Spectrum, progression and predictors of morbidity in perinatally HIV-infected adolescents on antiretroviral therapy

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Declaration

I, Lisa Jane Frigati, hereby declare that this thesis is my own work, both in concept and execution, apart from the guidance received from my supervisors and contributions by those acknowledged.

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Included Publications

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1. Frigati L, Jao J, Mahtab S, Asafu Agyei NA, Cotton M, Myer L, Zar H. **Insulin Resistance in South African Youth with perinatally acquired HIV**. AIDS Research and Human Retroviruses 2019, 35 (1) :56-62

I collected data, did data analysis and interpretation of results and wrote the manuscript. JJ and LM were involved with the original concept of the study. SM and NA collected data. SM offered expert advice in data analysis. JJ, LM, MC and HZ reviewed the manuscript and added conceptual and intellectual comments. All authors read the manuscript prior to submission.

2. Frigati L, Mahtab S, Nourse P, Ray P, Perrazzo S, Machemedze T, Agyei NA, Cotton M, Myer L, Zar H. **Prevalence of risk factors for chronic kidney disease in South African youth with perinatally acquired HIV**. Pediatric Nephrology. 2019, 34:313-318.

I collected data, did data analysis and interpretation of results and wrote the manuscript. SM collected data. SP performed the laboratory tests under the supervision of PR. TM offered expert advice in data analysis. PN, PR, LM, MC and HZ reviewed the manuscript and added conceptual and intellectual comments. All authors read the manuscript prior to submission.

3. Frigati, L. J., Brown, K., Mahtab, S., Githinji, L., Gray, D., Zühlke, L., Nourse, P., Stein, D. J., Hoare, J., Cotton, M. F., Myer, L. and Zar, H. J. **Multisystem impairment in South African adolescents with Perinatally acquired HIV on antiretroviral therapy (ART).** *J Int AIDS Soc*. 2019; 22:e25386.

I collected data, did data analysis and interpretation of results and wrote the manuscript. SM contributed towards data management. KB offered expert advice in data analysis. LZ read and interpreted the echocardiograms. LG and DG performed and interpreted spirometry. PN gave input on renal measures. DJS and JH gave input on neurocognitive concepts. HZ and LM conceived the study. MC, LM and HZ reviewed the manuscript and added conceptual and intellectual comments. All authors read the manuscript prior to submission.

4. Frigati LJ, Brown K, Cotton MF, Myer L, Zar HJ. **Hospitalization in South African**

Adolescents with Perinatally Acquired HIV on Antiretroviral Therapy. *Pediatr Infect Dis J*.2020; 39 (11): 1035-1039.

I collected data, did data analysis and interpretation of results and wrote the manuscript. KB was responsible for data management and gave advice on data analysis. HZ and LM conceived of the study. MC, LM and HZ reviewed the manuscript and added conceptual and intellectual comments. All authors read the manuscript prior to submission.

5. Frigati, L. J., Wilkinson KA, le Roux S, Brown K, Ruzive S, Githinji, L, Petersen W, Cotton, M. F., Myer, L. and Zar, H. J. **Tuberculosis infection and disease in South African perinatally HIV-infected adolescents on antiretroviral therapy: a cohort study.** Submitted to the Journal of International AIDS society August 2020.

I was the study doctor, assisted with collection of data; helped in planning the analyses; wrote the first draft of the manuscript and confirm that I had full access to all the data and takes final responsibility for the decision to submit for publication. KB was responsible for data management and oversight. SLR did the analysis with input from LJF. LM and HJZ conceived the CTAAC study and TB sub-study, and were responsible for study design, funding, implementation and overall leadership. KW, WP and SR were responsible for QFT testing on the study. LG was a study doctor and contributed to the initial study design. All authors contributed to and approved the final manuscript.

