) | 17.7 (3.2) | 19.5 (4.2) | <0.001 | 19.4 (3.5) | 21.1 (4.6) | <0.001 |
| Current history of cough on most days* | 74 (14.7) | 8 (7.6) | 0.050 | 51 (11.7) | 11 (11.7) | 0.995 |
| History of ever wheeze* | 55 (10.9) | 6 (5.7) | 0.100 | 31 (7.1) | 7 (7.5) | 0.913 |
| Wheeze in the last 12 months* | 31 (6.2) | 3 (2.8) | 0.174 | 19 (4.4) | 4 (4.3) | 0.961 |
| SOB in the last 12 months* | 3 (0.6) | 2 (1.9) | 0.181 | 6 (1.4) | 3 (3.2) | 0.218 |
| Digital clubbing | 18 (3.6) | 0 (0) | 0.048 | 18 (4.1) | 0 (0) | 0.045 |
| Respiratory rate, breaths/min | 21.5 (3.5) | 20.9 (5.2) | 0.142 | 21.0 (1.1) | 20.9 (0.9) | 0.565 |
| Oxygen saturation (%) | 98.6 (0.9) | 98.8 (0.4) | 0.036 | 98.3 (1.7) | 98.5 (0.7) | 0.199 |
| Tanner staging | | | | | | |
| I | 242 (48.1) | 34 (32.1) | 0.003 | 62 (14.3) | 11 (11.7) | 0.516 |
| II | 123 (24.5) | 30 (28.3) | 0.406 | 81 (18.6) | 15 (16.0) | 0.544 |
| III | 69 (13.7) | 18 (17.0) | 0.383 | 87 (20.0) | 23 (24.5) | 0.333 |
| IV | 39 (7.8) | 17 (16.0) | 0.007 | 110 (25.3) | 26 (27.7) | 0.633 |
| V | 23 (4.6) | 6 (5.7) | 0.633 | 83 (19.1) | 16 (17.0) | 0.643 |
| Missing | 7 (1.4) | 1 (0.9) | 0.713 | 12 (2.8) | 3 (3.2) | 0.819 |

[^] P-value t-test or chi-square; * symptoms are self-reported at each time point
SOB: shortness of breath; BMI: body mass index

Table 2: Summary of lung function at baseline, 12 and 24 months

| Variable mean (SD) | Baseline | | | 12 months | | | 24 months | | |
|------------------------|---------------|---------------|---------|---------------|--------------|---------|---------------|--------------|---------|
| | HIV+ n=503 | HIV- n=106 | P-value | HIV+ n=472 | HIV- n=96 | P-value | HIV+ n=435 | HIV- n=94 | P-value |
| zFEV ₁ | -1.0 (1.3) | -0.5 (1.0) | <0.001 | -0.9 (1.3) | -0.3 (1.0) | <0.001 | -0.8 (1.3) | -0.3 (1.0) | <0.001 |
| zFVC | -1.1 (1.3) | -0.8 (1.0) | 0.060 | -0.9 (1.2) | -0.3 (1.0) | <0.001 | -0.7 (1.2) | -0.3 (0.9) | 0.003 |
| zFEF ₂₅₋₇₅ | -0.4 (1.4) | 0.1 (1.0) | <0.001 | -0.5 (1.4) | -0.1 (1.1) | 0.015 | -0.5 (1.4) | -0.0 (1.1) | 0.004 |
| zFEV ₁ /FVC | 0.2 (1.4) | 0.9 (1.2) | <0.001 | -0.2 (1.3) | 0.0 (1.1) | 0.231 | -0.3 (1.2) | 0.0 (1.0) | 0.008 |
| BDR | n=483 | n=88 | | n=437 | n=91 | | n=402 | n=88 | |
| BDR (yes), n (%) | 78 (16.1) | 10 (11.4) | 0.253 | 45 (10.3) | 5 (5.5) | 0.155 | 25 (6.2) | 5 (5.7) | 0.849 |

P-values from two sample T-test, z-scores derived from GLI African -American reference values, BDR: bronchodilator responsiveness, positive if change in FEV₁≥12%

FVC: forced vital capacity, FEV₁: forced expiratory volume in 1 second, FEF₂₅₋₇₅: forced expiratory flow at 25% and 75% of FVC; SD: standard deviation

Table 3: Effect of HIV on lung function over two years (n=609)

| | Coefficient | 95% CI | P value[^] |
|-------------------------|--------------------|----------------|----------------------------|
| z-FEV ₁ | -0.52* | -0.76 to -0.27 | <0.001 |
| z-FVC | -0.37* | -0.60 to -0.14 | 0.001 |
| z-FEF ₂₅₇₅ | -0.42* | -0.68 to -0.17 | 0.001 |
| z-FEV ₁ /FVC | -0.41* | -0.64 to -0.19 | <0.001 |

[^] linear mixed-effects model; * Adjusted for age, sex, height, time

FVC: forced vital capacity; FEV₁: forced expiratory volume in 1second; FEF₂₅₇₅: forced expiratory flow at 25% and 75% of FVC

Table 4: Association of z-FEV₁, z-FVC and z-FEV₁/FVC at 24 months (n=515)

| Variable | z-FEV₁ | | z-FVC | | z-FEV₁/FVC | |
|-------------------------|--------------------------|----------------------------|--------------------|----------------------------|------------------------------|----------------------------|
| | Coefficient | P-value[^] | Coefficient | P-value[^] | Coefficient | P-value[^] |
| HIV infection | -0.12 | 0.510 | 0.01 | 0.946 | -0.34 | 0.036 |
| Time at 12 months | 0.04 | 0.296 | 0.25 | <0.001 | -0.51 | <0.001 |
| Time at 24 months | 0.17 | <0.001 | 0.41 | <0.001 | -0.61 | <0.001 |
| Tobacco smoke exposure* | 0.01 | 0.931 | 0.08 | 0.192 | -0.18 | 0.022 |
| Previous PTB | -0.27 | 0.024 | -0.27 | 0.015 | -0.01 | 0.904 |
| Previous LRTI | -0.37 | 0.003 | -0.30 | 0.013 | -0.27 | 0.020 |

[^] multivariate linear mixed model; * self-reported passive smoke exposure (active smoking had too few numbers therefore not reported)

LRTI: lower respiratory tract infection; PTB: pulmonary tuberculosis; FEV₁: forced expiratory volume in 1s, FVC: forced vital capacity

Table 5: Association of abnormal spirometry pattern and respiratory symptoms/signs over 24 months

| Respiratory symptom/sign* | n | Odd's ratio | P-value[^] | 95% CI |
|---|----------|--------------------|----------------------------|---------------|
| History of ever wheeze (yes) | 608 | 2.4 | 0.009 | 1.25 to 4.68 |
| Cough on most days (yes) | 607 | 2.7 | <0.001 | 1.62 to 4.62 |
| History of asthma ever (yes) | 607 | 3.1 | <0.001 | 1.64 to 5.83 |
| History of shortness of breath ever (yes) | 608 | 4.7 | 0.010 | 1.44 to 15.25 |
| Digital clubbing (yes) | 609 | 5.6 | 0.068 | 0.89 to 35.94 |

* All symptoms are self-reported except digital clubbing; [^] mixed-effects logistic regression; adjusted for HIV status and time

