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Longitudinal controlled attenuation parameter and liver stiffness in children with and without perinatal HIV infection in South Africa

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Objectives: Metabolic dysfunction-associated steatotic liver disease (MASLD) is an emerging cause of liver disease in HIV. Transient elastography (TE) with controlled attenuation parameter (CAP) measures liver stiffness as a marker of liver fibrosis and CAP as a measure of hepatic steatosis. Our aim was to evaluate longitudinal CAP and liver stiffness in children with perinatally acquired HIV (PHIV) on antiretroviral therapy (ART) from early life compared to children without HIV (HU).

Design: Prospective cohort study.

Methods: PHIV and HU were followed annually for two years. During the study, 60% of PHIV switched from older ART regimens to tenofovir disoproxil, lamivudine and dolutegravir (TLD). Longitudinal evolution of CAP and liver stiffness were investigated in two PHIV groups – on older ART and on TLD – compared to HU children using linear mixed effects models.

Results: 263 children and adolescents (112 PHIV, 151 HU) aged 7–20 years were followed. PHIV on older ART had CAP 8.61% (95% CI 4.42–12.97, $P < 0.001$) greater than HU and no significant difference in CAP between PHIV on TLD and HU. No significant difference in liver stiffness was found between PHIV on older ART regimens and PHIV on TLD compared to HU.

Conclusion: PHIV on older ART had higher CAP than HU, whereas in PHIV switched to TLD there was no difference in CAP compared to HU. There was no difference in liver stiffness between either PHIV group and HU. This suggests starting ART early in life might protect PHIV from developing hepatic fibrosis.

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Keywords: fatty liver, hepatic steatosis, lean, nonalcoholic fatty liver disease, paediatric

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Introduction

With the success of antiretroviral therapy (ART) in reducing mortality in children and adolescents living with perinatally acquired HIV (PHIV), chronic HIV-associated comorbidities are increasingly recognized [1]. Liver disease is the leading cause of non-HIV-related death in adults living with HIV, with steatotic liver disease emerging as an important contributor. The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) – previously known as nonalcoholic fatty liver disease – is higher in adults with HIV, with more rapid progression to steatohepatitis and fibrosis in leaner and more physically active individuals [2–4]. In PHIV, HIV infection and ART exposure are lifelong, which may lead to earlier onset of chronic HIV-associated comorbidities. Chronic systemic inflammation may lead to hepatic injury and contribute to development and progression of MASLD in HIV [4,5]. We previously found that PHIV switching to dolutegravir-containing ART regimens had reduced hepatic steatosis and lowered fasting triglycerides and cholesterol than PHIV remaining on older ART regimens with either protease inhibitors (PI) or nonnucleoside reverse transcriptase inhibitors (NNRTI) and that both zidovudine and lopinavir/ritonavir were associated with hepatic steatosis [6,7].

Although the pathophysiology of MASLD in HIV is not well defined, traditional risk factors such as obesity, dyslipidaemia and insulin resistance are important, regardless of HIV status. HIV-specific risk factors identified in adults include HIV viraemia, nucleoside reverse transcriptase inhibitor (NRTI) use and both increased or decreased CD4 counts, and may also be risk factors in children and adolescents with HIV [8–10]. In PHIV, the prevalence of MASLD ranges from 8 to 33% with identified risk factors being increased waist circumference, higher BMI and insulin resistance [11–14]. There is limited data available on MASLD in PHIV and no longitudinal data on changes over time.

Transient elastography (TE) is a rapid ultrasound-based technology that measures liver stiffness as a surrogate marker for liver fibrosis [15–18]. TE uses a transducer to generate an elastic shear wave in the liver and measures the speed of shear wave transmission to evaluate liver stiffness. Controlled attenuation parameter (CAP) measures attenuation of the ultrasound signal, which increases as fatty infiltration of liver tissue accumulates (73–76). CAP is more sensitive than ultrasound, which only detects moderate to severe steatosis [19]. An important limitation of noninvasive imaging including TE with CAP is lower sensitivity for early diagnosis of hepatic steatosis. TE has been validated in patients living with HIV and recently released South African guidelines recommend using both TE and CAP to screen those on long-term ART for hepatic steatosis and fibrosis [20].

The aim of this study was to evaluate the longitudinal trajectory of CAP and liver stiffness over a two year period in PHIV after early ART initiation compared to children without HIV (HU). We split PHIV measurements further into two exposure groups based on ART regimen at the time of the measurement. PHIV Group 1 comprised measurements when a child was on older ART regimens – nucleoside reverse transcriptase inhibitor/nonnucleoside reverse transcriptase inhibitor/protease inhibitor (NRTI/NNRTI/PI), – and PHIV Group 2 comprised measurements when on combination tenofovir disoproxil fumarate, lamivudine and dolutegravir (TLD). Each PHIV group was compared to HU.

Methods

We conducted a longitudinal prospective observational study. From April 2019 to October 2021, adolescents on ART were recruited from an existing cohort of PHIV and HU controls followed at the Family Centre for Research with Ubuntu (FAMCRU) at Tygerberg Hospital in Cape Town, South Africa. Participants were followed annually for two years; however, recruitment and follow-up were interrupted from March to October 2020 due to the COVID-19 pandemic and national lockdown. Follow-up assessments were performed from November 2020 to August 2023.

Adolescents had previously participated in the Children with HIV Early antiRetroviral (CHER) and P1060 clinical trials [21–23], along with HU from the same communities. The CHER trial compared early time limited ART until 1 or 2 years age to deferred ART as per standard of care at the time in infants with perinatal HIV 6–12 weeks of age. First-line ART was lopinavir-ritonavir, zidovudine and lamivudine. P1060, conducted through the International Maternal Paediatric Adolescent AIDS Clinical trial (IMPAACT) group, compared nevirapine to lopinavir-ritonavir as a third antiretroviral, in PHIV enrolling between 6 months and 3 years of age. All received zidovudine and lamivudine.

