Early View

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

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Take home message 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.

Abstract:

Despite the introduction of 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+) 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-standardization of lung function by sex, age and height.

In total 394 children (HU n=90, HEU n=162, HIV+ n=142) underwent PFT, median age 8.7 (IQR 7.7-9.8) years. HIV+ had ART initiated at a median age of 17.6 (8.0-36.7) weeks. FEV₁, FVC, FEV₁/FVC, Z-scores were similar in all groups. Plethysmography demonstrated air-trapping with increased total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV) and RV/TLC Z-scores in HIV+. There were no differences in alveolar volume, however diffusing capacity was increased in HIV+.

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+. 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+ [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 chronic obstructive airway disease (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+ 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+ 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+.

Methodology:

We analyzed cross-sectional data of baseline lung function in children with HIV (HIV+), 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 socio-economic, demographic and geographic population in the Cape Town metropole.

1. HIV+: 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 per milliliter at 4 weeks of age for the participants from the CHER trial [2], or in the 1060/1104s trial HIV+ 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 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+ and were previously enrolled in one of two randomized, 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-December 2014. HU were recruited from vaccination centers and outpatient clinics that served the HIV+ participants and were without known lung disease or recent lung infection. HEU were enrolled from the same household, family or community as the HIV+, these children were included in a later neurocognitive substudy [20]. All HU and HEU participants had HIV infection excluded by enzyme-linked immunosorbent assay (ELISA) for HIV antibodies upon recruitment to this study. PFT was performed annually from September 2013-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 on going.

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 deferred ART deferred according the 2006 World Health Organization ART guidelines[23]. Those randomized to early time-limited arms, began ART <12 weeks of age. First-line ART was lopinavir-ritonavir, zidovudine and lamivudine. Of 133 participants completing 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.

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 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+. HIV viral suppression was defined as a HIV viral load < 400 viral copies per milliliter.

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, 97204 Hoechberg, Leibnizstrasse 7) [24–26]. All participants underwent spirometry to measure forced expiratory flow in 1 second (FEV₁) and forced vital capacity (FVC). The best of a minimum of 3 acceptable spirometry maneuvers is reported. Body plethysmography was performed to calculate lung volumes and capacities including, total lung capacity (TLC), residual volume (RV), functional residual capacity (FRC), vital capacity (VC_{PLETH}). Single breath diffusion capacity testing was introduced from October 2013 to to measure the alveolar volume (VA), DLCO/VA and diffusing capacity of the lung for carbon monoxide (DLCO). DLCO has not normalized to haemoglobin concentration. . The mean of a minimum of 2 technically acceptable body plethysmography and single breathe CO diffusing capacity tests are reported.

Statistical analysis: Data were analyzed using Prism 9.0.1 (Graphpad). Clinical and anthropometric data was summarized using descriptive statistics. Z-standardization of lung function parameters was performed using Global Lung Initiative (GLI) reference data and software [27–29]. Z-scores of spirometry data was estimated using the “Other” ethnic reference population which has been demonstrated to best fit South African participants of african and mixed ethnic origin [30]. [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+, HEU to HIV+. Normally distributed data was compared using one-way ANOVA with post-hoc Tukey’s correction for multiple comparisons, and is summarized as mean with standard deviation. Data which are not normally distributed was 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+ pulmonary function Z -score differed significantly from HU or HEU, the mean difference and 95% confidence intervals or mean rank difference was 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 summarized in table 1. CWH 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 (VTP) and ART: Mothers with HIV and their infants received 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+ children received ART, mothers with CD4 counts below 250 cells/ml 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 pulmonary function testing 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 ART initiation (< 12 weeks of age), 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 (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+.

Spirometry: (table 2, figure 3)

Spirometry outcomes were similar for HU, HEU, HIV+ with similar mean Z-score of FEV₁, FVC, or FEV₁/FVC and no statistically significant difference between any of the groups for FEV₁, FVC, or FEV₁/FVC.

Plethysmography (table 2, figure 4)

TLC Z-score was higher in HIV+ than HU (mean difference 0.5101, 95% CI 0.1381 to 0.8820, p=0.0039) and HEU (mean difference 0.6966, 95% CI 0.3722 to 1.021, p<0.0001). RV Z-score was higher in HIV+ than HU (mean difference 0.3372, 95% CI 0.09434 to 0.5801, p=0.0034) and HEU (mean difference 0.3246, 95% CI 0.1127 to 0.5365, p=0.001). The mean RV/TLC Z-score was significantly higher in HIV+ than HU (mean difference 0.3341, 95% CI 0.01584 to 0.6524, p=0.0371) but not HEU (mean difference 0.2431, 95% CI -0.03450 to 0.5208, p=0.0995). RV/TLC Z-score was similar in HU and HEU. Furthermore, FRC Z-scores was higher in HIV+ than HU (mean difference 0.3840, 95% CI 0.03799 to 0.6579, p=0.0234). The increase in TLC, RV and FRC Z-scores, along with significantly higher RV/TLC in HIV+ indicates air trapping (figure 4). Earlier ART initiation was associated with increased TLC Z-score ($R^2 = 0.081$, p=0.0016), however there was no correlation between the timing of ART initiation and Z-scores of RV ($R^2 = 0.028$, p= 0.071)), RV/TLC ($R^2 = 0.027$, p=0.073)) or FRC ($R^2 = 0.017$, p=0.155)) (supplementary figure 1).

Single breath Diffusion Capacity testing: (table 2)

HIV+ had significantly higher DL_{CO} Z-score than HU (mean rank difference 31.05, p=0.0376) and HEU (mean rank difference 35.81, p=0.0026). The DL_{CO} Z-score was similar in HU and HEU. No correlation between timing of ART initiation and DL_{CO} Z-score in HIV+ was present (r=-0.125, p=0.22). There was no statistically significant difference in V_A 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+ 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.