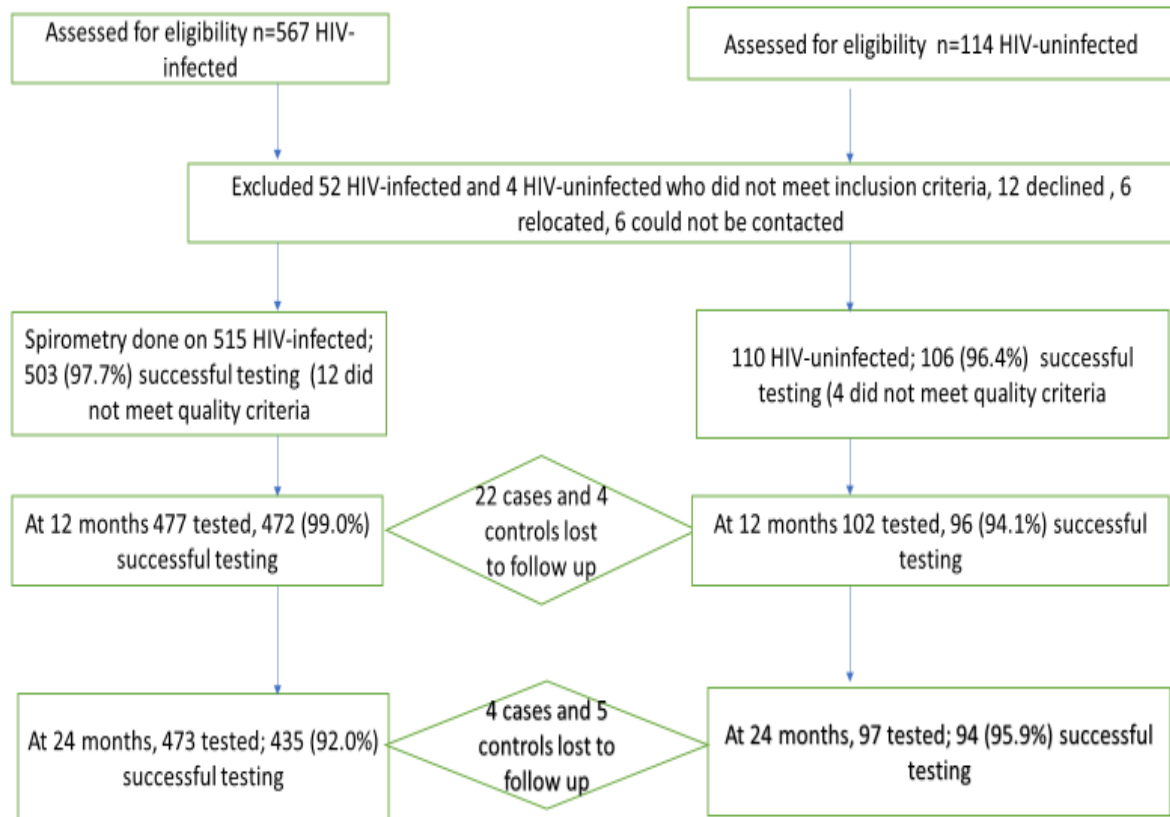


Figure 1: Description of the study cohort

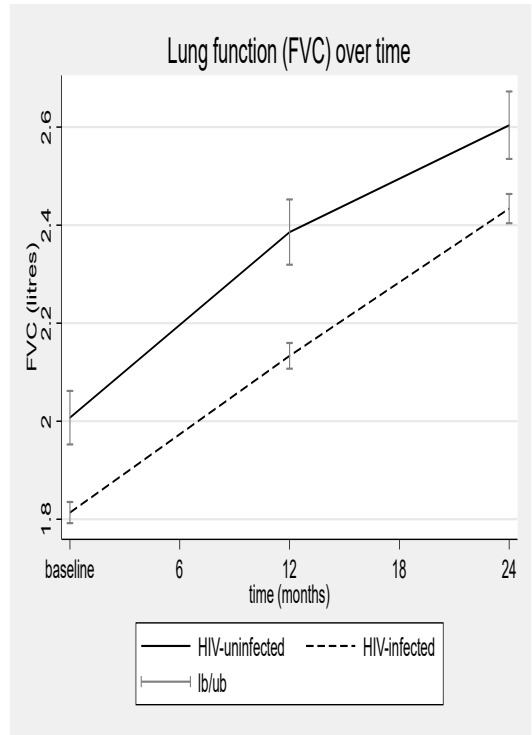
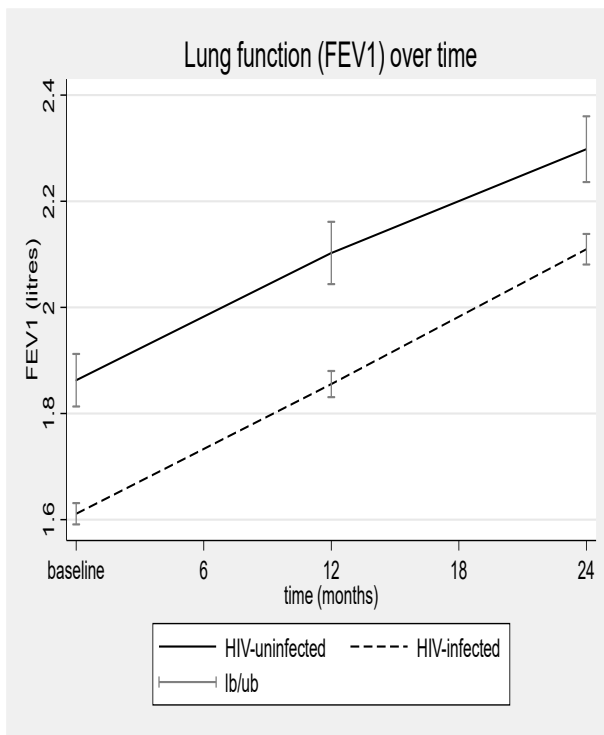
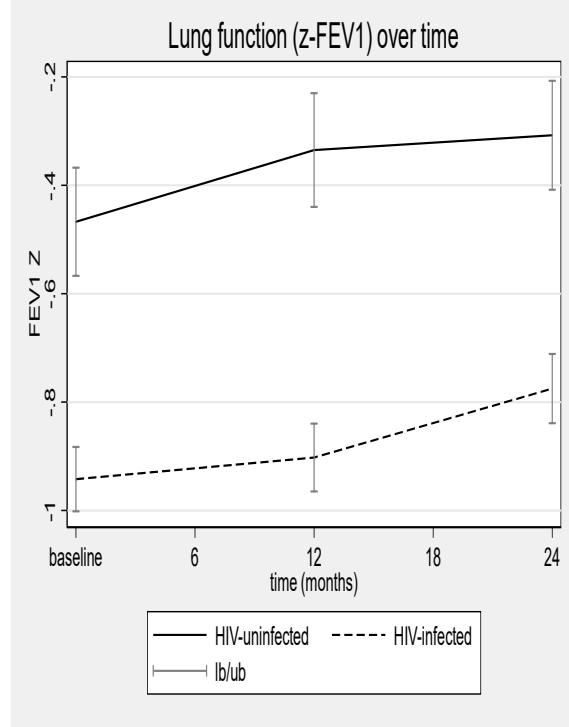
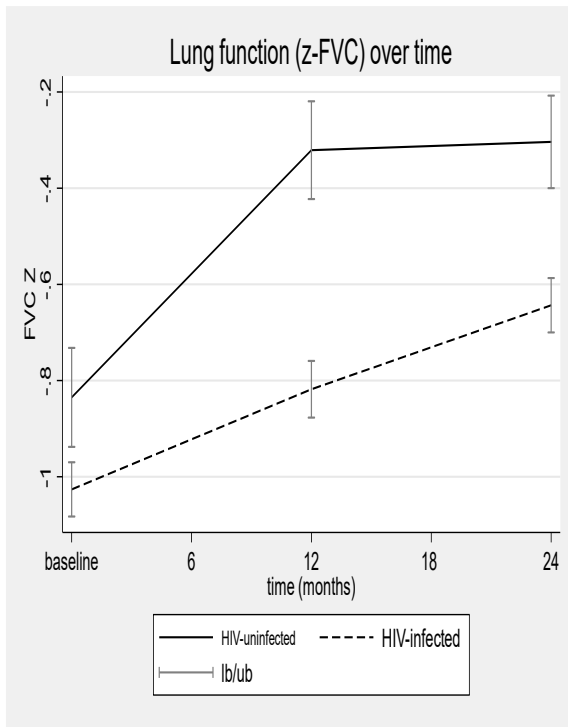
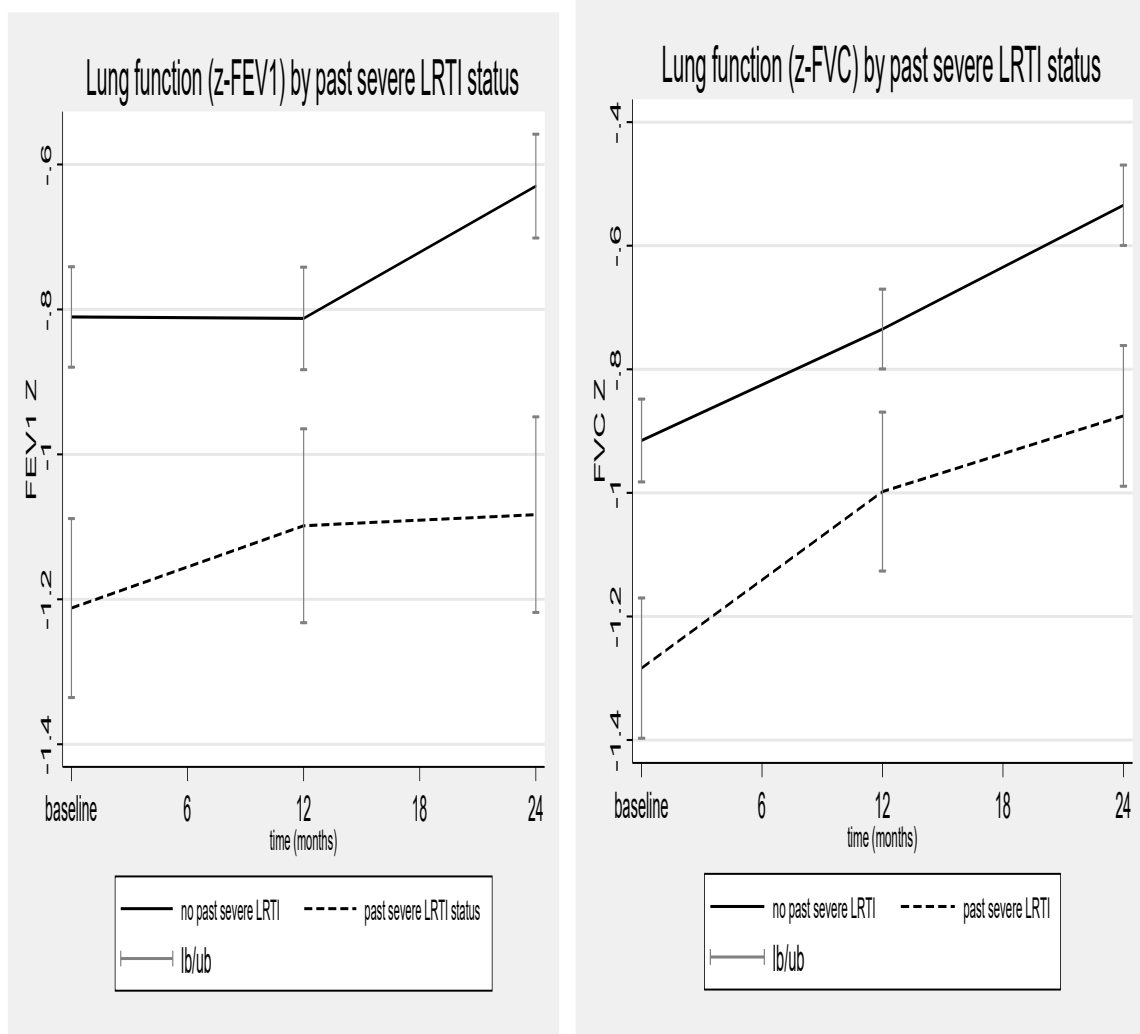


Figure 2: Line plots of the trend of lung function over two years (HIV+ n=503, HIV-106 at baseline, HIV+472, HIV-96 at 12 months, HIV+435, HIV-94 at 24 months)



| Variable mean (SD) | Baseline | | | 12 months | | | 24 months | | |
|-----------------------|----------------|----------------|-------------|----------------|----------------|-------------|----------------|----------------|-------------|
| | LRTI+ n=145 | LRTI- n=329 | P- value | LRTI+ n=134 | LRTI- n=312 | P- value | LRTI+ n=123 | LRTI- n=289 | P- value |
| zFEV ₁ | -1.2 (1.5) | -0.9 (1.3) | 0.004 | -1.1 (1.5) | -0.8 (1.2) | 0.034 | -1.1 (1.5) | -0.7 (1.2) | 0.002 |
| zFVC | -1.3 (1.4) | -1.0 (1.2) | 0.005 | -1.0 (1.5) | -0.8 (1.1) | 0.056 | -0.9 (1.3) | -0.6 (1.1) | 0.010 |

* T-test;

^missing LRTI data: 29 (5.8%) at baseline, 26 (5.5%) at 12 months, 23 (5.3%) at 24 months;

LRTI: lower respiratory tract infection

Figure 3: Lung function at baseline, 12 and 24 months by past severe LRTI status in the HIV-infected adolescents

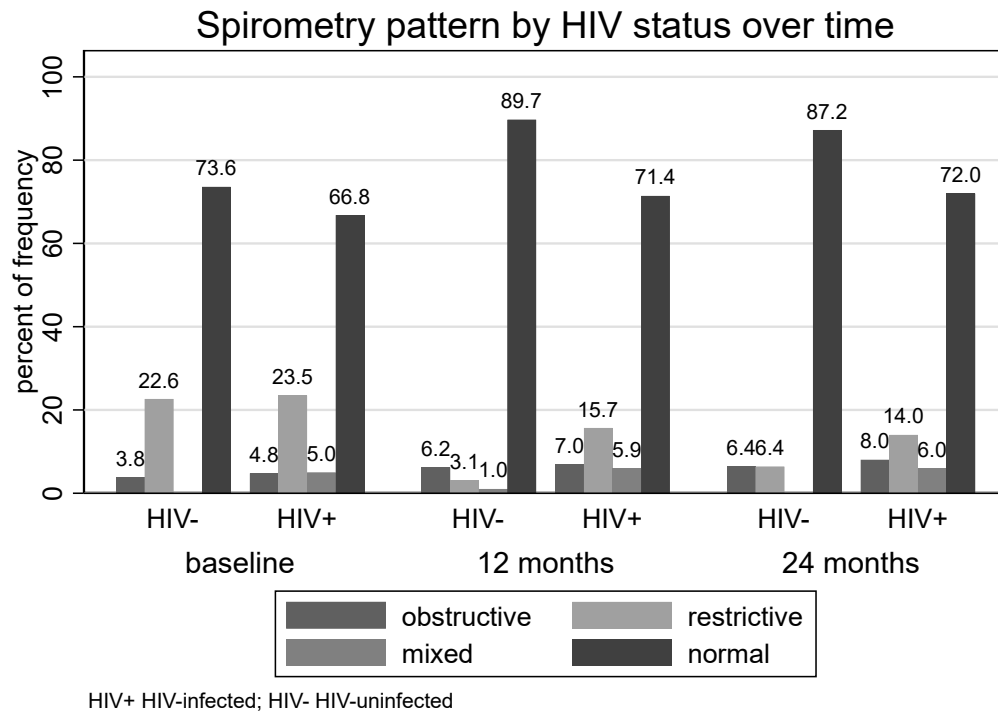


Figure 4: Spirometry pattern by HIV status at baseline, 12 and 24 months

Supplementary Material

Table 1S: Effect of HIV on lung function (n=609)