At enrolment in the present study, ART included an NRTI in 110 (98%); an NNRTI in 18 (16%); a PI in 89 (79%); and an integrase strand transfer inhibitor (INSTI) in 11 (10%) children and adolescents (Table 1). During the study, 67 (60%) children switched to TLD, in line with the 2019 ART Clinical Guidelines of the South African Department of Health [24].

All PHIV initiated ART in early childhood and historical clinical data was available since birth. Children without HIV were either perinatally HIV-exposed but uninfected or perinatally HIV-unexposed and came from a vaccine study linked to the CHER trial or from a neurocognitive substudy following P1060.

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Table 1. Baseline characteristics of the cohort, by HIV group. Median (IQR) or *n* (%), and *P*-values (chi-squared/*F*-test) are reported.

	PHIV ($n=112$)	HU ($n=151$)	*P*-value
Age:			
Age at entry (years) – median (IQR)	14.3 (13.2, 14.9)	12.8 (11.4, 14.8)	<0.001
Sex:			
Male, *n* (%)	53 (47)	81 (54)	0.416
Ethnicity:			
African, *n* (%)	101 (90)	101 (67)	<0.001
Mixed ethnicity, *n* (%)	11 (10)	50 (33)	
CAP			
CAP – median (IQR)	195 (173, 223)	182 (160, 203	<0.001
Children with hepatic steatosis, *n* (%)	10 (9)	4 (3)	0.049
Liver stiffness			
Liver stiffness – median (IQR)	5.0 (4.4, 5.9)	4.8 (4.4, 5.7)	0.257
Children with hepatic fibrosis (>7 kPa), *n* (%)	8 (7)	2 (1)	0.036
Metabolic indicators			
Weight – median (IQR) (kg)	46.0 (39.6, 51.3)	45.9 (35.1, 52.4)	0.734
Waist circumference – median (IQR) (cm)	66.6 (62.4, 72.6)	65.7 (61.1, 72.3)	0.892
BMI – median (IQR)	19.2 (17.3, 21.3)	18.3 (16.6, 21.1)	0.373
BMI *z*-score – median (IQR)	-0.02 (-0.81, 0.66)	0.01 (−0.71, 0.76)	0.576
HOMA – median (IQR)	2.20 (1.50, 3.60)	1.59 (1.10, 2.90)	<0.001
ALT (U/l) – median (IQR)	16 (12, 21)	13 (10, 16)	<0.001
AST (U/l) – median (IQR)	24 (21, 29)	23 (20, 27)	0.01
Insulin (mIU/l) – median (IQR)	10.65 (7.70, 16.75)	7.80 (5.45, 14.30)	<0.001
Cholesterol (mmol/l) – median (IQR)	4.11 (3.61, 4.71)	3.80 (3.29, 4.27)	<0.001
HDL (mmol/l) – median (IQR)	1.38 (1.20, 1.60)	1.39 (1.19, 1.61)	0.800
LDL (mmol/l) – median (IQR)	2.23 (1.83, 2.77)	2.11 (1.72, 2.54)	0.022
PLT ($\times 10^9$/l) – median (IQR)	331 (277, 393)	325 (261, 360)	0.178
$CD4^+$ cell count (cells/mm^3)			
<200, *n* (%)	0 (0)	–	
200–499, *n* (%)	9 (8)	–	
500–1000, *n* (%)	73 (65)	–	
>1000, *n* (%)	29 (26)	–	
Missing *n* (%)	1 (1)	–	
Viral load (copies/ml)			
<399, *n* (%)	105 (94)	–	
400–1000, *n* (%)	1 (1)	–	
>1000, *n* (%)	0 (0)	–	
Missing *n* (%)	3 (3)	–	
WHO stage			
Stage I, *n* (%)	9 (8)	–	
Stage II. *N* (%)	16 (14)	–	
Stage III, *n* (%)	48 (43)	–	
Stage IV, *n* (%)	38 (34)	–	
Missing, *n* (%)	1 (1)	–	
Drugs:			
Zidovudine, *n* (%)	79 (71)	–	
Lamivudine, *n* (%)	110 (98)	–	
Lopinavir/r, *n* (%)	79 (71)	–	
Nevirapine, *n* (%)	7 (6)	–	
Abacavir, *n* (%)	22 (20)	–	
Tenofovir disoproxil, *n* (%)	10 (9)	–	
Emtricitabine, *n* (%)	1 (1)	–	
Atazanavir, *n* (%)	4 (4)	–	
Darunavir, *n* (%)	1 (1)	–	
Dolutegravir, *n* (%)	11 (10)	–	
Raltegravir, *n* (%)	0 (0)	–	
Ritonavir, *n* (%)	5 (4)	–	
Efavirenz, *n* (%)	11 (10)	–	

HU, children without HIV; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitors; PHIV, children living with perinatally-acquired HIV. † Missing values are not included in the inferential analysis.

Children were followed for noncommunicable diseases (NCDs) at FAMCRU (R01HD083042). All children with a valid scan were eligible for inclusion; however, we excluded those with evidence of conditions that may lead to hepatic steatosis. Scans were only conducted if the child presented to clinic well with no transient infections. The study was approved by Stellenbosch University's Health Research Ethics Committee (S20/02/046). Written informed consent was obtained from a parent or legal guardian and written assent from all adolescent participants.

