



# Childhood lung function following perinatal HIV infection and early antiretroviral therapy initiation: a cross-sectional study

Andre Gie<sup>1</sup>, Julie Morrison<sup>1</sup>, David Maree<sup>2</sup>, Barbara Laughton <sup>1,3</sup>, Sara H. Browne <sup>4</sup>, Mark F. Cotton <sup>1,3</sup>, Pierre Goussard<sup>1</sup> and Steve Innes<sup>1,3,5</sup>

<sup>1</sup>Dept of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Tygerberg Hospital, Stellenbosch University, Cape Town, South Africa. <sup>2</sup>Dept of Medicine, Division of Pulmonology, Tygerberg Hospital, Stellenbosch University, Cape Town, South Africa. <sup>3</sup>Family Center for Research with Ubuntu (FAMCRU), Stellenbosch University, Cape Town, South Africa. <sup>4</sup>Dept of Medicine, University of California San Diego, San Diego, CA, USA. <sup>5</sup>Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa.

Corresponding author: Andre Gie ([agie@sun.ac.za](mailto:agie@sun.ac.za))



Shareable abstract (@ERSpublications)

Early ART benefits childhood lung function. Perinatally HIV-infected children with early ART have similar spirometry to HIV unexposed. However, they may be at risk of obstructive lung disease later in life due to small airway dysfunction. <https://bit.ly/3oIBGow>

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## Abstract

Despite the introduction of antiretroviral therapy (ART), HIV-associated pulmonary complications remain prevalent in children following perinatal HIV infection. In the post-ART era the incidence of opportunistic infections has decreased; however, non-infectious complications including diminished lung function are common. It is unclear whether early initiation of ART influences lung function later in life.

We performed a cross-sectional study examining pulmonary function tests (PFT) (spirometry, plethysmography, carbon monoxide diffusing capacity) in HIV-unexposed (HU), HIV-exposed-uninfected (HEU) and perinatally HIV-infected children on early ART (HIV<sup>+</sup>) recruited from the Cape Town arms of the CHER and IMPAACT 1060 trials. PFT was performed once children could participate (October 2013 to January 2020). Global Lung Initiative reference software was used for Z-standardisation of lung function by sex, age and height.

In total 394 children (HU n=90, HEU n=162, HIV<sup>+</sup> n=142) underwent PFT, median age 8.7 (IQR 7.7–9.8) years. HIV<sup>+</sup> had ART initiated at a median age of 17.6 (8.0–36.7) weeks. Forced expiratory volume in 1 s (FEV<sub>1</sub>), forced vital capacity (FVC) and FEV<sub>1</sub>/FVC Z-scores were similar in all groups. Plethysmography demonstrated air-trapping with increased total lung capacity (TLC), functional residual capacity, residual volume (RV) and RV/TLC Z-scores in HIV<sup>+</sup>. There were no differences in alveolar volume; however, diffusing capacity was increased in HIV<sup>+</sup>.

Our findings indicate that following perinatal HIV infection, early ART may attenuate HIV-associated lung disease and is associated with normal childhood spirometry. However plethysmography demonstrates that small airway dysfunction is more pronounced in HIV<sup>+</sup>. Longitudinal follow-up is required to assess if these children are at risk of obstructive airway disease later in life.

## Introduction

Lung disease remains a major complication of perinatal HIV infection. While antiretroviral therapy (ART) has decreased the incidence of opportunistic and recurrent infections, chronic lung disease and poor lung function persist in HIV-infected children [1–5]. Furthermore, the influence of perinatal HIV exposure on lung function later in life is not fully understood. HIV exposure without infection increases the risk of severe infection and immunological dysfunction early in life, which may disrupt lung function [6–8].

Lung involvement is thought to occur early in life with multiple mechanisms implicated. These include the human immunodeficiency virus itself, immunological dysfunction, pulmonary inflammation and respiratory infection [3, 9–11]. ART addresses several of these factors by decreasing the pulmonary viral



reservoir and limiting recurrent lung infections. Thus the manifestation of HIV-associated lung disease has changed with the introduction of ART. Lung disease is now subtle, as structural changes and pulmonary infection are being replaced by chronic functional impairment. However, despite advances in ART, lung disease remains a significant morbidity in HIV<sup>+</sup> [5, 12].

The effect of ART on HIV-associated lung disease and lung function remains unclear. There are no data examining the influence of the timing of ART initiation and childhood lung function. Available studies have examined children in whom ART was initiated late in childhood with lung function mostly performed in the second decade of life. These children had impaired lung function with small airway disease and airway obstruction being the predominant abnormalities [3, 13, 14]. This obstructive lung function phenotype tracked with age and did not improve over time [15].

Longitudinal studies of children with non-infectious lung disease suggest that a significant proportion of COPD has its origin early in life [16, 17]. Individuals who fail to reach peak expected lung function in the third decade of life are at increased risk for COPD. There is limited information on lung function in HIV<sup>+</sup> children, and current data may not reflect lung function in those who began ART early in life. Similarly, there is insufficient data to demonstrate the lung function trajectory in HIV<sup>+</sup> children and whether early ART influences this trajectory. Thus it remains to be seen whether the early introduction of ART allows for children with HIV to reach their full lung function potential.