| Variable | Coefficient | P-value | 95% CI |
|------------------------------|-------------|---------|----------------|
| zFEV₁ | | | |
| Time at one year | 0.10 | 0.261 | -0.08 to 0.28 |
| Time at two years | 0.16 | 0.081 | -0.02 to 0.34 |
| HIV | -0.52 | <0.001 | -0.78 to -0.25 |
| HIV# time at one year | -0.03 | 0.793 | -0.22 to 0.17 |
| HIV#time at two years | -0.03 | 0.764 | -0.17 to 0.23 |
| zFVC | | | |
| Time at one year | 0.49 | <0.001 | 0.31 to 0.66 |
| Time at two years | 0.54 | <0.001 | 0.36 to 0.72 |
| HIV | -0.25 | 0.050 | -0.50 to 0.00 |
| HIV#time at one year | -0.26 | 0.009 | -0.46 to -0.06 |
| HIV#time at two year | -0.14 | 0.154 | -0.34 to 0.05 |
| z-FEV₁/FVC | | | |
| Time at one year | -0.93 | <0.001 | -1.16 to -0.71 |
| Time at two years | -0.91 | <0.001 | -1.13 to -0.68 |
| HIV | -0.71 | <0.001 | -0.97 to -0.45 |
| HIV#time at one year | 0.56 | <0.001 | 0.32 to 0.81 |
| HIV#time at two year | 0.40 | 0.002 | 0.15 to 0.65 |

^ Random intercept mixed-effect model, adjusted for time and the effect of interaction (#) between time and HIV status; z-score derived from GLI 2012 African-American reference
FVC: forced vital capacity, FEV₁: forced expiratory volume in 1 second;

Table 2S: Comparison of spirometry patterns by HIV status at baseline, 12 and 24 months

| Spirometry pattern n (%) | Baseline | | | 12 months | | | 24 months | | |
|-----------------------------|---------------|---------------|---------|---------------|--------------|---------|---------------|--------------|----------|
| | HIV+ n=503 | HIV- n=106 | P-value | HIV+ n=472 | HIV- n=96 | P-value | HIV+ n=435 | HIV- n=94 | P-value* |
| Obstructive | 24 (4.8) | 4 (3.8) | 0.656 | 33 (7.0) | 6 (6.2) | 0.793 | 35 (8.1) | 6 (6.4) | 0.584 |
| Restrictive | 118 (23.5) | 24 (22.6) | 0.856 | 74 (15.7) | 2 (2.1) | <0.001 | 61 (14.0) | 6 (6.4) | 0.043 |
| Mixed | 25 (5.0) | 0 | 0.019 | 28 (5.9) | 1 (1.0) | 0.047 | 26 (6.0) | 0 | 0.015 |
| Normal | 336 (66.8) | 78 (73.6) | 0.174 | 337 (71.4) | 87 (89.7) | <0.001 | 313 (72.0) | 82 (87.2) | 0.002 |

* Chi square test; HIV: human immunodeficiency virus

Table 3S: Determinants of bronchodilator positivity over 24 months (n=538)

| Variable | Odds ratio | 95% CI | P value [^] |
|--|------------|--------------|----------------------|
| Age, years | 1.05 | 0.86 to 1.27 | 0.645 |
| Height, cm | 1.00 | 0.97 to 1.03 | 0.959 |
| Time at 12 months | 0.52 | 0.32 to 0.84 | 0.007 |
| Time at 24 months | 0.27 | 0.15 to 0.50 | <0.001 |
| HIV status (infected) | 1.46 | 0.60 to 3.52 | 0.402 |
| Sex (male) | 1.11 | 0.69 to 1.79 | 0.666 |
| History of wheeze ever (yes) | 1.70 | 0.86 to 3.38 | 0.130 |
| History of doctor diagnosed asthma (yes) | 1.96 | 1.02 to 3.77 | 0.043 |
| History of cough on most days (yes) | 1.30 | 0.72 to 2.33 | 0.389 |
| History of past LRTI requiring hospitalisation | 1.72 | 1.02 to 2.91 | 0.042 |
| History of past PTB | 1.15 | 0.68 to 1.93 | 0.598 |

* Mixed-effects logistic regression

LRTI: lower respiratory tract infection; PTB: pulmonary tuberculosis

Table 4Sa: Associations of z-FEV₁ at two years

| Variable | n | Univariate | | | Multivariate | | |
|-------------------|-----|-------------|----------------|---------|--------------|----------------|----------------------|
| | | Coefficient | 95% CI | P-value | Coefficient | 95% CI | P-value [^] |
| HIV status | 515 | -0.50 | -0.76 to -0.23 | <0.001 | -0.12 | -0.46 to 0.23 | 0.510 |
| Time at 12 months | 515 | 0.07 | -0.01 to 0.14 | 0.086 | 0.04 | -0.04 to 0.13 | 0.296 |
| Time at 24 months | 515 | 0.18 | 0.10 to 0.25 | <0.001 | 0.17 | 0.08 to 0.25 | <0.001 |
| Smoke exposure | 515 | 0.01 | -0.14 to 0.11 | 0.818 | -0.01 | -0.13 to 0.12 | 0.931 |
| Previous PTB | 504 | -0.41 | -0.61 to -0.20 | <0.001 | -0.27 | -0.50 to -0.03 | 0.024 |
| Previous LRTI | 463 | -0.47 | -0.71 to -0.23 | <0.001 | -0.37 | -0.62 to -0.13 | 0.003 |

[^] linear mixed models

FEV₁: forced expiratory volume in 1 second; PTB: pulmonary tuberculosis; LRTI: lower respiratory tract infection

Table 4Sb: Associations of z-FVC over two years

| Variable | n | Univariate | | | Multivariate | | |
|-------------------|-----|-------------|----------------|---------|--------------|----------------|----------------------|
| | | Coefficient | 95% CI | P-value | Coefficient | 95% CI | P-value [^] |
| HIV status | 515 | -0.34 | -0.59 to -0.09 | 0.007 | 0.01 | -0.31 to 0.33 | 0.946 |
| Time at 12 months | 515 | 0.26 | 0.18 to 0.34 | <0.001 | 0.25 | 0.17 to 0.33 | <0.001 |
| Time at 24 months | 515 | 0.42 | 0.34 to 0.49 | <0.001 | 0.41 | 0.33 to 0.50 | <0.001 |
| Smoke exposure | 515 | 0.02 | -0.11 to 0.14 | 0.795 | 0.08 | -0.04 to 0.21 | 0.192 |
| Previous PTB | 504 | -0.35 | -0.54 to -0.15 | <0.001 | -0.27 | -0.49 to -0.05 | 0.015 |
| Previous LRTI | 463 | -0.37 | -0.60 to -0.15 | <0.001 | -0.30 | -0.53 to -0.06 | 0.013 |

[^]linear mixed models

FVC: forced vital capacity; CI: confidence interval; PTB: pulmonary tuberculosis; LRTI: lower respiratory tract infection

Table 5S: Spirometry outcomes at baseline, 12 and 24 months by category of age at antiretroviral therapy initiation

| ART initiated before age 3 years | | | ART initiated after age 3 years | | |
|----------------------------------|-----|------|---------------------------------|------|---------|
| Baseline | n | mean | n | mean | P value |
| z-FEV ₁ | 185 | -0.9 | 309 | -1.0 | 0.347 |
| z-FVC | 185 | -1.1 | 309 | -1.1 | 0.837 |
| z-FEV ₁ /FVC | 185 | 0.4 | 309 | 0.1 | 0.053 |
| 12 months | | | | | |
| z-FEV ₁ | 173 | -0.8 | 290 | -1.0 | 0.115 |
| z-FVC | 173 | -0.8 | 290 | -0.9 | 0.206 |
| z-FEV ₁ /FVC | 173 | -0.1 | 290 | -0.2 | 0.497 |
| 24 months | | | | | |
| z-FEV ₁ | 158 | -0.8 | 268 | -0.9 | 0.422 |
| z-FVC | 158 | -0.6 | 268 | -0.8 | 0.279 |
| z-FEV ₁ /FVC | 158 | -0.3 | 268 | -0.3 | 0.767 |

FEV₁ forced expiratory volume in 1 sec; FVC forced vital capacity; z-scores from GLI 2012 African-American reference; ART antiretroviral therapy