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Transient elastography and controlled attenuation parameter

TE with CAP was used to simultaneously measure liver stiffness and CAP at each study visit using the Fibroscan (Echosens: Paris, France) with either the M or XL probe in obese participants after a minimum three hour fast [15–19,25,26]. Children had scans at baseline and each study visit over the follow-up period. All measurements were performed by one of two experienced operators who were not blinded to clinical data. A scan was considered reliable if 10 valid measurements were obtained (>60% successful and the interquartile range [IQR] was <30% of the median) [27].

Clinical variables

Demographic, anthropometric and clinical data collected included age, sex, weight, height, ethnicity, current and previous medical conditions, current and past medication including herbal or alternative remedies, Tanner pubertal staging, alcohol consumption and recreational substance usage. Current ART, including dates of regimen change, were obtained from clinical records. Body mass index (BMI) *z*-scores were calculated using World Health Organization AnthroPlus software (www.who.int/growthref/tools/en/). All adolescents were given basic information on healthy lifestyle at study enrolment and those with obesity or hepatic steatosis (CAP > 248 dB/m) [12,28] were offered referral to a dietitian.

Blood tests for glucose, insulin, platelets, triglycerides (TG), cholesterol [total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL)], alanine transaminase (ALT), aspartate transaminase (AST) and HIV RNA PCR viral load in PHIV were obtained after overnight fasting. If liver stiffness was >7 kPa or CAP was ≥238 dB/m or if the transaminases were elevated more than twice the upper limit of normal, further investigations were performed to exclude viral hepatitis, autoimmune hepatitis, Wilson's disease and alpha- 1-antitrypsin deficiency. Tests included hepatitis B surface antigen, hepatitis C antibody, autoantibodies (antinuclear, antismooth muscle and antiliver kidney), total immunoglobulin G (IgG), caeruloplasmin and alpha-1-antitrypsin level.

Confounders and interactions

Confounders were selected using a conceptual causal inference approach, represented by a directed acyclic graph ([DAG], Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/D256). Age was included as a potential time-varying confounder, whilst ethnicity and gender were included as a potential fixed confounders. Ethnicity was divided into two categories – African and mixed ethnicity [29,30]. Traditional risk factors of hepatic steatosis such as obesity, metabolic syndrome and blood lipids were excluded as are strongly influenced by HIV status in our resource-limited context [31–33], and may lie on the causal pathway (see DAG, Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/D256). Likewise, Tanner stage was deemed to be on the causal pathway. Interaction effects considered for inclusion were age by HIV group, gender by HIV group and age by gender.

Statistical analysis

We compared baseline characteristics between groups using the chi-squared test for categorical variables and the *F*-test for normal quantitative variables. Age and liver functions at the first Transient Elastography measurement were compared. Descriptive analyses included a graph of the mean CAP/liver stiffness by HIV group as a function of children's age.

In HIV group comparisons, we compared PHIV and HU, with significance declared at level $\alpha = 0.05$. We split PHIV measurements into two exposure groups based on ART regimen at the time of the measurement. PHIV Group 1 comprised measurements of children on older ART regimens (NRTI/ NNRTI/ PI), and PHIV Group 2 comprised measurements on combination tenofovir disoproxil, lamivudine and dolutegravir (TLD). Measurements taken within 4 months of switching regimen were included in PHIV Group 1 as we assumed that hepatic steatosis related to medications was unlikely to change in the initial time period. Children and adolescents already on TLD at study commencement only contributed measurements to PHIV Group 2, whilst children and adolescents who remained on older ART regimens only contribute measurements to PHIV Group 1. HIV-exposed and unexposed HU children were grouped together for analysis given their small sample sizes.

Two outcomes were considered: CAP and liver stiffness. Both outcomes were log-transformed because of skewness of data. The results were back-transformed, and reported as relative mean percentage differences between groups. These were modelled as a function of HIV status using linear mixed effects models with child-specific random effects. Single-predictor analysis explored the association between CAP/ liver stiffness and each variable of interest using a linear mixed model with random intercepts for each variable. Multipredictor analysis was conducted as detailed below.

We chose to evaluate random slope with age given the strong association between age and liver stiffness, previously found in children including our cohort [18,26,34,35]. We considered two random effects structures: random intercept only and random intercept and random age slope. We selected the model with minimum Akaike information criterion (AIC) from a restricted maximum likelihood (REML) fit [36]. The mean model included the HIV group (two levels), gender, age and ethnicity, as variables of interest; and the interaction effects age by HIV group, gender by HIV group and age by gender. Interaction effects were included if significant at 0.05 level. Potential confounders

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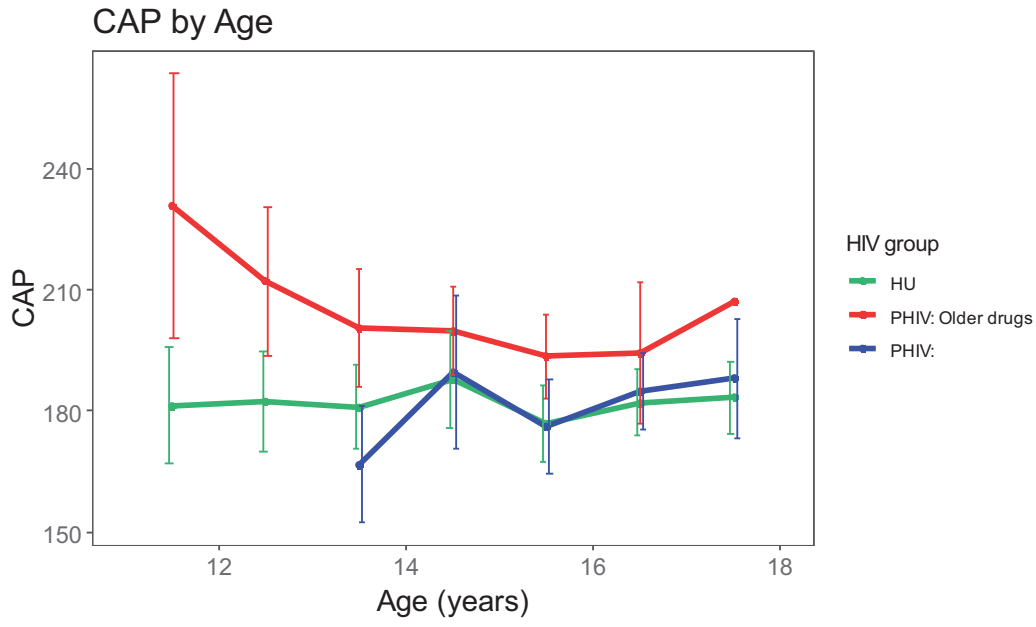