6. Frigati LJ, Ameyan W, Cotton MF, Gregson CL, Hoare J, Jao J, Majonga ED, Myer L, Penazzato M, Rukuni R, Rowland -Jones S, Zar HJ, Ferrand RA. **Chronic comorbidities in children and adolescents with perinatally acquired HIV infection in sub-Saharan Africa in the era of antiretroviral therapy**. *The Lancet Child & Adolescent Health* 2020.

RAF and I conceptualised and coordinated the Review. RAF wrote the chronic lung disease section with SR-J. RAF and EDM provided clinical pictures. SR-J wrote the pathogenesis section. I wrote the malignancy section. CLG and RR wrote the musculoskeletal section. EDM and HJZ wrote the cardiac disease section. JH wrote the neurocognitive and mental health section with input from myself and RAF. JJ and CLG wrote the renal and metabolic disease section. WA and LM wrote the policy section with input from RAF and myself. All authors contributed to editing and approved the final version of the Review.

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Abbreviations

ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ART	combination antiretroviral therapy
AZT	Zidovudine
BMI	body mass index
CD4	cluster of differentiation
CKD	chronic kidney disease
DDI	didanosine
DTG	dolutegravir
D4T	stavudine
FAC	fractional right ventricle area change
FEV_1	forced expiratory volume
FVC	forced vital capacity
GFR	glomerular filtration rate
HCLD	HIV associated chronic lung disease
HDL-C	high-density lipoprotein cholesterol
HIV-	HIV negative (used to refer to adolescents living without HIV)
HIV+	HIV positive (used to refer to adolescents living with HIV)
HIVAN	HIV associated nephropathy
HIV-VL	HIV viral load
HOMA-IR	homeostatic model assessment of insulin resistance
IGRA	Interferon gamma release assay
INH	Isoniazid
INSTI	integrase strand transfer inhibitor
IPT	Isoniazid preventive Therapy
IR	insulin resistance
LDL-C	low-density lipoprotein cholesterol
LLN	lower limit of normal
LV	left ventricle
LVSF	left ventricular shortening fraction
LPV/r	lopinavir/ritonavir
mPAP	mean pulmonary artery pressure
Mtb	Mycobacterium tuberculosis
NNRTI	non- nucleoside reverse transcriptase inhibitor

NRTI	nucleoside reverse transcriptase inhibitor
PCP	*Pneumocystis jerovici* pneumonia
PHIV+	adolescents living with perinatally acquired HIV
PI	protease inhibitor
PY	person-years
PYO	person-years observation
QFT	QuantiFERON
RV	right ventricle
TAPSE	tricuspid annular plane systolic excursion
TB	Tuberculosis
TC	total cholesterol
TDF	Tenofovir disoproxil fumarate
TG	triglycerides
TPT	TB preventive therapy
WC	waist circumference
WHO	World Health Organization
y-IHDS	youth-International HIV Dementia Scale
YLPHIV	youth living with perinatally acquired HIV

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Abstract

Spectrum, progression and predictors of morbidity in perinatally HIV-infected adolescents on antiretroviral therapy

Background: Long term survival of children living with HIV due to improved early access to antiretroviral therapy (ART) is contributing to a growing population of adolescents living with perinatally acquired HIV (PHIV+) at risk of developing chronic multisystem comorbidity. There is limited knowledge on the overall burden, progression and causes of morbidity in PHIV+ adolescents, especially in resource limited settings. Much of what is known about morbidity in PHIV+ adolescents relates to single organ system pathology and there is a lack of a holistic approach to PHIV+ adolescents and their overall health.

The aim of this PhD project was therefore to investigate the spectrum and determinants of chronic morbidity, the progression of disease and intercurrent illness in PHIV+ adolescents on ART over a 4-year period.