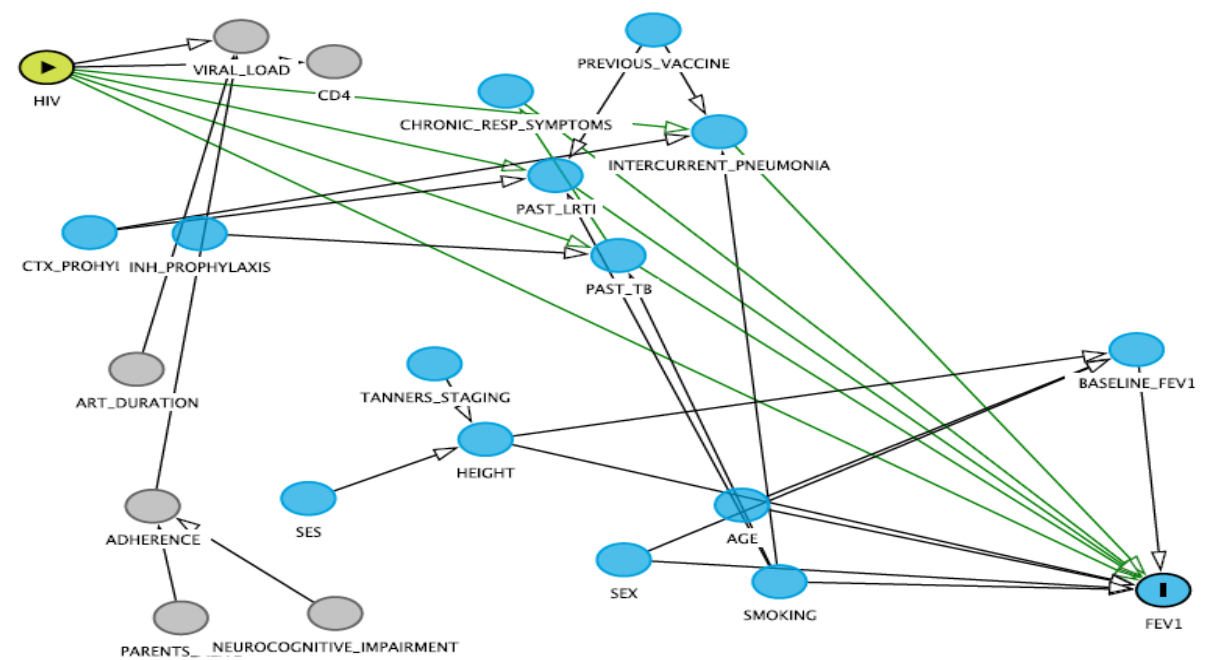


Figure 1S: Directed Acyclic Graph to determine minimum covariates in the model

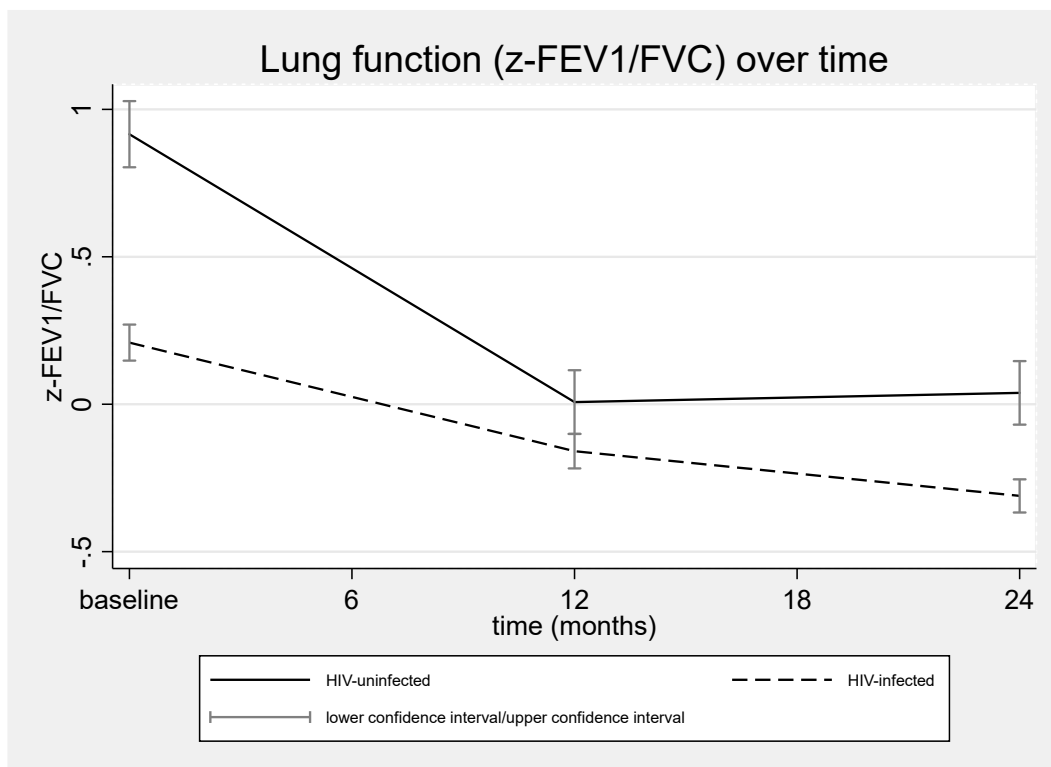


Figure 2S: Trend of lung function (z-FEV₁/FVC) over two years

FEV₁ forced expiratory volume in 1 sec; FVC forced vital capacity; z-scores from GLI 2012 African-American reference

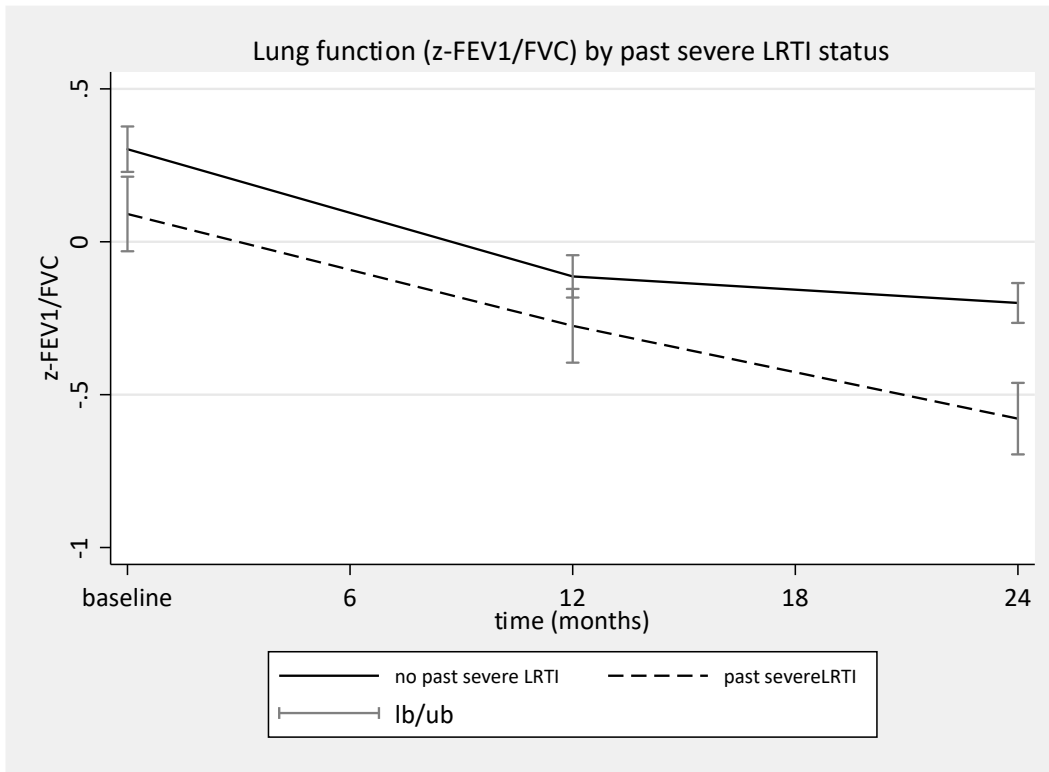
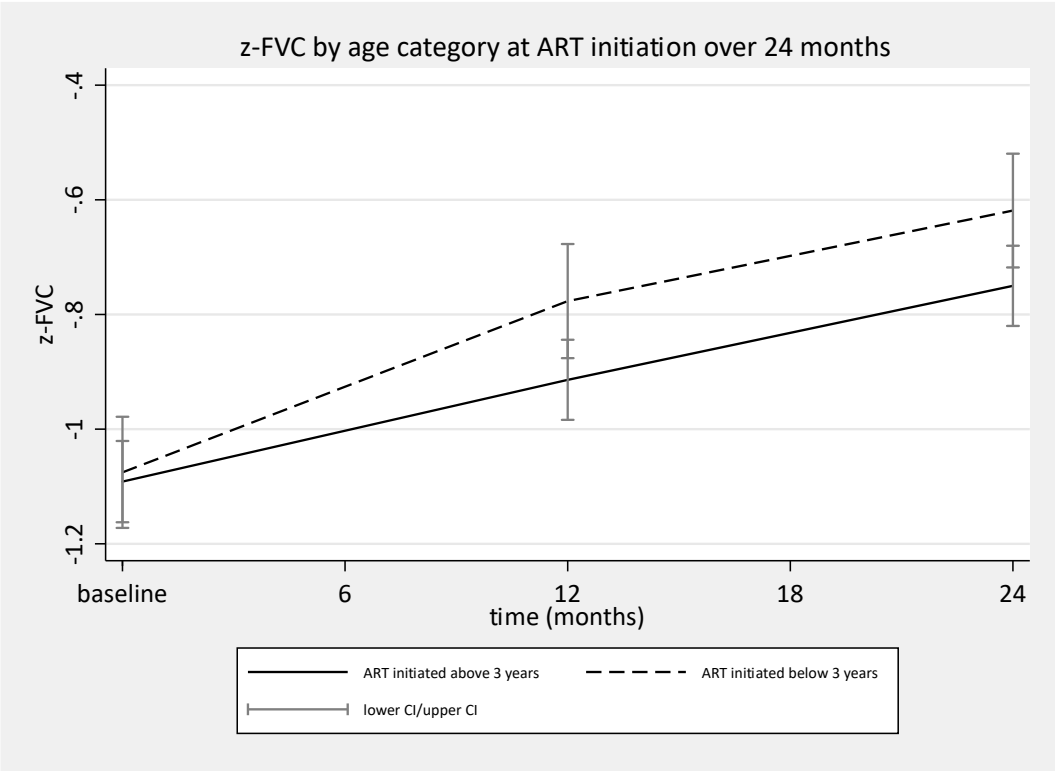
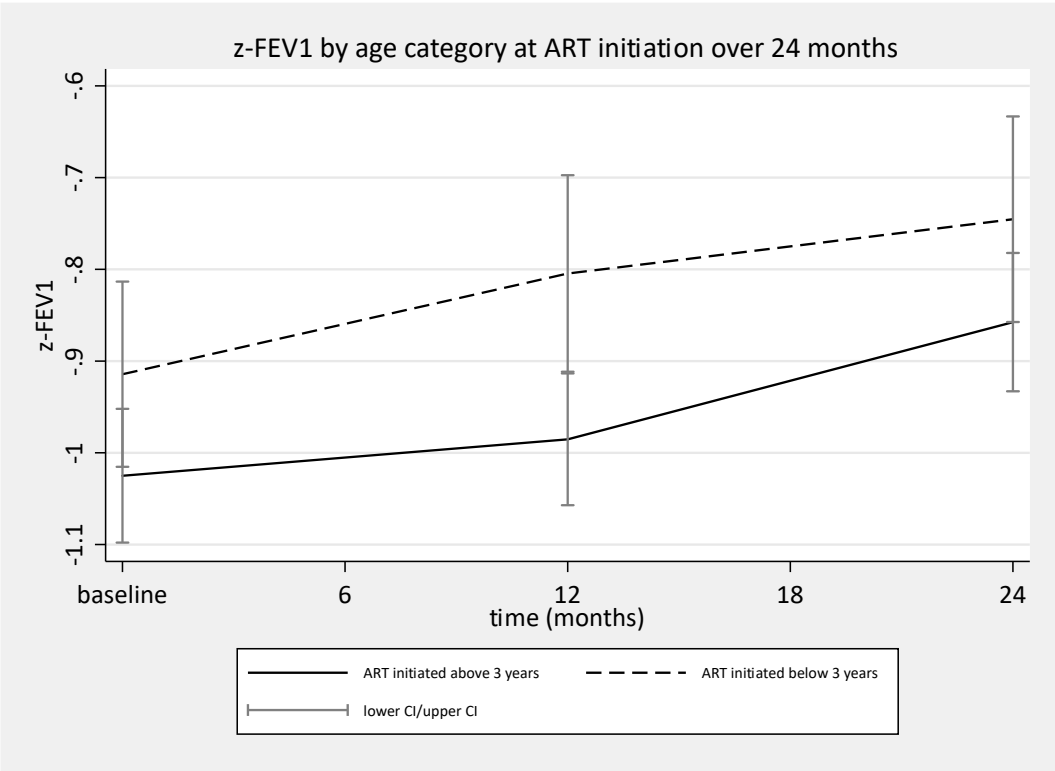


Figure 3S: Z-FEV₁/FVC by past severe LRTI status

FEV₁ forced expiratory volume in 1 sec; FVC forced vital capacity; z-scores from GLI 2012 African-American reference, LRTI lower respiratory tract infection, lb lower 95% confidence interval, ub upper 95% confidence interval