Fig. 1. Unadjusted mean (with 95% CI) controlled attenuation parameter (CAP) by HIV group and age comparing HU (green) with PHIV on older ART regimens (red) and PHIV on TLD (blue). Means are calculated for each yearly age band. CAP, controlled attenuation parameter; HU, children without HIV; PHIV, children living with perinatally-acquired HIV; TLD, tenofovir disoproxil, lamivudine and dolutegravir.

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were included if significant at 0.20 level using the likelihood ratio test (LRT) in backward stepwise model selection. We also fitted the polynomials age-squared and age-cubed as part of the mean model. These variables were tested for significance using the likelihood ratio test, and were removed if not significant at 0.05 level. The final model was re-fitted using REML.

Statistical analysis was performed using RStudio [37]. Since ethnicity differed across HIV groups, in sensitivity analyses we checked whether inclusion changed the coefficient of interest by >10%. As some children had exposure to alcohol, smoking and recreational drugs, we checked if these variables changed the coefficient of interest by >10% in sensitivity analysis. We performed additional sensitivity analysis with BMI z-score, to confirm our understanding of its relationship with CAP/liver stiffness.

Results

Characteristics of children

A total of 263 children (112 PHIV, 151 HU) with median age 13.7 years at enrolment and 49% female, were analysed. Children had recorded liver measurements between the ages of 7.9 years and 19.5 years old. There were 573 liver measurements over the study period (287 PHIV, 286 HU), with a median of 2 (interquartile range, IQR 1, 3) measurements per child. The median (IQR) follow-up time was 1.9 (IQR 1.0, 2.1) years. The median age at ART initiation in PHIV was 2.7 months old (IQR 1.8, 8.5).

At baseline (Table 1), PHIV on average had their first liver measurement at an older age than HU. Median CAP was higher in PHIV at baseline ($P < 0.001$), and the proportion with hepatic steatosis (CAP $\geq$ 248 dB/m) was significantly higher in PHIV (9%) compared to HU (3%). Liver stiffness was similar between groups, however the proportion of children with hepatic fibrosis (liver stiffness measure > 7 kPa) was greater in PHIV (7%) compared to HU (1%). PHIV had significantly higher baseline HOMA and cholesterol than HU. The proportion of mixed ethnicity vs. African children differed across the two groups ($P < 0.001$). No participants had underlying liver disease and all screened for hepatitis B and C tested negative.

During the study, 67 (60%) PHIV switched from older ART regimens (Group 1) to TLD (Group 2). The median age of switching was 15.4 years old (IQR 14.7, 15.8). Of the 287 PHIV liver measurements, 187 were conducted on older ART regimens, contributing to PHIV Group 1. The remaining 100 measurements were conducted after switching to TLD, contributing to PHIV Group 2.

Overall, PHIV had a high level of viral suppression, with a mean of 96% virally suppressed over time. Figure 2, Supplemental Digital Content, http://links.lww.com/QAD/D257 shows the proportion of PHIV with viral suppression over time.

Controlled attenuation parameter

Unadjusted CAP in PHIV on older ART regimens remained significantly higher than HU and PHIV on TLD (Fig. 1). In univariable analysis (Table 2), CAP in

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Table 2. Controlled attenuation parameter (CAP) by HIV group using linear mixed effects models.

Predictors	Unadjusted estimated mean percentage difference (95% CI)	*P*-value	Adjusted estimated mean percentage difference (95% CI)	*P*-value
PHIV: older ART regimens (vs. HU)	8.61 (4.42–12.97)	<0.001	8.61 (4.42–12.97)	<0.001
PHIV: TLD (vs. HU)	0.88 (−3.65–5.63)	0.707	0.88 (−3.65–5.63)	0.707
Age (years)	−19.21 (−45.11–18.94)	0.279		
Age square	2.61 (−29.12–48.54)	0.891		
Ethnicity: mixed	0.18 (−4.26–4.82)	0.939		
Gender: female	1.8 (−1.97–5.71)	0.353		

Unadjusted estimates show univariable analysis of each variable on CAP. Adjusted results show the mean percentage difference (with 95% CI) on CAP between PHIV on older ART regimens, PHIV on TLD, and HU. The model with random intercept only was optimal. HU, children without HIV; PHIV, children living with perinatally-acquired HIV; TLD, tenofovir disoproxil, lamivudine and dolutegravir.

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PHIV on older ART regimens was 8.61% higher than HU (95% CI 4.42–12.97, $P < 0.001$). There was no significant difference between PHIV on TLD and HU. Age, ethnicity and gender had no significant association with CAP in univariable analysis.