To address the lack of knowledge on the effect of early ART and perinatal HIV exposure on childhood lung function, we compared childhood lung function in children with HIV and early ART, perinatal HIV exposure without infection, and children not exposed to HIV. Our hypothesis was that the early ART introduction would limit HIV-associated lung disease and permit normal development of childhood lung function in HIV<sup>+</sup>.

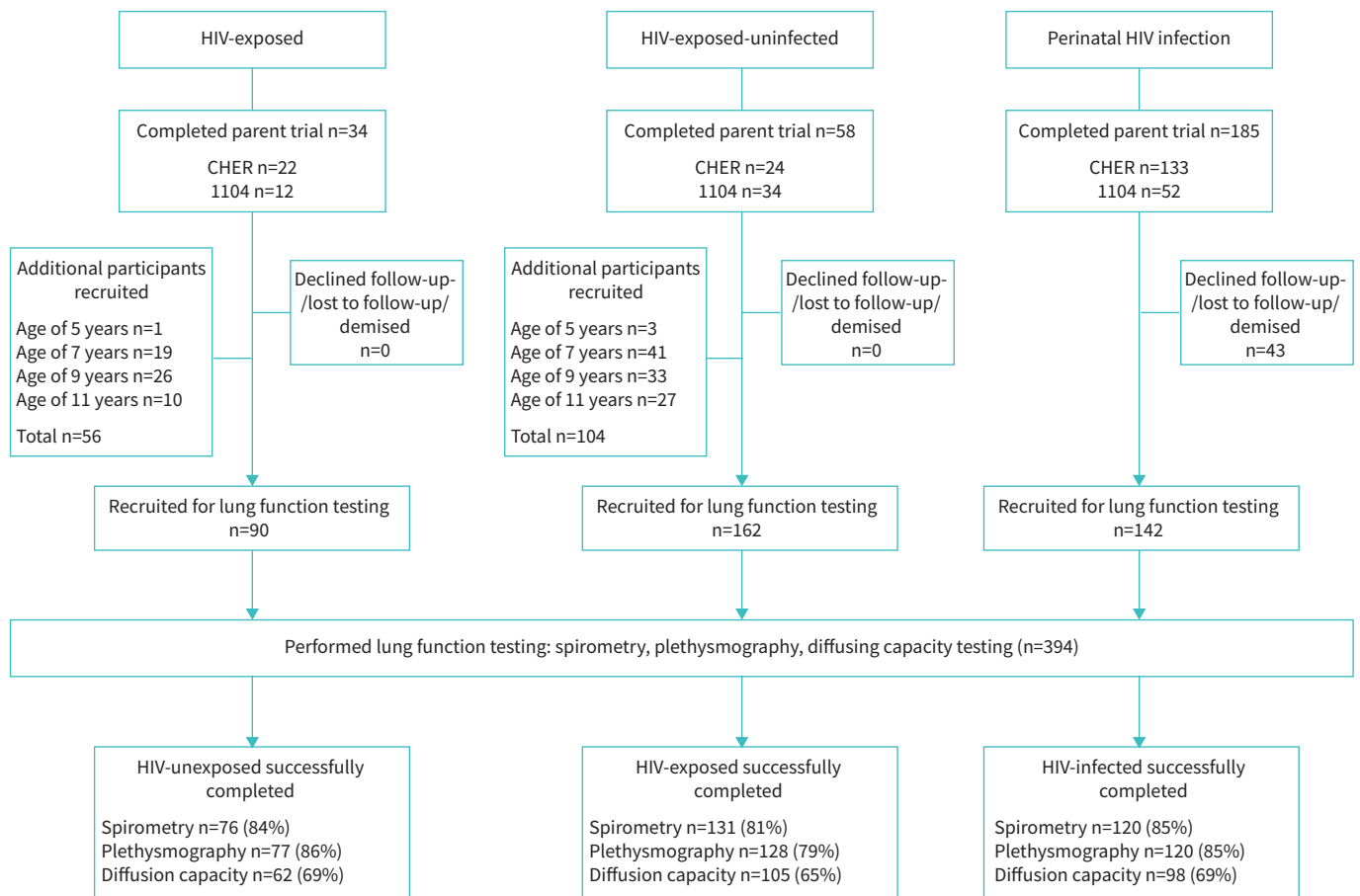
### Methodology

We analysed cross-sectional data of baseline lung function in children with HIV (HIV<sup>+</sup>), HIV-exposed-uninfected (HEU) and HIV-unexposed (HU) children at Tygerberg Children's Hospital (Cape Town, South Africa), an academic referral hospital in a middle-income country with a high prevalence of HIV infection. Children were recruited from community clinics from a similar socioeconomic, demographic and geographic population in the Cape Town metropole.