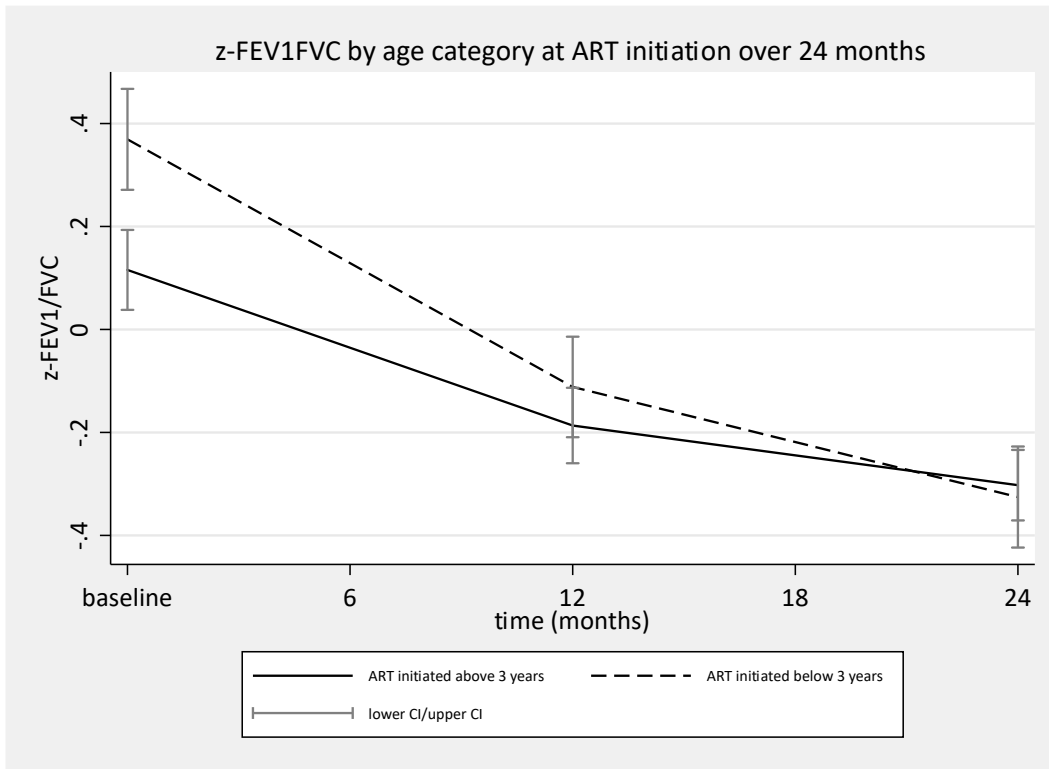


Figure 4S: Trend of z-FEV₁, z-FVC and z-FEV₁/FVC over 2 months by age category at ART initiation

FEV₁ forced expiratory volume in 1 sec; FVC forced vital capacity; z-scores from GLI 2012 African-American reference; lb lower 95% confidence interval, ub upper 95% confidence interval

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Chapter 6:

Summary and Recommendations

6.1 Summary

In this study, we investigated the spectrum and determinants of lung function in perinatally HIV-infected adolescents on antiretroviral therapy (ART) including longitudinal changes.

The following key findings were reported: -

1. HIV-infected adolescents, well established on ART had lower lung function (volume, flow, compliance and diffusion capacity) and increased airway resistance and lung clearance index at baseline compared to HIV-uninfected adolescents. The low lung function could have been caused by the impact of HIV and opportunistic infections. Further, stunted growth may have contributed to low lung volumes due to suboptimal lung growth as HIV-infected adolescents were shorter than HIV-uninfected at all time points.
2. Spirometry outcomes tracked over two years. Z-FEV₁, z-FVC and z-FEV₁/FVC tracked lower over two years in HIV-infected adolescents compared to the uninfected. This may have been attributable to the early effect of HIV or opportunistic infections on the developing lung. Adolescents who had ART initiated before 3 years of age had higher z-FVC, Z-FEV₁ and z-FEV₁/FVC over time compared to those who had ART initiated above 3 years. Further, those who began ART before 3 years of age had significantly lower rates of history of severe LRTI compared to those who began ART after 3 years. This emphasises the need for early initiation of ART to optimise lung health. Furthermore, it highlights the need for lung function surveillance in children and adolescents with HIV to diagnose cases of early COPD or accelerated decline in

lung function after the period of physiological lung growth in early childhood up to early adulthood.

3. A lower rate of bronchodilator responsiveness was reported in the HIV-infected adolescents at two years (6.4%) compared to baseline (16.4%), supporting the development of irreversible airway obstruction; this has been reported in similar cohorts in sub-Saharan Africa. [1-4] In previous studies, [2, 3] mosaic attenuation on chest computed tomography (CT) scan was reported, most likely caused by post-infectious bronchiolitis obliterans, as the predominant finding, which is characterised by irreversible lower airway obstruction.
4. There was no difference in six-minute walk test outcomes between HIV-infected adolescents on ART and the HIV-uninfected participants, probably reflecting that the HIV-infected cohort in our study was generally well and showing the impact of ART on preserving exercise tolerance, or cardiopulmonary function in general. Published studies with high rates of exercise intolerance were done in cohorts not established on ART. [5, 6]
5. Participants reported minimal chronic respiratory symptoms in our study cohort, compared to other similar age cohorts in sub-Saharan Africa, [1-2, 7] probably because participants under-reported symptoms. However, this may be on account of a more severe spectrum of chronic lung disease and lung function in the other cohorts who were not established on ART. These differences may also be as a result of cohort selection; our cohort had a mean ART duration of nine years, with ART initiated on average by the age of five years, in comparison to the other cohorts whose ART duration ranged from a few months to six years and age of ART initiation was in later childhood, affirming the importance of early initiation of ART.

6. Although lung function was lower in our HIV-infected adolescents than HIV-negative matched controls at enrolment, HIV-infected adolescents lung function was still within normal limits, unlike other adolescent cohorts that reported a higher rate of respiratory symptoms and impaired lung function. More than 65% of HIV-infected adolescents in our study cohort had a normal spirometry pattern at baseline, 12 and 24 months. Again, this reflects that our participants were generally well and had a long duration on ART.
7. Previous PTB and previous hospitalisation for LRTI were common in HIV-infected adolescents and were associated with lower lung function. High rates of PTB and LRTIs have also been reported in HIV-infected children and adolescents. [1, 2] This highlights the impact of early LRTI and PTB on the developing lung and the need to strengthen prevention strategies.
8. Lower respiratory tract infection requiring hospitalisation was associated with increased lung clearance index (LCI) and low diffusion capacity for carbon monoxide (DLCO); showing the impact of HIV and LRTI on small airways and alveolar-capillary interface.
9. Impaired cardiopulmonary function was detected in 13% of HIV-infected adolescents and 8% of HIV-uninfected adolescents, $p=0.136$. The lack of reference values for cardiac function assessment in African population may have overestimated the cardiopulmonary dysfunction. Previous severe LRTI and body mass index were predictors of impaired cardiopulmonary function.

6.2 Strengths and limitations

Study strengths include a large cohort of HIV-infected adolescents well established on ART, and with high cohort retention and close follow-up. We also included an age-matched

comparator group of HIV-uninfected adolescents. Further, comprehensive lung function testing including diffusion capacity testing, compliance and resistance testing with forced oscillation technique, six-minute walk test, volume measurements with nitrogen multiple breath washout tests and spirometry and bronchodilator testing, were used to describe the spectrum of lung function patterns. Longitudinal measurement of lung function over two years also strengthened the study by showing the trajectory of lung function in HIV-infected adolescents. In this study population, we highlighted areas to prevent and manage lung disease.

Limitations include that these findings may not be generalisable in populations with low ART coverage, however, ART coverage is increasing globally and in Africa. [8] Other study limitations include that smoking data was self-reported rather than a quantitative measurement of nicotine. This may have underestimated the impact of passive and active smoking on lung function in our cohort. Respiratory symptoms were also self-reported, hence recall bias may have been a factor, but the same question was repeated in subsequent visits. Computed tomography chest scans were not done on all participants to limit cost and radiation exposure; thus, this study does not show the structural pathology associated with impaired lung function in HIV-infected adolescents. Further, cardiopulmonary function testing using independent assessment of lung and cardiac function by spirometry, six-minute walk test and echocardiography may not have been as sensitive as a combined measure of cardiopulmonary function such as formal cardiopulmonary exercise testing that would measure oxygen consumption and carbon dioxide excretion by sampling inspired and expired gas during exercise. [9] This however is expensive and not widely available.

6.3 Recommendations

1. Longer studies continuing through late adulthood are needed to characterise the progression of lung function in HIV-infected adolescents. Such longitudinal studies would investigate the impact of lower lung function in HIV-infected adolescents on respiratory health into adulthood and would also delineate if accelerated decline of airflow and volume or COPD occurs earlier in this population compared to the uninfected population.
2. Longitudinal studies involving HIV-exposed uninfected children, HIV-infected children on ART and HIV-unexposed uninfected children are needed to delineate the impact of HIV prenatally on the lung. With the scaling up of prevention of mother to child treatment (PMTCT) programmes and the increasing population of HIV-exposed uninfected children, these are likely to be important studies with the three comparator groups.
3. Chest CT, lung biopsy and histopathology of lung specimens would be useful to better understand the pathology of low lung function phenotype in HIV-infected adolescents on ART. Studies on lung microbiome in HIV-infected and uninfected adolescents are needed to delineate the effect of airway colonisation by microbes on lung function.
4. Longitudinal use of lung function spanning to adulthood may offer an objective measure of lung health in HIV-infected adolescents and identify abnormalities that may be amenable to treatment.
5. Cardiac magnetic resonance imaging (MRI) may provide a more sensitive assessment for right ventricle function and pulmonary hypertension than echocardiography. Similarly, arterial gas analysis and cardiac output index measurement and cardiopulmonary exercise technology may better assess cardiopulmonary function.

6. Public health interventions to prevent PTB or LRTI such as immunisation or antimicrobial prophylaxis and promoting optimal nutrition may be important interventions to prevent development of low lung function or preserve lung function in HIV-infected children and adolescents. Such practices would include cotrimoxazole and isoniazid prophylaxis, avoidance of passive or active tobacco smoke, better nutrition and accessible quality health care for pregnant mothers and children. In addition, optimising childhood immunisation and specific immunisation strategies for HIV-infected adolescents (such as booster pneumococcal conjugate vaccine) need further study. All these strategies must be strengthened for HIV-infected children and adolescents.
7. With the increasing population of perinatally HIV-infected children surviving into adolescence, health systems to provide integrated care to this vulnerable group must be strengthened to provide comprehensive care beginning from early infancy.