Multivariable analysis (Table 2) excluded all potential confounders and interactions using the likelihood ratio test. The final model was therefore identical to the univariable model.

Liver stiffness

Unadjusted liver stiffness increased slightly with age in PHIV on older ART regimens and HU (Fig. 2). No significant difference between groups is visible. In univariable analysis (Table 3), age and sex were significantly associated with liver stiffness. However, HIV group was not significantly associated with liver stiffness.

In multivariable analysis, no significant difference in liver stiffness was found between PHIV on older ART regimens and PHIV on TLD compared to HU, after adjusting for age, age squared and gender (Table 3).

Sensitivity analysis

Ethnicity, alcohol and recreational drug use were not significant confounders in the sensitivity analysis for CAP or liver stiffness, and therefore were omitted. Likewise, BMI *z*-score was not a significant confounder, with little change in the HIV group coefficients and results, supporting our reasons for exclusion.

Discussion

Statement of principal findings

In this study, PHIV on older ART regimens had an 8.61% higher CAP than HU. In those on TLD, there was no difference in CAP between PHIV and HU. We observed

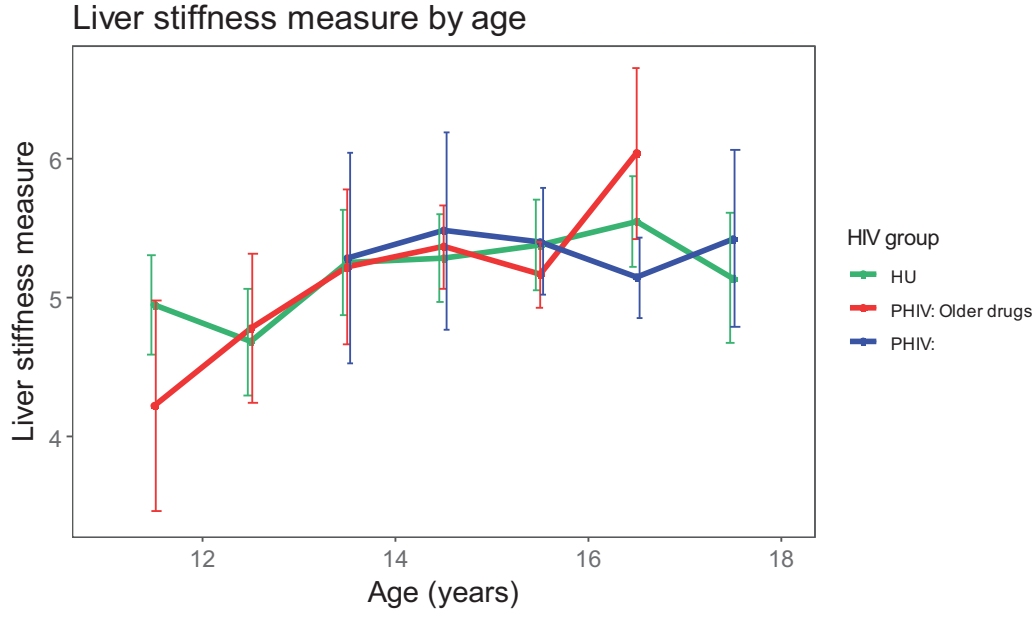


Fig. 2. Unadjusted mean (with 95% CI) of liver stiffness measure by HIV group and age comparing HU (green) with PHIV on older ART regimens (red) and PHIV on TLD (blue). Means are calculated for each yearly age band. TLD, tenofovir disoproxil, lamivudine and dolutegravir.

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Table 3. Liver stiffness by HIV group using linear mixed effects models.

Predictors	Unadjusted estimated mean percentage difference (95% CI)	*P*-value	Adjusted estimated mean percentage difference (95% CI)	*P*-value
PHIV: older ART regimens (vs. HU)	2.98 (−1.61–7.78)	0.207	1.08 (−3.38–5.74)	0.641
PHIV: TLD (vs. HU)	3.32 (−2.08–9.02)	0.232	−1.51 (−6.68–3.95)	0.580
Age (years)	183.42 (84.01–336.54)	<0.001	14.75 (4.27–26.29)	0.005
Age square	−42.73 (−62.23 to −13.17)	0.009	−0.4 (−0.74 to −0.06)	0.020
Ethnicity	−1.19 (−6.11–3.99)	0.644		
Gender: female	−9.83 (−13.41 to −6.12)	<0.001	−10.16 (−13.65 to −6.52)	<0.001

Unadjusted estimates show univariable analysis of each variable on liver stiffness. Adjusted results show the mean percentage difference (with 95% CI) on liver stiffness between PHIV on older ART regimens, PHIV on TLD, and HU. The model with random intercept only was optimal. HU, children without HIV; PHIV, children living with perinatally-acquired HIV; TLD, tenofovir disoproxil, lamivudine and dolutegravir.

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no difference in liver stiffness between PHIV on either older or newer ART regimens or HU. However, as the confidence interval is wide, we cannot exclude a small but clinically significant difference in liver stiffness between groups. To our best knowledge this is the first report of longitudinal change in CAP and liver stiffness in PHIV children and adolescents.

Early ART from birth has been standard of care for years now, now beginning prenatally. With the increasing recognition of long-term noncommunicable diseases and comorbidities in PHIV, ART adverse effects will be an increasingly important consideration in selecting optimal ART. Chronic inflammation in individuals living with HIV persists on suppressive ART and might contribute to development and progression of MASLD [4,38]. Although in our study PHIV switched to TLD had similar CAP to HU, future weight gain might reverse the observed favourable change in CAP.