1. HIV<sup>+</sup>: perinatal HIV infection diagnosed by a positive polymerase-chain-reaction (PCR) test for HIV-1 DNA and a plasma HIV-1 RNA viral load  $>1000$  copies·mL<sup>-1</sup> at 4 weeks of age for the participants from the CHER trial [2], or in the 1060/1104s trial HIV<sup>+</sup> by having been diagnosed with HIV infection or having had an AIDS defining illness within 60 days of life, and a baseline HIV-1 RNA viral load above 5000 copies·mL<sup>-1</sup> at enrolment [18].
2. Perinatal HIV exposure (HEU): maternal HIV infection without perinatal infection of the infant. HIV infection excluded between 12 and 24 weeks of age by a negative PCR for HIV-1 DNA.
3. Control-HIV unexposed (HU); no maternal HIV infection or postnatal HIV exposure. HIV infection was excluded by PCR testing for HIV-1 DNA at 24 weeks of age.

All HIV<sup>+</sup> were previously enrolled in one of two randomised, controlled trials (CHER and IMPAACT 1060) and were recruited upon exit of the parent trial (CHER, September 2013; IMPAACT 1060, September 2015), and followed until able to participate in pulmonary function testing (PFT) [2, 18, 19]. 34 of the HU and 58 of the HEU were recruited from one of the parent trials (figure 1). Additional age-matched HU (n=56) and HEU (n=104) were recruited between October 2013 and December 2014. HU were recruited from vaccination centres and outpatient clinics that served the HIV<sup>+</sup> participants and were without known lung disease or recent lung infection. HEU were enrolled from the same household, family or community as the HIV<sup>+</sup> children; these children were included in a later neurocognitive substudy [20]. All HU and HEU participants had HIV infection excluded by ELISA for HIV antibodies upon recruitment to this study. PFT was performed annually from September 2013 to March 2020. Informed consent was obtained for all participants. In this study we report the results of the first PFT of these children. The analysis on longitudinal lung function data is ongoing.

The design, recruitment, methodology and outcomes of CHER and IMPAACT 1060 have previously been published [2, 19, 21, 22]. CHER compared early time-limited ART to ART deferred according to the 2006 World Health Organisation ART guidelines [23]. Those randomised to early time-limited arms began ART at  $<12$  weeks of age. First-line ART was lopinavir-ritonavir, zidovudine and lamivudine. Of 133 participants completing the CHER trial (2011), 31 declined study participation, moved away or had died. Of the remaining participants, 12 were lost to follow-up prior to PFT.



**FIGURE 1** Participants recruited to perform pulmonary function tests and proportion who successfully completed spirometry, plethysmography and diffusing capacity testing.

IMPAACT 1060 compared nevirapine to lopinavir-ritonavir as a third antiretroviral in addition to zidovudine and lamivudine in children with HIV between 6 months and 3 years of age [18, 19]. These children then participated in a later neurocognitive substudy.

This study was performed under the approval and oversight of Stellenbosch Human Research Ethics Committee (#N12/11/076).

### Anthropometry

Weight and height were measured at the time of PFT (supplementary methods). Weight-for-age, height-for-age Z-scores were calculated according to World Health Organization (WHO) Child Growth Standards using the WHO Anthro Survey Analyser (WHO AnthroPlus: <https://www.who.int/growthref/tools/en/>)

### Haematological investigations

HIV viral load and CD4 count was performed annually in HIV<sup>+</sup>. HIV viral suppression was defined as a HIV viral load <400 viral copies·mL<sup>-1</sup>.

### Pulmonary function testing

PFT was performed at Tygerberg Hospital by a single trained respiratory technologist, according to American Thoracic Society and European Respiratory Society standards and guidelines using the Jaeger MasterScreen-PFT and Master Screen Body with Labmanager version 5.32.0.5 (Carefusion Germany 234 GmbH, Hoechberg, Germany) [24–26]. All participants underwent spirometry to measure forced expiratory flow in 1 s (FEV<sub>1</sub>) and forced vital capacity (FVC). The best of a minimum of three acceptable spirometry manoeuvres is reported. Body plethysmography was performed to calculate lung volumes and capacities

including total lung capacity (TLC), residual volume (RV), functional residual capacity (FRC) and vital capacity ( $VC_{PLETH}$ ). Single-breath diffusion capacity testing was introduced from October 2013 to measure the alveolar volume ( $V_A$ ), diffusing capacity of the lung for carbon monoxide ( $D_{LCO}/V_A$  and  $D_{LCO}$ ).  $D_{LCO}$  was not normalised to haemoglobin concentration. The mean of a minimum of two technically acceptable body plethysmography and single-breath CO diffusing capacity tests is reported.

### Statistical analysis

Data were analysed using Prism 9.0.1 (GraphPad). Clinical and anthropometric data were summarised using descriptive statistics. Z-standardisation of lung function parameters was performed using Global Lung Initiative (GLI) reference data and software [27–29]. Z-scores of spirometry data were estimated using the ‘Other’ ethnic reference population which has been demonstrated to best fit South African participants of African and mixed ethnic origin [30]. Plethysmography and diffusing capacity Z-scores were estimated using Caucasian reference values as no data for other ethnic groups are available. QQ plots were used to visually examine the distribution of quantitative lung function data. We performed multiple comparisons between all groups: HU to HEU, HU to HIV<sup>+</sup> and HEU to HIV<sup>+</sup>. Normally distributed data were compared using one-way ANOVA with *post hoc* Tukey’s correction for multiple comparisons and is summarised as mean±SD. Data which are not normally distributed were compared using the Kruskal–Wallis test with *post hoc* Dunn’s correction for multiple comparisons and are reported as median with interquartile range. Where HIV<sup>+</sup> pulmonary function Z-score differed significantly from HU or HEU, the mean difference and 95% confidence intervals or mean rank difference were calculated. *Post hoc* linear regression was performed to examine if an association between ART initiation and pulmonary function outcome was present.