Methods: This was a prospective study of participants enrolled in the Cape Town Adolescent Antiretroviral Cohort (CTAAC), a longitudinal cohort study, that recruited 515 PHIV+ adolescents and 110 HIV negative (HIV-) adolescents matched by age from 7 health care sites in Cape Town, South Africa. Eligibility criteria included PHIV+ adolescents who were aged 9-14 years, who had been on ART for at least 6 months and were aware of their HIV status. All adolescents and caregivers gave informed consent/assent. Participants were followed 6-monthly with questionnaires, clinical examination with detailed pulmonary (lung function), neurocognitive (magnetic resonance imaging and a battery of neurocognitive tests), cardiovascular (echocardiogram and ECG) and laboratory investigations. Analyses for each specific objective of the PhD were developed. Three analyses used data from the enrolment visit and were primarily descriptive and two were longitudinal and examined the incidence of hospitalizations, QuantiFERON conversion (an interferon gamma release assay used to measure Mycobacterium tuberculosis infection) and Tuberculosis (TB) disease.

Results: Five hundred fifteen PHIV+ and 109 HIV- participants had a median follow-up of 4.1 years (IQR: 3.7–4.6). At enrollment, PHIV+ adolescents had a median duration of ART of 7.6 years (IQR: 4.6–9.2), median CD4 count of 713 cells/mm^3 (IQR: 561.0–957.5) and 387 (75%) had a viral load of <50 copies/mL. Neurocognitive impairment was present in more than half of the PHIV+ cohort (56.3% vs. 45.3% in HIV-, *p*=0.05) but renal impairment was rare (2.3% in PHIV+ vs. 2.1% in HIV-, *p*=0.89).

Microalbuminuria was also rare (8.0 in PHIV+ vs. 9.0% in HIV-, *p*=0.80). Respiratory or cardiac impairment were more common in PHIV+ adolescents than in HIV- participants (27.1% vs. 14.7%, *p*=0.01 and 46.1% vs. 33.7%, *p*=0.03, respectively). Multisystem impairment (defined as impairment of ≥ 3 systems) was uncommon, with only 10% of PHIV+ adolescents having 4-system impairment. Metabolic abnormalities, such as insulin resistance (IR), were relatively common but IR rates did not differ compared to HIV- adolescents (18 vs. 20%, *p*= 0.17). Incidence rates for hospitalization were 6.6 per 100-person-years (PY) in PHIV+ adolescents, three times that of HIV- adolescents. Sixty percent of hospitalization episodes were due to non-infectious causes and 24% due to infectious causes, of which pneumonia and TB were the predominant causes. PHIV+ adolescents had a substantially higher incidence of TB disease than HIV- adolescents (2.2/100 PY, 95% CI 1.6-3.1 vs. 0.3/100 PY, 95% CI 0.04-2.2), despite a similar rate of TB infection, as measured by QuantiFERON positivity. TB disease was associated with low CD4 counts and high viral loads in PHIV+ adolescents.

Conclusion: Chronic single system morbidity experienced by PHIV+ adolescents on ART was common and merits further study, as this population begins to engage in adult lifestyle factors, such as smoking and alcohol use, that may compound these abnormalities. However, multisystem morbidity was relatively rare. In addition, in a relatively small percentage of adolescents there were subclinical metabolic abnormalities (IR and microalbuminuria) that may result in increased morbidity especially with regards to diabetes and cardiovascular disease in later life. The high burden of hospitalization and intercurrent disease, mainly due to TB, could be prevented by proven strategies, such as TB preventive therapy and ensuring adherence to optimal ART regimens.

Format of PhD

Four of the chapters (chapters 2-5) of this thesis are presented as published manuscripts. Chapter 6 is presented as a manuscript that has been submitted for publication. A published manuscript is also included as Annex 1.

Chapter 1 contains the introduction, including literature review and methodology.
Chapter 2 (published manuscript) describes metabolic morbidities, including insulin resistance and metabolic syndrome at study enrolment.
Chapter 3 (published manuscript) describes the prevalence of risk factors for renal disease at study enrolment.
Chapter 4 (published manuscript) describes multisystem comorbidity at study enrolment.
Chapter 5 (published manuscript) describes incidence and causes of hospitalizations over time.
Chapter 6 (submitted manuscript) describes the incidence of TB infection and disease over time.
Chapter 7 is a summary of the PhD findings and recommendations.