Comparison with other studies was difficult due to differences in diagnostic modalities for hepatic steatosis and fibrosis diagnosis and large differences in ages of participants, with some including only children and others adolescents. Also, antiretrovirals, treatment duration and virologic suppression varied. The only published longitudinal studies investigating liver disease in PHIV relied on liver enzyme abnormalities, with or without ultrasound imaging to diagnose hepatic steatosis. A multicentre cohort study of Asian adolescents with persistent hepatic enzyme abnormalities found 8% of PHIV had evidence of hepatic steatosis on imaging. In multivariable analysis, both insulin resistance and raised baseline ALT, but not obesity or ART history, were associated with hepatic steatosis and/or fibrosis [13]. The prevalence of hepatic steatosis in this longitudinal study (9%) is the same as observed in our cross-sectional study [7]. Cross sectional studies of liver disease in European and US PHIV children and youth found a higher prevalence of hepatic steatosis ranging from 17–33% [11,12,14]. Risk factors for hepatic steatosis were higher BMI or waist circumference, but not ART regimen, HIV viral suppression or other HIV-related factors. However all studies were small with few PHIV participants and controls.

In a longitudinal US study, APRI and FIB4 indices were higher in youth with HIV and progressed over a two year follow up period, suggesting subclinical fibrosis in 13% of PHIV individuals [39]. Although youth known with hepatitis B or C were excluded, there was neither routine testing nor imaging to assess for hepatic steatosis. Although HIV was a predictor of fibrosis score progression, there was no association between either score or ART regimen. In contrast to our study, many youth with HIV had low CD4 counts and unsuppressed viral loads, both of which were associated with an APRI score consistent with hepatic fibrosis. It is possible that because of early ART and consistent virological suppression in our study, liver stiffness in PHIV was similar to HU controls. Although our cohort was younger than the US cohort (median 14.3 vs. 17.3 years at baseline), we observed a lack of progression of liver stiffness compared to HU controls even at an older age. In cross sectional studies investigating liver disease in children with HIV in Latin America and Poland, effective ART, including PI therapy, was protective against high APRI, whereas uncontrolled HIV viraemia was a risk factor for elevated APRI [40,41].

In adults living with HIV, a longitudinal study of the role of change in BMI and ART on hepatic steatosis progression found that hepatic steatosis may develop despite BMI within the normal range. Also, INSTIs and tenofovir alafenamide increased the risk of progression, whereas tenofovir disoproxil was associated with slower progression of hepatic steatosis [42]. In contrast, we had observed improvement in CAP, which might be partly due to simultaneously switching to TLD and stopping zidovudine and lopinavir/ritonavir, both being associated with hepatic steatosis in our cohort and other studies [7,10,11].

Strengths and limitations

Our prospective longitudinal cohort includes some of the first children globally to start ART soon after birth with minimal HIV disease and preserved CD4 counts [22,23]. Early ART is now standard-of-care and longitudinal data collection from this unique cohort offers a window into the likely future of the world's early-treated PHIV generation. Early-treated PHIV differ from their predecessors as they

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have largely been spared cumulative organ damage due to repeated opportunistic and intercurrent infections. However, they face many decades of cumulative ART exposure and HIV-associated immune activation. Further, as steatotic liver disease likely varies in genetic susceptibility, pubertal timing and diet, this unique South African cohort allows study of liver disease in early-treated PHIV in Sub-Saharan Africa. Through including HU controls from the same local community, we could undertake valid comparisons in a population with similar environmental exposures [44].

Study limitations include the relatively short follow up period of only two years, limiting our ability to observe long-term trends in liver stiffness and CAP. Nonetheless, the two-year timeframe is sufficient to examine the difference in liver stiffness between HIV groups. Our recruitment and follow up was also interrupted by the COVID pandemic, resulting in many missed follow-up visits at one year and a protracted recruitment period. Despite this break, we were able to complete two year follow up visits as planned and most participants completed all visits. In addition, mixed effects models are robust in dealing with missing data and imbalanced follow-up times. A further limitation is the uniformity of ART regimens in early life and later heterogeneity, which prevented comparison of the possible impact of different ART medications on hepatic steatosis, other than in those switching to TLD. Although PHIV who switched to TLD had lower CAP, dolutegravir has been associated with weight gain. Therefore, longer follow up is important to evaluate trends over time. We could not adjust for unmeasured confounders. However, given that controls came from the same community as PHIV, it is unlikely that these will have a significant confounding effect. Likewise, in adults living with HIV alcohol use is prevalent and may contribute to nonadherence to ART [43]. Alcohol consumption may be a contributing factor to the development of hepatic steatosis in some PHIV adolescents and youth, however as our study included children with a median age of 13.7 years, our participants had low alcohol consumption and recreational drug use. It is also possible that differences in liver stiffness between children exposed to HIV and not infected and children who were not exposed and not infected with HIV might have been missed due to small numbers.

Conclusions

In this cohort of children starting ART in early life, PHIV children and adolescents on older ART regimens had higher CAP than HU children and adolescents. However in PHIV switched to TLD there was no difference in CAP compared to HU individuals. There was no difference in liver stiffness between PHIV started on early ART and HU individuals. Further studies are needed to determine changes in CAP and liver stiffness in PHIV individuals and evaluate the possible impact different ART regimens may play in the long term. Liver stiffness and CAP requires longer follow-up to identify long-term trends.