### Results

We recruited a total of 394 children for PFT (figure 1). Baseline demographic and clinical characteristics are summarised in table 1. HIV<sup>+</sup> were lighter for age than HU ( $p=0.0186$ ) and shorter for age than HEU ( $p=0.0187$ ). The anthropometry of HU and HEU was similar.

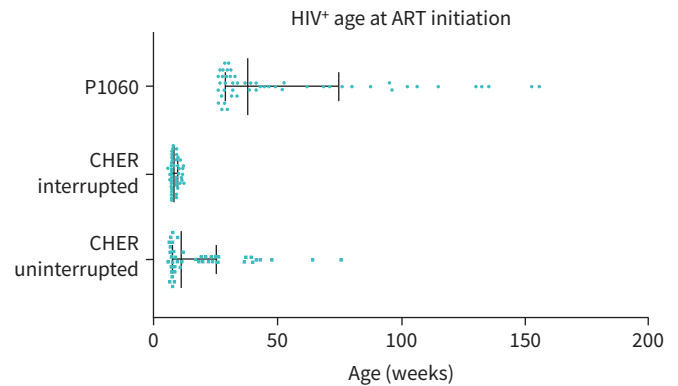
### Vertical transmission prophylaxis and ART

Mothers with HIV and their infants received vertical transmission prophylaxis (VTP) according to local guidelines. Zidovudine was administered to mothers from 34 weeks’ gestation and to neonates for 7 days with a single dose of antepartum nevirapine during labour and to the newborn infant [31]. All HIV<sup>+</sup> children received ART, and mothers with CD4 counts below 250 cells·mm<sup>-3</sup> received combination ART. The median age of ART initiation was 17.6 (range 5.86–155.86, IQR 8.0–36.7) weeks and median duration from initiation of ART to PFT was 7.65 (IQR 7.35–8.37) years (table 1). Of the CHER participants, 46 had uninterrupted ART (median age 10.86 weeks, IQR 7.57–25.50); of these 32 had early

TABLE 1 Characteristics of participants

	HIV-unexposed (HU)	HIV-exposed (HEU)	HIV-infected (HIV <sup>+</sup> )	HU versus HIV <sup>+</sup> (p-value)	HEU versus HIV <sup>+</sup> (p-value)
Subjects n	90	162	142		
Age years	8.8 (7.8–10.5)	9.0 (7.9–10.6)	8.4 (7.6–8.8)	$p=0.0004$	$p<0.0001$
Ethnicity n (%)	African 90 (100)	African 147 (91) Mixed 15 (9)	African 129 (91) Mixed 13 (9)		
Height cm	128.3 (122.5–138.8)	129.5 (124.1–139.0)	123.8 (119.0–128.5)	$p<0.0001$	$p<0.0001$
Height-for-age Z-score	$-0.56\pm 1.1$	$-0.53\pm 1.09$	$-0.87\pm 1.00$	$p=0.0848$	$p=0.0187$
Weight kg	27.75 (23.50–34.98)	27.2 (23.9–33.2)	24.3 (22.0–26.7)	$p<0.0001$	$p<0.0001$
Weight-for-age Z-score	$-0.09\pm 1.22$	$-0.22\pm 1.19$	$-0.51\pm 0.99$	$p=0.0186$	$p=0.0758$
Age at ART initiation weeks			17.6 (8.0–36.7)		
Time interval years, first ART exposure to PFT			7.65 (7.35–8.37)		
CD4 count at PFT (n=126) cells·mm <sup>-3</sup>			1065 (786–1298)		
HIV viral load at PFT (n=108) copies·mL <sup>-1</sup>			39 (19–302)		
HIV viral suppression at PFT (%)			100 (92.6)		

Data are presented as mean±SD if normally distributed, median (interquartile range) if not normally distributed, unless otherwise stated. HIV viral suppression was defined as a HIV viral load <400 viral copies·mL<sup>-1</sup>. Compared with either one-way ANOVA with *post hoc* Tukey’s multiple comparison test (normal distribution), Kruskal–Wallis with *post hoc* Dunn’s multiple comparison test (not normally distributed) and Chi-square (categorical data). No statistically significant differences between HU and HEU. ART: antiretroviral therapy; PFT: pulmonary function test.



**FIGURE 2** Age at antiretroviral therapy (ART) initiation in perinatally HIV-infected (HIV<sup>+</sup>) children. Data displayed as median (interquartile range).