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APPENDICES

Appendix 1: Respiratory Questionnaire

(Please ask the participant)

| PAST RESPIRATORY ILLNESS | |
|--------------------------|--|
| 1. | <p>Has any doctor, nurse or caregiver EVER told you that you have any of the following:</p> <p><input type="checkbox"/> Chest infection/pneumonia</p> <p><input type="checkbox"/> Bronchitis</p> <p><input type="checkbox"/> Asthma</p> <p><input type="checkbox"/> TB</p> <p><input type="checkbox"/> None of the above</p> |
| 2. | <p>How many times in the past 12 months have you been admitted to hospital with a chest infection?</p> <p><input type="checkbox"/> (write in number)</p> |
| 3. | <p>How many times in the past 12 months have you been treated at your clinic for a chest infection?</p> <p><input type="checkbox"/> (write in number)</p> |

| WHEEZE AND TIGHTNESS IN CHEST | |
|-------------------------------|---|
| 1. | <p>Have you <u>ever</u> had a whistling or wheezing noise in the chest at any time in the past?</p> <p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No (if no, skip to Q 6)</p> |
| 2. | <p>Have you had wheezing or whistling in the chest <u>in the past 12 months</u>?</p> <p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No (if no, skip to Q 6)</p> |
| 3. | <p>How many attacks of wheezing have you had <u>in the past 12 months</u>?</p> <p><input type="checkbox"/> None</p> <p><input type="checkbox"/> 1 to 3</p> <p><input type="checkbox"/> 4 to 12</p> <p><input type="checkbox"/> More than 12</p> |
| 4. | <p><u>In the past 12 months</u>, how often, on average, has your sleep been disturbed due to wheezing?</p> <p><input type="checkbox"/> Never</p> <p><input type="checkbox"/> Less than 1 night per week</p> |

| | | |
|----|--|---|
| | | <input type="checkbox"/> One or more nights per week |
| 5. | <u>In the past 12 months</u> , has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 6. | Have you ever had asthma? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 7. | <u>In the past 12 months</u> , has your chest sounded wheezy during or after exercise? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 8. | <u>In the past 12 months</u> , have you had a dry cough at night, apart from a cough associated with a cold or chest infection? | <input type="checkbox"/> Yes <input type="checkbox"/> No |

ALL QUESTIONS ARE ABOUT PROBLEMS WHEN YOU DO NOT HAVE A COLD OR FLU

| | | |
|-----|---|--|
| 9. | Have you <u>ever</u> had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the flu? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q 14) |
| 10. | <u>In the past 12 months</u> , have you had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the flu? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q 14) |
| 11. | <u>In the past 12 months</u> , has this nose problem been accompanied by itchy-watery eyes? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 12. | In which of <u>the past 12 months</u> did this nose problem occur? (Please tick any which apply) | <input type="checkbox"/> January <input type="checkbox"/> February <input type="checkbox"/> March <input type="checkbox"/> April <input type="checkbox"/> May <input type="checkbox"/> June <input type="checkbox"/> July <input type="checkbox"/> August <input type="checkbox"/> September <input type="checkbox"/> October <input type="checkbox"/> November <input type="checkbox"/> December |
| 13. | <u>In the past 12 months</u> , how much did this nose problem interfere with your daily activities? | <input type="checkbox"/> Not at all <input type="checkbox"/> A little <input type="checkbox"/> A moderate amount <input type="checkbox"/> A lot |

| ALLERGY SYMPTOMS | | |
|------------------|--|---|
| 14. | Have you <u>ever</u> had hay fever? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 15. | Have you <u>ever</u> had an itchy rash which was coming and going for at least six months? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q20) |
| 16. | Have you had this itchy rash at any time <u>in the past 12 months</u> ? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q20) |
| 17. | Has this itchy rash <u>at any time</u> affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 18. | Has this rash cleared completely at any time <u>during the past 12 months</u> ? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 19. | <u>In the past 12 months</u> , how often, on average, have you been kept awake at night by this itchy rash? | <input type="checkbox"/> Never <input type="checkbox"/> Less than 1 night per week <input type="checkbox"/> One or more nights per week |
| 20. | Have you <u>ever</u> had eczema? | <input type="checkbox"/> Yes <input type="checkbox"/> No |

| SHORTNESS OF BREATH | | |
|---------------------|--|--|
| 21. | Have you ever had an attack of shortness of breath when you were at rest? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q25) |
| 22. | If Yes, has this occurred in the last 12 months? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 23. | Are you more short of breath when exercising compared to other people your age? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 24. | How many times a week do you do enough exercise or physical effort to make you breathe hard? | <input type="checkbox"/> Never or occasionally <input type="checkbox"/> Once or twice per week <input type="checkbox"/> Three or more times per week |

| COUGH AND PHLEGM | | |
|------------------|--|--|
| 25. | Do you currently have a cough on most days? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q29) |
| 26. | When you cough, do you usually bring up phlegm from your chest? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q29) |
| 27. | Have you brought up phlegm every day for at least three months during the last year? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q29) |
| 28. | How many years have you brought up phlegm in this way? | Years: _____ |
| 29. | Have you been woken by an attack of coughing in the last 12 months? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 30. | Do you usually cough when you get up in the morning? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q33) |
| 31. | If Yes, does this occur on most mornings for at least three months in a row each year? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 32. | Do you easily bring up phlegm from your chest when you get up in the morning? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 33. | Have you been woken by an attack of coughing when you did not have a cold in the last 12 months? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 34. | Have you ever coughed up blood? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q37) |
| 35. | If Yes, how much blood did you cough up? | <input type="checkbox"/> More than a cup <input type="checkbox"/> Teaspoon <input type="checkbox"/> Speckles of blood <input type="checkbox"/> Not sure |
| 36. | If Yes, when last did you cough up blood? | <input type="checkbox"/> Within the last week <input type="checkbox"/> Within the last month <input type="checkbox"/> Within the last year <input type="checkbox"/> More than a year ago <input type="checkbox"/> Can't remember |

| BREATHING | | |
|-----------|--|--|
| 37. | Do you ever have trouble breathing? | <input type="checkbox"/> Yes <input type="checkbox"/> No (if no, skip to Q43) |
| 38. | If Yes, how often do you have this trouble? | <input type="checkbox"/> Continuously (your breathing is never quite right) <input type="checkbox"/> Repeatedly (but it always gets completely better between episodes) <input type="checkbox"/> Rarely (less than once a month) |
| 39. | Are you troubled by shortness of breath when walking fast or walking up a hill? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 40. | Have you ever been troubled by coughing a lot when you run or just after you have stopped running? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 41. | Does cold air make you cough a lot? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 42. | Do you sometimes cough in bed at night when you do not have a cold? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 43. | Does anyone ever complain that you snore at night? | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| 44. | If Yes, how often does this happen? | <input type="checkbox"/> Most night <input type="checkbox"/> Some nights <input type="checkbox"/> Hardly ever |

Appendix 2: Human Research Ethics Committee, University of Cape Town (REC Ref: 051/2013)

UNIVERSITY OF CAPE TOWN
FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FHS0001637; IR300001636)
This serves as notification of annual approval, including any documentation described below.

Approved Annual progress report Approved ultimate renewal date: 28/02/19
 Not approved See attached comments

Signature Chairperson of the HREC: [signature] signature removed
Date Signed: 17/1/19

Comments in form from the HREC:

Principal Investigator to complete the following:

1. Protocol information

| | |
|---|--|
| Date (when submitting this form) | 04 January 2018 |
| HREC REF Number | 018/2016 Current Ethics Approval was granted until: 28/02/2018 |
| Protocol title | LUNG FUNCTION IN HIV-INFECTED ADOLESCENTS ON ANTIRETROVIRAL THERAPY IN CAPE TOWN, SOUTH AFRICA |
| Protocol number (if applicable) | |
| Are there any sub-studies linked to this study? | <input checked="" type="checkbox"/> No |
| If yes, could you please provide the HREC Ref's for all sub-studies? (Note: A separate FHS016 must be submitted for each sub-study) | |
| Principal investigator | Prof. Heather Zar |
| Department / Office Internal Ref Address | Department of Paediatrics, 5 th floor ICH Building |

28 June 2017 Page 1 of 6 FHS016

Order: Please complete the Closure form (FHS010) if the study is completed within the approval period.

MANMAN RESEARCH ETHICS COMMITTEE
17 JAN 2018
HEALTH SCIENCES FACULTY UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN
FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

1.1 Does this protocol receive US Federal funding? Yes No

1.2 If the study receives US Federal Funding, does the annual report require full committee approval? Yes No

Note: Any annual approvals for Full Committee review MUST be submitted on the monthly HREC submission dates.

If yes in 1.2 please complete section 1.5 below for invoicing purposes

1.5 Annual Approval for full committee review - R 3430 (inclusive of vat)

For invoicing purposes, please provide:

Sponsor's name
Contact person
Address
Telephone number
E-mail Address

2. List of documentation for approval

3. Protocol status (tick ✓)

Open to enrolment
 Closed to enrolment (tick ✓)
 Research-related activities are ongoing
 Research-related activities are complete, long-term follow-up only
 Research-related activities are complete, data analysis only
 Main study is complete but sub-study research-related activities are ongoing
 Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

| | |
|---|-----|
| Number of participants enrolled to date | 001 |
| Number of participants enrolled since last HREC Progress report (excluding renewal) | 0 |
| Additional number of participants still required | 0 |

28 June 2017 Page 2 of 6 FHS016

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)



5. Refusals

| | |
|---|---|
| Total number of refusals (participants invited to join the study, but refused to take part) | 4 |
|---|---|

6. Cumulative summary of participants

| | |
|--|--------------------------------------|
| Total number of participants who provided consent | 661 |
| Number of participants determined to be ineligible (i.e. after screening) | 63 |
| Number of participants currently active on the study | 588 |
| Number of participants completed study (without events leading to withdrawal) | 0 |
| Number of participants withdrawn at participants' request (i.e. changed their mind) | 23 |
| Number of participants withdrawn by PI due to toxicity or adverse events | NONE |
| Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance) | 0 |
| Number of participants lost to follow-up. Please specify below on reasons for loss of follow-up | 37 |
| Prior to first enrolment visit at Red Cross Hospital (relocated from 2013) | |
| 5 relocated out of Cape Town | |
| 7 no longer willing | |
| 32 unable to contact | |
| 1 withdrawn by doctor at primary clinic | |
| 9 insufficient level of disclosure of HIV status | |
| 1 older than age of study eligibility | |
| 1 control participant tested HIV positive | |
| Number of participants no longer taking part for reasons not listed above. Please provide reasons below: | 37 (24 HIV positive and 13 controls) |
| Since first enrolment visit at Red Cross Hospital | |
| 16 no longer willing | |
| 17 relocated | |
| 1 withdrawn by Dr. at primary clinic | |
| 1 participant requested to be withdrawn as was participating in another study | |
| 1 control tested HIV positive | |
| 1 participant died | |

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report, as well as any relevant comments/cause you would like to report to the HREDC.



Baseline, 6 month, 12 month, 18 month, 24 month and 30 month visits have been completed with retention rate from 88% to 92%. 36 month, 42 month and 48 month visit is ongoing.

8. Protocol violations and exceptions (tick ✓ all that apply)

| |
|--|
| <input checked="" type="checkbox"/> No prior violations or exceptions have occurred since the original approval |
| <input type="checkbox"/> Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved |
| <input type="checkbox"/> Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review |

9. Amendments (tick ✓ all that apply)

| |
|---|
| <input checked="" type="checkbox"/> No prior amendments have been made since the original approval |
| <input type="checkbox"/> Prior amendments have been reported since the last review and have already been approved |
| <input type="checkbox"/> New protocol changes/amendments are requested as part of this continuing review (See note below) |

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS008). Specific changes in the amended protocol and consent/assent forms must be bolded, italicized or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREDC). Please comment on whether causality to any study procedure or intervention could be established. No adverse events noted or anticipated.



10.2 Have participants received appropriate treatment/follow-up/referral when indicated (e.g. in the case of abnormal or incidental physical findings, disease or anxiety)?

Yes No Not applicable

If yes, please describe:

Participants who have abnormal lung function and abnormal chest radiograph have been referred to primary doctor and sub-specialists' clinics for review.

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. MCC, FDA)?

Yes No Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?

Yes No Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.

| | | | | |
|-------------|----------------------|------------------------------|-----------------------------|---|
| Agency Name | Report attached | <input type="checkbox"/> Yes | <input type="checkbox"/> No | <input type="checkbox"/> Not applicable |
| | DSMB report attached | <input type="checkbox"/> Yes | <input type="checkbox"/> No | <input type="checkbox"/> Not applicable |

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?