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Conflicts of interest

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References

1. Frigati LJ, Ameyan W, Cotton MF, Gregson CL, Hoare J, Jao J, *et al*. **Chronic comorbidities in children and adolescents with perinatally acquired HIV infection in sub-Saharan Africa in the era of antiretroviral therapy.** *Lancet Child Adolesc Heal* 2020; **4642**:1–11.
2. van Welzen BJ, Mudrikova T, El Idrissi A, Hoepelman AIM, Arends JE. **A review of non-alcoholic fatty liver disease in HIV-infected patients: the next big thing?** *Infect Dis Ther* 2019; **8**:33–50.
3. Kapoor N, Audsley J, Rupali P, Sasadeusz J, Paul TV, Thomas N, *et al*. **A gathering storm: HIV infection and nonalcoholic fatty liver disease in low and middle-income countries.** *AIDS* 2019; **33**:1105–1115.
4. Lake JE, Overton T, Naggie S, Sulkowski M, Loomba R, Kleiner DE, *et al*. **Expert panel review on nonalcoholic fatty liver disease in persons with human immunodeficiency virus.** *Clin Gastroenterol Hepatol* 2022; **20**:256–268.
5. Kaspar MB, Sterling RK. **Mechanisms of liver disease in patients infected with HIV.** *BMJ Open Gastroenterol* 2017; **4**:e000166.
6. Rose PC, De La Rey Nel E, Cotton MF, Otwombe K, Browne SH, Frigati LJ, *et al*. **Decreased hepatic steatosis in south african adolescents with perinatal HIV switching to dolutegravir-containing regimens.** *Pediatr Infect Dis J* 2023; **42**:564–572.
7. Rose PC, Nel ED, Cotton MF, Pitcher RD, Otwombe K, Browne SH, *et al*. **Prevalence and risk factors for hepatic steatosis in children with perinatal HIV on early antiretroviral therapy compared to HIV-exposed uninfected and HIV-unexposed children.** *Front Pediatr* 2022; **10**:893579.
8. Dallapiazza M, Amorosa VK, Localio R, Kostman JR, Lo V, Iii R. **Prevalence and risk factors for significant liver fibrosis among HIV-monoinfected patients.** *BMC Infect Dis* 2010; **10**:116.
9. Mohr R, Schierwagen, Robert, Schwarze-Zander C, Boesecke C, Wasmuth J-C, *et al*. **Liver fibrosis in HIV patients receiving a modern cART.** *Medicine (Baltimore)* 2015; **94**:1–7.
10. Perazzo H, Cardoso SW, Yanavich C, Nunes P, Morata M, Gorni N, *et al*. **Predictive factors associated with liver fibrosis and steatosis by transient elastography in patients with HIV mono-infection under long-term combined antiretroviral therapy.** *J Int AIDS Soc* 2018; **21**:e25201.
11. Rubio ÃA, Monpoux F, Huguon E, Rosenthal-allieri M, Deville A, Rosenthal ÃAE, *et al*. **Noninvasive procedures to evaluate liver involvement in HIV-1 vertically infected children.** *J Pediatr Gastroenterol Nutr* **(14)**:2009:599–606.
12. Aepfelbacher JA, Balmaceda J, Purdy J, Mattingly A, Zambell K, Hawkins K, *et al*. **Increased prevalence of hepatic steatosis in young adults with lifelong HIV.** *J Infect Dis* 2019; **220**:266–269.
13. Sudjaritruk T, Bunupuradah T, Id LA, Kosalaraksa P, Srinakarin J, Chaijitraruch N, *et al*. **Nonalcoholic fatty liver disease and hepatic fibrosis among perinatally HIV-monoinfected Asian adolescents receiving antiretroviral therapy.** *PLoS One* 2019; **14**:e0226375.
14. Carrasco I, Olveira A, Lancharro Á, Escosa L, Mellado MJ, Busca C, *et al*. **Prevalence of nonalcoholic fatty liver disease using noninvasive techniques among children, adolescents and youths living with HIV.** *AIDS* 2022; **36**:805–814.
15. De Ledinghen V, Le Bail B, Rebouissoux L, Fournier C, Foucher J, Caste L, *et al*. **Liver stiffness measurement in children using fibroscan: feasibility study and comparison with fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy.** *J Pediatr Gastroenterol Nutr* 2007; **45**:443–450.
16. Fitzpatrick E, Quaglia A, Vimalesvaran S, Basso MS, Dhawan A. **Transient elastography is a useful noninvasive tool for the evaluation of fibrosis in paediatric chronic.** *J Pediatr Gastroenterol Nutr* 2013; **56**:72–76.
17. Lee CK, Perez-atayde AR, Mitchell PD, Raza R, Afdhal NH, Jonas MM. **Serum biomarkers and transient elastography as predictors of advanced liver fibrosis in a United States cohort: the Boston Children's Hospital Experience.** *J Pediatr* 2013; **163**:1058–1064.e2.
18. Engelmann G, Gebhardt C, Wenning D, Wühl E, Hoffmann GF, Selmi B, *et al*. **Feasibility study and control values of transient elastography in healthy children.** *Eur J Pediatr* 2012; **171**:353–360.
19. Desai NK, Harney S, Raza R, Al-ibraheemi A, Shillingford N, Mitchell PD, *et al*. **Comparison of controlled attenuation parameter and liver biopsy to assess hepatic steatosis in pediatric patients.** *J Pediatr* 2016; **173**:160–164.e1.
20. Sonderup M, Gogela N, Spearman CW. **Guidelines for the use of transient elastography in South Africa.** *South African Gastroenterol Rev* **(1)**:2019:29–37.
21. Palumbo P, Lindsey JC, Hughes MD, Cotton MF, Bobat R, Meyers T, *et al*. **Antiretroviral treatment for children with peripartum nevirapine exposure.** *N Engl J Med* 2010; **363**:1510–1520.
22. Cotton MF, Violari A, Otwombe K, Panchia R, Dobbels E, Rabie H, *et al*. **Early time-limited antiretroviral therapy versus deferred therapy in South African infants infected with HIV: results from the children with HIV early antiretroviral (CHER) randomised trial.** *Lancet* 2013; **382**:1555–1563.
23. Violari A, Cotton MF, Gibb DM, Babiker AG, Steyn J, Madhi SA, *et al*. **Early antiretroviral therapy and mortality among HIV-infected infants.** *N Engl J Med* 2008; **359**:2233–2244.
24. South African National Department of Health. 2019 ART clinical guidelines. 2019. Available at: https://www.health.gov.za/wp-content/uploads/2020/11/2019-art-guideline.pdf [Accessed 12 May 2024].
25. Goldschmidt I, Streckenbach C, Dingemann C, Pfister ED, di Nanni A, Zapf A, *et al*. **Application and limitations of transient liver elastography in children.** *J Pediatr Gastroenterol Nutr* 2013; **57**:109–113.
26. Mjelle AB, Mulabecirovic A, Havre RF, Rosendahl K, Juliusson PB, Olafsdottir ÃE, *et al*. **Normal liver stiffness values in children: a comparison of three different elastography methods.** *J Pediatr Gastroenterol Nutr* 2019; **68**:706–712.
27. Castera L, Forns X, Alberti A. **Noninvasive evaluation of liver fibrosis using transient elastography q.** *J Hepatol* 2008; **48**:835–847.
28. Ferraioli G, Calcaterra V, Lissandrin R, Guazzotti M, Maiocchi L, Tinelli C, *et al*. **Noninvasive assessment of liver steatosis in children: the clinical value of controlled attenuation parameter.** *BMC Gastroenterol* 2017; **17**:61.