ART initiation (<12 weeks of age) and 24 had deferred ART initiation (median age 33.79 weeks, IQR 22.25–41.07). 46 CHER participants had planned early interrupted ART according to the study protocol (median interruption duration 225 days, IQR 129–354) (figure 2). The median age of ART initiation of P1060 participants was 37.79 weeks (IQR 28.97–74.65) (figure 2). Viral load data were available in 108 (76.1%) with 100 being virologically suppressed (median viral load 39 copies·mL<sup>-1</sup>, IQR 19–302).

#### Lung function tests

Of the 394 recruited participants, 327 (83%) successfully completed spirometry, 325 (82%) completed plethysmography and 265 (67%) diffusing capacity (figure 1). Z-scores were calculated for lung function outcomes to account for the influence of age, height and sex and allow comparison between HU, HEU and HIV<sup>+</sup>.

#### Spirometry (table 2, figure 3)

Spirometry outcomes were similar for HU, HEU and HIV<sup>+</sup> with similar mean Z-score of FEV<sub>1</sub>, FVC or FEV<sub>1</sub>/FVC and no statistically significant difference between any of the groups for FEV<sub>1</sub>, FVC or FEV<sub>1</sub>/FVC.

#### Plethysmography (table 2, figure 4)

TLC Z-score was higher in HIV<sup>+</sup> than HU (mean difference 0.5101, 95% CI 0.1381–0.8820, p=0.0039) and HEU (mean difference 0.6966, 95% CI 0.3722–1.021, p<0.0001). RV Z-score was higher in HIV<sup>+</sup> than HU (mean difference 0.3372, 95% CI 0.09434–0.5801, p=0.0034) and HEU (mean difference 0.3246, 95% CI 0.1127–0.5365, p=0.001). The mean RV/TLC Z-score was significantly higher in HIV<sup>+</sup> than HU (mean difference 0.3341, 95% CI 0.01584–0.6524, p=0.0371) but not HEU (mean difference 0.2431, 95% CI -0.03450–0.5208, p=0.0995). RV/TLC Z-score was similar in HU and HEU. Furthermore, FRC Z-scores were higher in HIV<sup>+</sup> than HU (mean difference 0.3840, 95% CI 0.03799–0.6579, p=0.0234). The increase in TLC, RV and FRC Z-scores, along with significantly higher RV/TLC in HIV<sup>+</sup>, indicates air-trapping (figure 4). Earlier ART initiation was associated with increased TLC Z-score (R<sup>2</sup>=0.081, p=0.0016); however, there was no correlation between the timing of ART initiation and Z-scores of RV (R<sup>2</sup>=0.028, p=0.071), RV/TLC (R<sup>2</sup>=0.027, p=0.073) or FRC (R<sup>2</sup>=0.017, p=0.155) (supplementary figure S1).

#### Single-breath diffusion capacity testing (table 2)

HIV<sup>+</sup> had significantly higher D<sub>LCO</sub> Z-score than HU (mean rank difference 31.05, p=0.0376) and HEU (mean rank difference 35.81, p=0.0026). The D<sub>LCO</sub> Z-score was similar in HU and HEU. No correlation between timing of ART initiation and D<sub>LCO</sub> Z-score in HIV<sup>+</sup> was present (r=-0.125, p=0.22). There was no statistically significant difference in V<sub>A</sub> Z-scores between groups.

#### Discussion

In this study, we demonstrate that following perinatal HIV infection, forced expiratory lung function and diffusing capacity are preserved in HIV<sup>+</sup> children in whom ART is initiated early in life. However, plethysmography findings indicate that small airway obstruction continues to occur in children with HIV despite early ART.

**TABLE 2** Successfully completed lung function tests of HIV-unexposed (HU), HIV-exposed-uninfected (HEU) and perinatally HIV-infected children on early ART (HIV<sup>+</sup>)