Yes No

If yes, please explain:

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:

Increased

Decreased

Shown no change

If there has been a change, please explain:

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.



none

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)

Yes No

If yes, please explain and if necessary attach a revised conflict of interest statement (Section 87 in the New Protocol Application Form FHS013).

14. Signature

My signature certifies that the study is complete and correct.

Signature of PI: signature removed

Appendix 3: Consent Form

Parent /Legal Guardian/Caregiver Information Sheet and Consent Form

Cape Town Adolescent Antiretroviral Cohort (CTAAC)

Your child is being invited to take part in this clinical research study because he/she is an HIV-infected adolescent receiving antiretroviral therapy (ART). This study that is being done at Red Cross Children's Hospital in the School of Child and Adolescent Health, University of Cape Town. Before you decide if you want to be a part of this study, we want you and your child to know more about the study.

This form gives you information about your child's participation in this study. The research staff will talk with you and your child about this information. You are free to ask questions about this study and discuss any concerns with the staff. If you agree to take part in this study, you will be asked to sign this consent form and your child may be asked to sign a separate assent form. You will be given copies of these forms.

WHY IS THIS STUDY BEING DONE?

We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. The study will take place over 3 years during which we will follow-up your child and monitor his/her health. At any time during this study, if we find that your child has a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHO CAN TAKE PART IN THE STUDY?

To participate, adolescents must be between the ages of 9 and 14 years, and must have been on ART for at least 6 months and must live in Cape Town. Approximately 600 adolescents will take part in this study.

WHAT DO I HAVE TO DO IF I TAKE PART?

Your child will need to attend study visits held at Red Cross War Memorial Children's Hospital every 6 months for the next 3 years.

Enrolment and annual (yearly) visits

The first study visit at Red Cross War Memorial Children's Hospital will last 4-5 hours. At the first visit and once a year, your child will be asked to undergo the following measurements:

1. You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, HIV treatment history, and other aspects of health, both in the past and at present.
2. Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting 10 minutes approximately, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.
3. Your child will be asked to give blood. We will collect about 15 milliliters (3 teaspoons) of blood once a year. This blood will be used to test for HIV in the body (viral load), the body's response to HIV infection (CD4 cell count), the fats in the blood (lipogram), the body's degree of health or general inflammation (Full blood count, Liver and Kidney function, C-reactive protein, Calcium, Magnesium Phosphate, Albumin), TB infection and autoantibodies like ANA, anti-cardiolipin antibody and anti-dsDNA. Antibodies are made by the body to help fight infection. Sometimes rather than fighting infection these antibodies attack the body and are called autoantibodies. There are specific autoantibodies that cause specific diseases like Lupus. It is possible to have these autoantibodies even before one gets sick with a disease and these antibodies can sometimes be present without actually causing any disease. If permission is given some of this blood may be stored for future tests and studies.

4. Your child will be asked to undergo echocardiography. This is a video of the child's heart. This video is called an echocardiogram or "echo". For this, we will put some gel and a probe on your child's chest. The doctor will see your child's heart move on a screen. A short video of this movement will be recorded. We will also check your heart rate and rhythm (the way in which your heart is beating). We will also look at the arteries in your child's neck to see if they are harder or thicker than usual. This will take less than 5 minutes. We will only do this test at the first visit, at the two year visit and the last study visit.
5. Your child will be asked to undergo endothelial pulse amplitude tonometry (Endo-PAT). This is a painless procedure very similar to having blood pressure measured, using a special machine. This will measure the thickness of your child's veins and how easily the blood flows through the veins. This test will be done at the first visit and then yearly.
6. Your child will be asked to have a chest x-ray. This is a painless procedure to take a picture of your child's lungs. This helps to check if your child has a lung problem which needs treatment. An X-ray will be done at the first visit and yearly.
7. Your child will be asked to provide a urine sample to test for kidney function and to test for substances that may affect his/ her health and if permission is given a sample will be stored for future testing. Urine will be tested for commonly abused drugs and exposure to smoking. If any of these are detected in the urine we will inform you and your child of these results, you will both receive counselling and you will be referred to the Cape Town Drug Counselling Centre for treatment.
8. Your child will be asked to give us some stool (kaka) that we will collect in a container. The stool (kaka) is being collected so that we can study it to see what it is made up of and to see if the medicines your child has taken has any effects on the stool (kaka). This can help us to understand how your child is growing and also understand any illnesses that they may have. To collect the stool, we will put a container in the toilet. Your child will sit on the toilet as they usually do to

provide the stool. They will leave it in the bathroom and the study nurse will collect it afterwards.

9. Your child will be asked to undergo lung function testing. This involves breathing into a special machine to see how the lungs are working. Your child will do 3 different blowing tests. One test measures inflammation in the lungs and takes only 1 or 2 minutes. A second test measures the size of the lungs and how well they are working and involves breathing oxygen for the middle part of the test. The third test measures the flow of air out of the lungs when your child blows as hard and fast as she/he can. If your child cannot blow air well out of their lungs we will give your child an asthma medicine (bronchodilator) to open their chest. After 5 minutes we will repeat the blowing test, to see if this treatment helps your child and what kind of lung problem your child might have.
10. Your child will be asked to do a 6 minute walk test to measure their ability to do exercise. This involves your child walking back and forth on a flat surface such as a hallway for 6 minutes. We will monitor their oxygen level and blood pressure before and after the test.
11. Your child may be asked to undergo sputum induction to test for TB and other infections. This involves having a nebuliser treatment to open your child's chest and make him / her cough. We will then ask your child to cough some phlegm up into a container or suction him/ her to get phlegm if he/ she cannot cough this up. The phlegm will be sent to the laboratory for tests for TB and other germs.
12. Your child will be asked to have a nasal swab done. This procedure involves putting a small stick with cotton wool at the tip into your child's nose to collect mucus. We will test this for bacteria and infection.
13. Your child will be asked to undergo a bedside ultrasound, it involves putting a little bit of gel on your child's stomach and chest and a probe that will take a video of your child's organs, and this is similar to the ultrasound that is done when you are pregnant and is quick and painless.

14. Your child will be asked to undergo another ultrasound of the joints with a bit of gel and the probe on their knuckles, wrist and the heel of their foot. It is also quick and painless though the gel may be cold.
15. Your child will be asked to have a hearing test done. This involves your child putting in earphones and listening to beeping sounds at different volumes and telling us when he/she hears the sounds. This will help us to test whether or not your child has a hearing problem. This is a quick and painless test.

Six-monthly visits

Between the yearly study visits your child will be asked to participate in a shorter study visit. This visit will last approximately 1-2 hours and will include the following:

1. You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, HIV treatment history, and other aspects of health.
2. Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting about 10 minutes, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

We will also ask for permission to look at information from your child's health care records at the clinic he/ she currently attends, and other facilities he/she may have attended. We will get information about your child's past health in the past, including whether they attended a clinic or hospital, the treatment received, and laboratory results over time. This information is important to help us understand the results of the tests done as part of this study.

We are not taking over your child's routine health care or ART; he/she should still attend all their regular follow up visits at your local health facility. We will inform your child's doctor of any abnormal results we may find during the study.

We may also ask you to participate in other studies that are related to this research. Participation in this additional research will have a separate consent process and another form, similar to this one.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?

Some of the questions asked in the questionnaire may make you or your child feel uncomfortable; this may include questions on smoking, alcohol and drug use and sexual behaviour. Our interviewers are trained in asking sensitive questions and you or your child can choose not to answer any questions if you don't want to.

The clinical examination may make your child feel uncomfortable. The examination will be in a private room, and if your child prefers you or another caregiver can be present.

Some of the study measurements are associated with slight discomfort. Specifically:

1. Blood drawing: Your child will experience discomfort from the needle when blood is taken. Where possible this blood test will be done at the same time as other blood tests and using an anaesthetic cream to dull the pain from the needle. Only a small amount of blood will be taken.
2. Endo-PAT: This will be similar to having a blood pressure done- that is for 5 minutes, a tight bandage will be placed on the upper part of both arms while measurements are done on the fingers. This is slightly uncomfortable but not painful.
3. Echocardiography: It is not painful, but will need your child to lie still for about 15 minutes. Gel will be used to help make the pictures clearer and this will be slightly cold.
4. Lung function testing: Lung function testing is not painful. Your child may feel a little dizzy for a short time when they blow out hard and fast. All the testing will be done by a trained technologist who is experienced in guiding young people in these tests. Breathing oxygen feels similar to breathing room air and is safe.

5. 6 minute Walk Test: The object of this test is to walk as far as possible in 6minutes. This is a painless test but your child may feel short of breath. We will monitor their oxygen level and blood pressure before and after the test and if your child feels tired they can stop to rest.
6. Chest X-ray: This is painless but may cause your child some discomfort as it requires sitting still for less than 5 minutes. An X-ray will be done at the start and at the end of the study, unless your child has an illness for which an X-ray is needed.
7. Induced Sputum: Your child may experience minor side effects such as increased coughing, vomiting, wheezing or mild bleeding from the nose. A trained nurse will do this in a special room.
8. Nasal swab: This is a painless procedure but may cause some sneezing that will stop quickly.
9. Bedside ultrasound: The gel might be slightly cold but it won't hurt.

WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

Your child's health will be carefully monitored during the study so any new health problems may be picked up early. If we find your child has a problem that needs to be treated, we will refer him/her to an appropriate facility. The information we learn through this study may help to improve the management of HIV-infected children in the future.

WILL I RECEIVE ANY PAYMENT?

You will receive R80 for the shorter visits and R200 for the longer visits when you and your child visit the study to compensate you for transport costs and time associated with the study.

CAN I AND/OR MY CHILD REFUSE TO TAKE PART IN THE STUDY?

If you or your child do not wish to participate, you or your child can refuse now or at any time in the future. Your child may also refuse to participate despite you having given

consent for him/her participation. Even if you and your child decide not to take part, your child will receive the same health care, including ART, through your local health facility.

CONFIDENTIALITY

Every effort will be made to ensure that your child's information is protected. The study team will keep your child's study information confidential. Your child will be given a study number. The questionnaire and study specimens will be labelled with this study number and not with his/ her name. As a participant in this study it is very important to be able to contact you and therefore we will need to collect detailed tracing information like your address and at least two phone numbers where we might get hold of you. Please take note that even when contacting friends or neighbours we will never give them the reason that we are looking for you.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about this study you may ask the study staff or you may contact:

Professor Heather Zar at (021) 658 5111 or the study Medical Officer at (021) 658 5520

If you have any questions about your rights as a research participant, you may contact the following member of the Human Research Ethics Committee in the Faculty of Health Sciences at the University of Cape Town:

Prof Marc Blockman at (021) 406 6338

_____ DD/MMM/YYYY at - -H- -
Witness' Name (Print) Witness' Signature Date and Time

Participant Enrolment number: _____

Please complete the following:

The caregiver of this child confirms that the biological mother / father (please circle) of this child is alive but does not take ANY responsibility for the care of or decision-making for this child.

Not applicable - neither of the child's biological parents are alive.

Not applicable - the child's biological parent signed consent.

Parent / Legal Guardian/ Caregiver Information Sheet and Consent Form

HIV negative controls

Lung function sub-study of Cape Town Adolescent Antiretroviral Cohort

Your child is being invited to take part in a clinical research study that is being done at Red Cross Children's Hospital in the School of Child and Adolescent Health, University of Cape Town. Before you decide if you want to be a part of this study, we want you and your child to know more about the study.