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 was similar in HU and HE 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+ [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 FEV₁ and FEV₁/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_{pleth} 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+. 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 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 pulmonary function testing such as plethysmography should be considered.

The results of diffusing capacity testing were unexpected. DL_{CO} 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, DL_{CO} in HIV+ children with late ART initiation (mean age 5 years) was significantly lower than HU [13]. Interpretation of DL_{CO} 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.

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 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+ 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 2nd and 3rd year of life and may have suffered long term sequelae. Due 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-standardization using GLI reference data. The Z-standardization of PFT parameters allows for the comparison of lung function between groups and potentially between different studies. The generalization 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 is 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 CWH 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 maneuvers, 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 hours of birth, inhibiting the discrimination between intrauterine and intra-partum HIV infection. Finally, the heterogeneous HIV+ 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 confounding the results. Specifically this study is not able to account for the influence of lung infection and tuberculosis, for which CWH 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+ 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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	HIV unexposed (HU) n=90	HIV exposed (HEU) n=162	HIV infected (HIV+) n=152	HU vs. HIV+ (p-value)	HEU vs. HIV+ (p-value)
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 (%)	African n=90 (100)	African n=147 (91) Mixed n=15 (9)	African n=129 (91) Mixed n=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 (1.1)	-0.53 (1.09)	-0.87 (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 (1.22)	-0.22 (1.19)	-0.51 (0.99)	p=0.0186	p=0.0758
Age at ART initiation (weeks)			17.6 (8.0-36.7)		
Time interval, first ART exposure to PFT (years)			7.65 (7.35-8.37)		
CD4 count at PFT (n=126)			1065 (786-1298)		
HIV viral load at PFT n=108 (copies/ml)			39 (19-302)		
HIV viral suppression at PFT (%)			100 (92.6)		

Table 1: Characteristics of participants. Data are mean (SD) if normally distributed, median (IQR) if not normally distributed. HIV viral suppression was defined as a HIV viral load < 400 viral copies per milliliter. 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), Chi-square (categorical data). No statistically significant differences between HU and HEU.

Spirometry					
	HU (n=76)	HEU (n=131)	HIV+ (n=120)	HU vs. HIV+	HEU vs. HIV+
FEV ₁ 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 ₁ 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 ₁ /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 ₁ /FVC Z-score	-0.031 (1.284)	0.301 (1.205)	0.1551 (1.076)	p=0.5297	p=0.5891
Plethysmography					
	HU (n=77)	HEU (n=128)	HIV+ (n=120)	HU vs. HIV+	HEU vs. HIV+
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 _{PLETH})(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 _{PLET} Z-score	-2.179 (0.8135)	-2.281 (1.061)	-2.157 (1.077)	p=0.9884	p=0.6037
Diffusing Capacity					
	HU (n=62)	HEU (n=105)	HIV+ (n=98)	HU vs. HIV+	HEU vs. HIV+

DL _{CO}	14.75 (13.22-16.87)	14.77 (12.69-17.01)	13.93 (12.46-16.09)	p=0.1802	p=0.2837
DL _{CO} Z-score	0.231 (-0.25 to 0.76)	0.176 (-0.38 to 0.82)	0.50 (0.01-1.28)	p=0.0375	p=0.0026
Alveolar volume (V _A)	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 _A Z-score	-0.44 (-1.15 to 0.18)	-0.34 (-1.0 to 0.52)	-0.19 (-0.68 to 0.36)	p=0.1480	p>0.9999
DLCO/ V _A	6.400 (5.788-6.783)	6.010 (5.395-6.680)	6.565 (6.005-7.230)	p=0.1564	p<0.0001

Table 2: Successfully completed lung function tests of HIV unexposed (HU), HIV-exposed uninfected (HEU) and perinatally HIV infected child early ART (HIV+). Data are mean (SD) if normally distributed, median (IQR) if not normally distributed. No statistically significant differences between HU and HEU

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Figure legends:

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

Figure 2: Age at antiretroviral therapy (ART) initiation in perinatally HIV infected (HIV+) children. Data displayed as median (IQR).

Figure 3: Spirometry (FEV₁, FVC, FEV₁/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 (SD)), compared with ordinary one-way ANOVA with Tukey post hoc multiple comparison test, non-normal distributed data (median (IQR)) compared with Kruskal-Wallis test and post-hoc Dunn's correction for multiple comparisons. FEV₁=forced expiratory volume in 1 second, FVC= forced vital capacity

Figure 4: Plethysmography Z-scores for HIV-unexposed (HU), HIV-exposed uninfected (HEU) and HIV+ children. Data displayed as mean (SD), comparison of groups with ordinary one-way ANOVA and Tukey post hoc multiple comparison test. Single data point of TLC, RV/TLC in HEU not included in figure as, $Z < -5$, $Z > 7$ respectively. TLC=total lung capacity, RV= residual volume, FRC= functional residual capacity, * $p < 0.05$, ** $p < 0.001$

Supplementary figure: Linear regression of timing of antiretroviral therapy initiation and childhood pulmonary function outcome following perinatal HIV infection. Data displayed as individual values with regression line and 95% confidence intervals.

Supplementary figure: Correlation between timing of ART and plethysmography outcomes in perinatally HIV infected infants

Supplementary methodology: Childhood lung function following perinatal HIV infection and early antiretroviral initiation; a cross-sectional study

Anthropometry was performed using standardized methods prior to lung function testing.

- 1) Weight was measured with a calibrated scale with the participant wearing light underclothes.
- 2) Standing height was measured with a stadiometer when the participant was not wearing shoes.