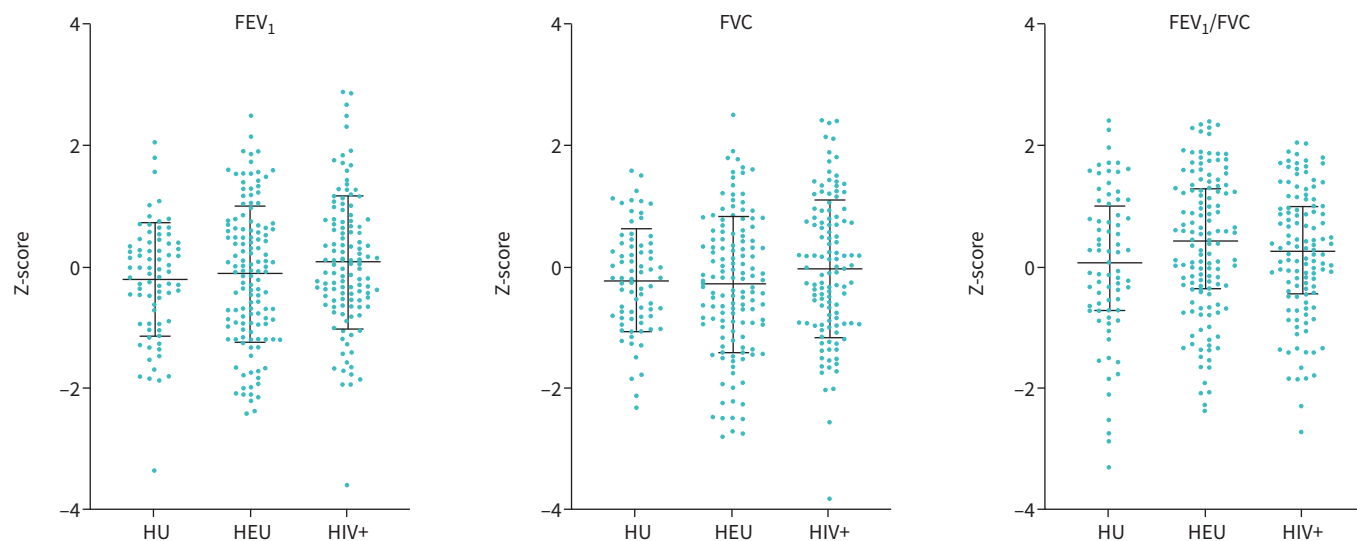
	HU	HEU	HIV <sup>+</sup>	HU versus HIV <sup>+</sup> (p-value)	HEU versus HIV <sup>+</sup> (p-value)
<b>Spirometry</b>					
Subjects n	76	131	120		
FEV <sub>1</sub> absolute L	1.380 (1.205–1.648)	1.430 (1.200–1.710)	1.250 (1.110–1.450)	p=0.0057	p=0.0001
FEV <sub>1</sub> Z-score	-0.892±0.932	-0.786±1.125	-0.570±1.083	p=0.1001	p=0.2456
FVC absolute L	1.550 (1.32–1.813)	1.570 (1.340–1.860)	1.355 (1.195–1.598)	p=0.0009	p=0.0002
FVC Z-score	-0.894±0.898	-0.953±1.196	-0.669±1.207	p=0.3695	p=0.1199
FEV <sub>1</sub> /FVC absolute	0.89 (0.853–0.940)	0.914 (0.863–0.962)	0.909 (0.879–0.954)	p=0.1771	p>0.9999
FEV <sub>1</sub> /FVC Z-score	-0.031±1.284	0.301±1.205	0.1551±1.076	p=0.5297	p=0.5891
<b>Plethysmography</b>					
Subjects n	77	128	120		
Total lung capacity (TLC) L	2.481±0.487	2.467±0.561	2.258±0.3524	p=0.0041	p=0.0018
TLC Z-score	-0.2194±1.050	-0.406±1.178	0.2906±0.9855	p=0.0039	p<0.0001
Residual volume (RV) L	0.8612±0.2584	0.8746±0.2537	0.8734±0.2257	p=0.9373	p=0.9992
RV Z-score	0.6125±0.7607	0.6251±0.7185	0.9497±0.6517	p=0.0034	p=0.001
RV/TLC	34.75±8.153	35.56±9.210	38.69±8.441	p=0.0058	p=0.0139
RV/TLC Z-score	1.399±0.8341	1.489±1.059	1.733±0.8190	p=0.0371	p=0.0995
Functional residual capacity (FRC) L	1.28 (1.13–1.55)	1.33 (1.15–1.59)	1.18 (1.06–1.36)	p=0.0135	p=0.0001
FRC Z-score	0.3676±0.9391	0.4456±0.8829	0.7156±0.8926	p=0.0234	p=0.0504
Vital capacity (VC <sub>PLETH</sub> ) L	1.540 (1.285–1.860)	1.530 (1.290–1.825)	1.380 (1.195–1.598)	p=0.0011	p=0.0008
VC <sub>PLETH</sub> Z-score	-2.179±0.8135	-2.281±1.061	-2.157±1.077	p=0.9884	p=0.6037
<b>Diffusing capacity</b>					
Subjects n	62	105	98		
D <sub>LCO</sub>	14.75 (13.22–16.87)	14.77 (12.69–17.01)	13.93 (12.46–16.09)	p=0.1802	p=0.2837
D <sub>LCO</sub> Z-score	0.231 (-0.25–0.76)	0.176 (-0.38–0.82)	0.50 (0.01–1.28)	p=0.0375	p=0.0026
Alveolar volume (V <sub>A</sub> ) L	2.370 (2.068–2.800)	2.380 (2.095–2.790)	2.11 (1.938–2.315)	p=0.0005	p<0.0001
V <sub>A</sub> Z-score	-0.44 (-1.15–0.18)	-0.34 (-1.0–0.52)	-0.19 (-0.68–0.36)	p=0.1480	p>0.9999
D <sub>LCO</sub> /V <sub>A</sub>	6.400 (5.788–6.783)	6.010 (5.395–6.680)	6.565 (6.005–7.230)	p=0.1564	p<0.0001

Data are presented as mean±SD if normally distributed, median (interquartile range) if not normally distributed, unless otherwise stated. No statistically significant differences between HU and HEU. ART: antiretroviral therapy; FEV<sub>1</sub>: forced expiratory volume in 1 s; FVC: forced vital capacity; D<sub>LCO</sub>: diffusing capacity of the lung for carbon monoxide.