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29. Matsha TE, Hassan MS, Kidd M, Erasmus RT. **The 30-year cardiovascular risk profile of South Africans with diagnosed diabetes, undiagnosed diabetes, prediabetes or normoglycaemia: the Bellville, South Africa pilot study.** *Cardiovasc J Afr* 2012; **23**:5–11.
30. Quintana-Murci L, Harmant C, Quach H, Balanovsky O, Zaporozhchenko V, Bormans C, *et al*. **Strong maternal khoisan contribution to the South African coloured population: a case of gender-biased admixture.** *Am J Hum Genet* 2010; **86**:611–620.
31. Shiau S, Webber A, Strehlau R, Patel F, Coovadia A, Kozakowski S, *et al*. **Dietary inadequacies in HIV-infected and uninfected school-aged children in Johannesburg, South Africa.** *J Pediatr Gastroenterol Nutr* 2017; **65**:332–337.
32. Rossouw ME, Cornell M, Cotton MF, Esser MM. **Feeding practices and nutritional status of HIV-exposed and HIV-unexposed infants in the Western Cape.** *South Afr J HIV Med* 2016; **17**:398.
33. Davies C, Vaida F, Otwombe K, Cotton MF, Browne S, Innes S. **Longitudinal comparison of insulin resistance and dyslipidemia in children with and without perinatal HIV infection in South Africa.** *AIDS* 2023; **37**:523–533.
34. Lewindon PJ, Balouch F, Pereira TN, Puertolas-Lopez MV, Noble C, Wixey JA, *et al*. **Transient liver elastography in unsedated control children: Impact of age and intercurrent illness.** *J Paediatr Child Health* 2016; **52**:637–642.
35. Tokuhara D, Cho Y, Shintaku H. **Transient elastography-based liver stiffness age-dependently increases in children.** *PLoS One* 2016; **11**:e0166683.
36. Fitzmaurice G, Laird N, Ware J. *Applied longitudinal analysis*. 2nd ed.Wiley & Sons; 2011.
37. R Studio Team. RStudio: integrated development environment for R. PBC, Boston, MA: RStudio; 2021.
38. Zicari S, Sessa L, Cotugno N, Ruggiero A, Morrocchi E, Concato C, *et al*. **Immune activation, inflammation, and non-AIDS comorbidities in HIV-infected patients under long-term ART.** *Viruses* 2019; **11**:E200.
39. Kapogiannis BG, Leister E, Siberry GK, Dyke RB Van, Rudy B, Flynn P, *et al*. **Prevalence of and progression to abnormal noninvasive markers of liver disease (APRI and FIB-4) among US HIV-infected youth.** *AIDS* 2016; **30**:889–898.
40. Pokorska-śpiewak M, Stańska-perka A, Popielska J, Coupland U, Zawadk K, Szczepańska-putz M. **Prevalence and predictors of liver disease in HIV-infected children and adolescents.** *Nat Sci Rep* 2017; **7**:12309.
41. Siberry GK, Cohen RA, Harris DR, Cruz LS, Oliveira R, Peixoto MF, *et al*. **Prevalence and predictors of elevated aspartate aminotransferase-to-platelet ratio index in Latin American perinatally HIV-infected children.** *Pediatr Infect Dis J* 2014; **33**:177–182.
42. Bischoff J, Gu W, Schwarze-Zander C, Boesecke C, Wasmuth JC, van Bremen K, *et al*. **Stratifying the risk of NAFLD in patients with HIV under combination antiretroviral therapy (cART).** *eClinicalMedicine* 2021; **40**:101116.
43. Seth A, Sherman K. **Fatty liver disease in persons with HIV infection.** *Top Antivir Med Antivir Med* 2019; **27.**:75–82.
44. Peer N, Balakrishna Y, De Villiers A, Naidoo P. **Differential associations of cardio-metabolic diseases by population group, gender and adiposity in South Africa.** *PLoS One* 2018; **13**: e0202899.

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