This form gives you information about your child's participation in this study. The research staff will talk with you and your child about this information. You are free to ask

questions about this study and discuss any concerns with the staff. If you agree to take part in this study, you will be asked to sign this consent form and your child may be asked to sign a separate assent form. You will be given copies of these forms.

WHY IS THIS STUDY BEING DONE?

We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. When collecting this type of information about HIV, it is important to compare the findings of the HIV positive group with a control group that are HIV negative, like your child. The study will take place over 3 years during which we will follow-up your child and monitor his/her health. At any time during this study, if we find that your child has a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHO MAY TAKE PART IN THIS STUDY?

You may participate in this study if your child is between the ages of 9 and 14 years old, HIV negative and living in Cape Town. Approximately 600 adolescents will take part in this study.

HIV testing procedure:

Since your child is a **HIV negative participant** in this study, with your permission we would like to do a simple HIV screening test to make sure we are comparing data from HIV positive children to HIV negative children like your child. It is also important for you and your child to know your HIV status to ensure early treatment if you test positive. This test will be done at your first study visit, prior to doing any other investigations.

If your child is confirmed HIV negative, he/she will be enrolled into this study with your permission as parent/legal guardian/ caregiver. If as a result of your participation in this research study your child is initially diagnosed as positive, the results will be given to your child privately if your child is older than 12 years; they may choose to share this result with you. If your child is less than 12 years, the results will be revealed to you. Your child will then be referred by the study doctor to the appropriate health facility for immediate counselling and appropriate treatment.

Having an HIV test done can cause feelings of anxiety and worry. These kinds of feelings are normal. We will take every step possible to ensure that you are comfortable with having your child take the HIV test. We will perform an HIV rapid test, which will require your child to have a finger prick for a drop of blood. The test results are immediately available.

As part of this procedure, you and your child will be counselled both prior to taking the test and afterwards, regardless of the test outcome.

If your child has already had a recent HIV test, he/she will not need to redo the test to participate in this study, but with your permission, we will have to get this information from the clinic at which he/she was tested.

WHAT DO I HAVE TO DO IF I TAKE PART?

Your child will need to attend study visits at Red Cross War Memorial Children's Hospital every 6 months for the next 3 years.

Enrolment and annual (yearly) visits

The first study visit at Red Cross War Memorial Children's Hospital will last 4-5 hours. At the first visit (enrolment) and once a year, your child will be asked to undergo the following measurements:

16. You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, and other aspects of health, both in the past and at present.
17. Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting 10 minutes approximately, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.
18. Your child will be asked to give blood. We will collect about 15 millilitres (3 teaspoons) of blood once a year. This blood will be used to test for the body's degree of health or general inflammation (Full blood count).

19. Your child will be asked to undergo echocardiography. This is a video of the child's heart. This video is called an echocardiogram or "echo". For this, we will put some gel and a probe on your child's chest. The doctor will see your heart move on a screen. A short video of this movement will be recorded. We will also check your heart rate and rhythm (the way in which your heart is beating). We will also look at the arteries in your child's neck to see if they are harder or thicker than usual. This will take less than 5 minutes. We will only do this test at the first visit, at the two year visit and the last study visit.
20. Your child will be asked to have a chest x-ray. This is a painless procedure that involves taking a picture of your child's lungs. This helps to check if your child has a lung problem which needs treatment. An X-ray will be done at the start of the study and yearly.
21. Your child will be asked to undergo lung function testing. This involves breathing into a special machine to see how the lungs are working. Your child will do 3 different blowing tests. One test measures inflammation in the lungs and takes only 1 or 2 minutes. A second test measures the size of the lungs and how well they are working and involves breathing oxygen for the middle part of the test. The third test measures the flow of air out of the lungs when your child blows as hard and fast as she/he can. If your child cannot blow air well out of their lungs we will give your child an asthma medicine (bronchodilator) to open their chest. After 5 minutes we will repeat the blowing test, to see if this treatment helps your child and what kind of lung problem your child might have.
22. Your child will be asked to do a 6 minute walk test to measure their ability to do exercise. This involves your child walking back and forth on a flat surface such as a hallway for 6 minutes. We will monitor their oxygen level and blood pressure before and after the test.

Six- monthly visits

Between the yearly study visits your child will be asked to participate in a shorter study visit. This visit will last approximately 1-2 hours and will include the following:

3. You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, and other aspects of health.
4. Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting about 10 minutes, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

We will also ask for permission to look at information from your child's health care records at the clinic he/ she currently attends, and other facilities he/she may have attended. We will get information about your child's past health, including whether they attended a clinic or hospital, the treatment received, and laboratory results over time. This information is important to help us understand the results of the tests done as part of this study.

We are not taking over your child's routine health care; he/she should still attend all their regular follow up visits at your local health facility. We will inform your child's doctor of any abnormal results we may find during the study.

We may also ask you to participate in other studies that are related to this research. Participation in this additional research will have a separate consent process and another form, similar to this one.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?

Some of the questions asked in the questionnaire may make you or your child feel uncomfortable; this may include questions on smoking. Our interviewers are trained in asking sensitive questions and you or your child can choose not to answer any questions if you don't want to.

The clinical examination may make your child feel uncomfortable. The examination will be in a private room, and if your child prefers you or another caregiver can be present.

Some of the study measurements are associated with slight discomfort. Specifically:

10. Blood drawing: Your child will experience discomfort from the needle when blood is taken. Where possible this blood test will be done at the same time as other blood tests and using an anaesthetic cream to dull the pain from the needle. Only a small amount of blood will be taken.
11. Echocardiography: It is not painful, but will need your child to lie still for about 15 minutes. Gel will be used to help make the pictures clearer and this will be slightly cold.
12. Lung function testing: Lung function testing is not painful. Your child may feel a little dizzy for a short time when they blow out hard and fast. All the testing will be done by a trained technologist who is experienced in guiding young people in these tests. Breathing oxygen feels similar to breathing room air and is safe.
13. 6 minute Walk Test: The object of this test is to walk as far as possible in 6minutes. This is a painless test but your child may feel short of breath. We will monitor their oxygen level and blood pressure before and after the test and if your child feels tired they can stop to rest.
14. Chest X-ray: This is painless but may cause your child some discomfort as it requires sitting still for less than 5 minutes. An X-ray will be done at the start and at the end of the study, unless your child has an illness for which an additional X-ray is needed.

WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

Your child's health will be carefully monitored during the study so any new health problems may be picked up early. If we find your child has a problem that needs to be treated, we will refer him/her to an appropriate facility. The information we learn through this study may help to improve the management of HIV-infected children in the future.

WILL I RECEIVE ANY PAYMENT?

You will receive R80 for the shorter visits and R200 for the longer visits when you and your child come to visit the study to compensate you for transport costs and time associated with the study.

CAN I AND/OR MY CHILD REFUSE TO TAKE PART IN THE STUDY?

If you or your child do not wish to participate, you or your child can refuse now or at any time in the future. Your child may also refuse to participate despite you having given consent for their participation. Regardless of whether or not you and your child participate, your child will receive the same health care through your local health facility.

CONFIDENTIALITY

Every effort will be made to ensure that your child's information is protected. The study team will keep your child's study information confidential. Your child will be given a study number. The questionnaire and study specimens will be labelled with this study number and not with his/ her name. As a participant in this study it is very important to be able to contact you and therefore we will need to collect detailed tracing information like your address and at least two phone numbers where we might get hold of you. Please take note that even when contacting friends or neighbours we will never give them the reason that we are looking for you.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about this study you may ask the study staff or you may contact:

Professor Heather Zar at (021) 658 5111 or the study Medical Officer at (021) 658 5520

If you have any questions about your rights as a research participant, you may contact the following member of the Human Research Ethics Committee in the Faculty of Health Sciences at the University of Cape Town:

Prof Marc Blockman at (021) 406 6338

Appendix 4: Lung Function CRF

| Study Details | | |
|---------------|-------------------------------|---|
| 1 | Time arrived (24 hour clock) | HH : HH |
| 2 | Time finished (24 hour clock) | HH : HH |
| 3 | Date of Birth | DD/MMM/YYYY |
| 4 | Height | cm |
| 5 | Weight | kg |
| 6 | Room temperature | °C |
| 7 | Pressure | hPa |
| 8 | Humidity | % |
| 8 | Position | <input type="checkbox"/> Sitting <input type="checkbox"/> Standing |
| 9 | Additional information: | |

| Equipment Calibration – EasyOnePro | | | | | | |
|------------------------------------|---------|--------------------------|-----|--------------------------|----------------|-------------|
| Date | Time | Within 3 % | | | File Reference | |
| DD/MMM/YYYY | HH : HH | <input type="checkbox"/> | yes | <input type="checkbox"/> | no | See Cal Log |

| Spirometry - Pre | | | | | |
|---------------------------------|---------|----------|----------|-----------|--|
| Test | FVC | FEV1 | FEV1/FVC | FEF25-75% | |
| Time | HH : HH | Comments | | | |
| Total number of attempts: _____ | | | | | |

| See attached results for values | | | | | |
|--|----|---|----|---------|--|
| Quality | | | | Comment | |
| Effort | 15 | <input type="checkbox"/> Maximal <input type="checkbox"/> Submaximal | 16 | | |
| Acceptable* <i>*meets SOP criteria: start and end of test, free from artefact</i> | 17 | <input type="checkbox"/> Yes <input type="checkbox"/> No | 18 | | |
| Repeatable <i><150ml difference best two FVC and FEV1</i> | 19 | <input type="checkbox"/> Yes <input type="checkbox"/> No | 20 | | |

| Bronchodilator | |
|-------------------------------|-----------|
| Time given (24 hour clock) | HH : HH |
| Dose of bronchodilator | _____ mcg |

| Spirometry - Post | | | | |
|---------------------------------|---------|----------|----------|-----------|
| Test | FVC | FEV1 | FEV1/FVC | FEF25-75% |
| Time | HH : HH | Comments | | |
| Total number of attempts: _____ | | | | |

| See attached results for values | | | |
|--|---|--|---------|
| Quality | | | Comment |
| Effort | <input type="checkbox"/> Maximal <input type="checkbox"/> Submaximal | | |
| Acceptable* <i>*meets SOP criteria: start and end of test, free from artefact</i> | <input type="checkbox"/> Yes <input type="checkbox"/> No | | |
| Repeatable <i><150ml difference best two FVC and FEV1</i> | <input type="checkbox"/> Yes <input type="checkbox"/> No | | |

| CO Diffusion Capacity | | | | |
|---------------------------------|--------------------|---------------------|-------------------------------|--------|
| Test | DLCO [ml/min/mmHg] | DLadj [ml/min/mmHg] | DLCO/VA (KCO) [ml/min/mmHg/L] | TLC sb |
| Time | HH : HH | Comments | | |
| Hb (Actual) | | | | |
| Total number of attempts: _____ | | | | |

| Multiple Breath Washout | | | | | |
|---------------------------------|------------|----------|----|----|-----------|
| Test | FRC litres | LCI | M0 | M1 | VT litres |
| Time | HH : HH | Comments | | | |
| Total number of attempts: _____ | | | | | |
| See attached results for values | | | | | |

| | |
|-------------------------------|-----------------|
| Date CRF completed | DD / MMM / YYYY |
| Name of person completing CRF | |
| Signature | |