Spirometry was similar in all groups indicating that the lung health was generally good without severe lung disease or function impairment. Expiratory flow and volume were similar in HU and HEU demonstrating that perinatal HIV and antiretroviral exposure for VTP does not disrupt the development of childhood lung function despite an increased risk of pneumonia in childhood [7]. Encouragingly, unlike previous PFT studies where ART was initiated later in childhood, there was no indication of expiratory airflow limitation in HIV<sup>+</sup> [13, 32, 33]. In contrast to our study, participants in previous childhood PFT studies were older at HIV diagnosis (5 to 12 years), ART initiation (5 to 12 years) and lung function testing (11 to 14 years) [13, 14, 32–34]. Airflow limitation with decreased FEV<sub>1</sub> and FEV<sub>1</sub>/FVC was more common and severe in children with delayed HIV diagnosis [33]. Our study findings suggest that in low- and middle-income countries, early diagnosis of perinatal HIV infection and initiation of ART may attenuate airflow limitation later in life in children with HIV infection.

The lack of expiratory flow and volume abnormalities may be due to the limited sensitivity of spirometry to detect small airway obstruction in children [35, 36]. Plethysmography can detect air-trapping in the absence of abnormal expiratory flow and may be more sensitive to detect small airway dysfunction in children than spirometry [37]. The use of plethysmography allowed our study to identify increased static RV and RV/TLC, features of small airway obstruction not found on spirometry. The mean VC<sub>PLETH</sub> of all groups was lower than expected. It is unclear whether this represents airflow obstruction in all groups or whether this is a limitation of the reference values that were used for this study. Small airway obstruction may be due to small airway injury or reactive airways. Air-trapping was significantly more severe in HIV<sup>+</sup>. Apart from air-trapping due to small airway obstruction, increased RV/TLC is found with certain structural abnormalities including cysts and bullae. However, given the limited abnormal spirometry and increased RV/TLC in all groups, significant structural lung disease is unlikely. We speculate that environmental factors such as exposure to air pollution or the burning of bio-fuels may have led to small airway injury in

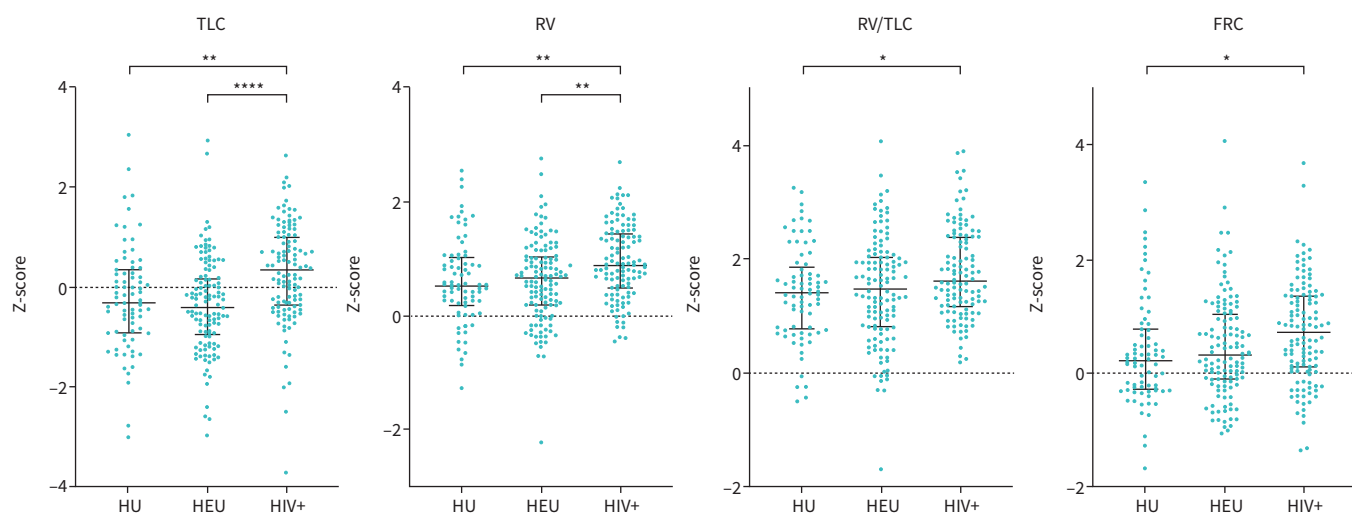




**FIGURE 3** Spirometry ( $FEV_1$ , FVC,  $FEV_1/FVC$ ) Z-scores of HIV-unexposed (HU), HIV-exposed-uninfected (HEU) and perinatally HIV-infected ( $HIV^+$ ) children. Z-scores calculated using Global Lung Initiative reference data and software. Normally distributed (mean $\pm$ SD), compared with ordinary one-way ANOVA with Tukey *post hoc* multiple comparison test, non-normally distributed data (median (interquartile range)) compared with Kruskal–Wallis test and *post hoc* Dunn's correction for multiple comparisons.  $FEV_1$ : forced expiratory volume in 1 s; FVC: forced vital capacity.

our study population and that  $HIV^+$  exaggerates this injury. Furthermore, our findings indicate that in children at risk of obstructive airway pathology, spirometry may not detect early small airway disease; therefore advanced PFT such as plethysmography should be considered.

The results of diffusing capacity testing were unexpected.  $D_{LCO}$  was higher in  $HIV^+$  despite a similar  $V_A$ . A potential explanation for this is increased pulmonary blood volume, either due to raised haemoglobin or increased alveolar capillary density. Given the good overall lung health in our population, pulmonary haemorrhage and intra-alveolar blood is highly unlikely. In contrast to our study,  $D_{LCO}$  in  $HIV^+$  children with late ART initiation (mean age 5 years) was significantly lower than HU [13]. Interpretation of  $D_{LCO}$  in  $HIV^+$  children is complicated by the paucity of data. Future studies should examine the diffusing capacity in  $HIV^+$  and include measurements of alveolar capillary volume and haemoglobin at the time of testing.



**FIGURE 4** Plethysmography Z-scores for HIV-unexposed (HU), HIV-exposed-uninfected (HEU) and  $HIV^+$  children. Data displayed as mean $\pm$ SD, comparison of groups with ordinary one-way ANOVA and Tukey *post hoc* multiple comparison test. Single data point of HEU Z-score for TLC and RV/TLC is not included in the figure as  $Z < -5$  and  $Z > 7$ , respectively. TLC: total lung capacity; RV: residual volume, FRC: functional residual capacity. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ .

The longitudinal lung function trajectory in children with HIV is unclear. It is not known whether children with HIV follow a lower trajectory throughout life, have a plateau or have a more rapid decline of lung function. A longitudinal study in a similar population to ours, median age of 4.3 years at ART initiation, demonstrated that children with HIV follow a similar, albeit lower, spirometry trajectory to HU children [15]. However, these children started with diminished lung function, and to date, no longitudinal studies have examined lung function following the early initiation of ART. Our study adds valuable lung function data of HIV<sup>+</sup> children in the first decade of life. We found that spirometry is not disrupted by HIV infection, provided ART is initiated in the first years of life. Thus, the children in our study have a similar start of their lung function trajectory to HU and the opportunity to follow a normal trajectory, potentially allowing children with HIV to reach their full lung function potential.

ART was initiated early relative to practice at the time of the parent studies. However, a number of children in the CHER deferred ART arm and P1060 trial initiated ART in the second and third year of life and may have suffered long-term sequelae. Owing to the limited number of children in our study initiating ART after the first year of life, we were not able to compare lung function of children initiated on ART in the first year of life to those with later ART initiation. Future cohort trials should compare lung function of children with HIV with early ART initiation to those with initiation in the second year of life to provide a better understanding of the influence of timing of ART initiation and childhood lung function. Specifically studies should examine whether extremely early initiation of ART, within the first days of birth, improves childhood lung function. Studies of extremely early ART have shown encouraging results to decrease the HIV viral reservoir; however, there are no data on the lung function of these children [38, 39].

In order to account for the influence of age, height and sex on pulmonary function we performed Z-standardisation using GLI reference data. The Z-standardisation of PFT parameters allows for the comparison of lung function between groups and potentially between different studies. The generalisation of normal lung function as calculated by the GLI reference data is limited by the lack of reference data for all ethnic groups. However, we were cautious to define lung disease based on the upper and lower limits of normal ( $Z < -1.64$ ,  $Z > 1.64$ ) of a reference group different to our study population. Furthermore, only Caucasian reference data are available for plethysmography and diffusing capacity, limiting the capability to comment on normality in these lung functions.

Our study had numerous strengths. We recruited a large number of participants from the same population and were able to compare lung function of HIV<sup>+</sup> to HE and HU to account for the effect of perinatal HIV and VTP exposure on childhood lung function. We performed extensive lung function testing to assess forced expiratory manoeuvres, static lung volumes and diffusing capacity testing giving unique insights into lung physiology and function.

Limitations include the lack of data of clinical information including respiratory symptoms and disease, antenatal and second-hand tobacco smoke exposure and the limited HU participants recruited in infancy, preventing examination of the influence of early life pulmonary infection on childhood lung function. A further limitation is that HIV PCR testing was not performed within the first 24 h of birth, inhibiting the discrimination between intrauterine and intra-partum HIV infection. Finally, the heterogeneous HIV<sup>+</sup> population of our study due to the different inclusion and ART initiation criteria of CHER and IMPAACT 1060 limits the insight into the correlation of timing of ART initiation and childhood lung function and may have introduced selection and survival bias. Specifically, this study is not able to account for the influence of lung infection and tuberculosis, for which HIV<sup>+</sup> are at increased risk, on lung function.

### Conclusion

In this study we demonstrate that early ART initiation, following perinatal HIV infection, is associated with normal childhood spirometry with no forced expiratory airflow deficit. However, despite early ART, small airway dysfunction is more pronounced in HIV<sup>+</sup> than HIV-exposed and HIV-unexposed children. Longitudinal examination of lung function is required to evaluate whether airflow limitation develops later in life.

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