Notes on Terminology and Sample Size

Throughout the PhD thesis (including the PhD title) the abbreviation for adolescents living with perinatally-acquired HIV may differ according to journal preference. The abbreviation of adolescents living without HIV was also according to journal preference. The student acknowledges that this may not always align with current preferred terminology.

The term ‘adolescent’, defined by the World Health Organization (WHO) as a person between the ages 10-19 years, is used throughout but the authors acknowledge that some participants were 9 years of age at study enrolment.

Sample size and follow up time for each chapter also differ as for each specific objective a different number of participants had results available for the specific tests required for that objective.

Chapter 1: Introduction

1. Literature review

1.1 Introduction

Adolescents with perinatally-acquired HIV (PHIV+) are a growing population that face unique health challenges. In many countries, there are existing cohorts of children that have been followed since their HIV diagnosis, into adolescence and beyond. Their specific challenges have been well documented. However in sub-Saharan Africa (SSA), where the majority of adolescents with perinatally-acquired HIV live, and where this population will continue to grow as more children access ART, the health concerns facing this vulnerable population are less well characterized.

The Cape Town Antiretroviral Cohort (CTAAC) began recruitment of PHIV+ adolescents in 2013, thereby establishing a cohort of adolescents with perinatal HIV. These adolescents continue to receive care at their primary clinics or hospitals but are seen biannually by the CTAAC study. Cohorts of PHIV+ adolescents and older youth with perinatal HIV are also being followed in the United States of America (US), South and Central America, the United Kingdom, Europe and other African and Asian countries. Examples of these include the Paediatric HIV/AIDS Cohort Study (PHACS) and the Adolescent Master Protocol (AMP) cohorts, the European Pregnancy and Pediatric HIV Cohort Collaboration (EPPICC) and the Therapeutic Research, Education and AIDS Training in Asia Pediatric HIV observational Database (TApHOD). The research from these cohorts has mostly focused on end-organ disease, for example chronic lung disease, HIV-related renal disease, neurocognitive and cardiovascular issues.

In adults, HIV is now seen as a chronic disease and the rise of non-AIDS defining illnesses (NADIs), non-communicable diseases (NCDs) and multi-morbidity have been described in resource rich as well as other less well-resourced countries.[1,2] There is a lack of data in the PHIV+ adolescent population where multi-morbidity may have far greater significance over the life course if left unchecked and opportunities for intervention may result in greater gains than in adults.

It is especially important to explore the burden of infectious morbidity in PHIV+ adolescents in settings where infectious morbidity is high. The interplay between this infectious morbidity and that seen with NADIs and NCDs may have specific implications that are not evident in adolescents in resource rich settings.

The aim of this work was therefore to describe the burden of health-related issues in PHIV+ adolescents, exploring not only the various organ systems but how impairment of these systems is interrelated in individuals, including metabolic issues that are not system specific, and lastly how this translates into morbidity resulting in hospitalizations. In addition, in SSA, it is evident that infectious morbidity plays a greater role than in high income countries and a further objective of the work was to describe the burden of infectious morbidity, especially Tuberculosis (TB).

Describing a more comprehensive spectrum and progression of morbidity in these adolescents will allow for the designing of better health care services for this population. Further research could build on this to determine the most appropriate screening strategies and interventions to ensure that the life-course of this unique population, in this setting, can be optimized.

The remainder of Chapter 1 will focus on reviewing the literature on morbidity in adolescents with perinatally-acquired HIV, including the epidemiology, spectrum of morbidity, intercurrent hospitalizations, mechanisms of morbidity and multi-morbidity.

1.2 PHIV+ adolescents

1.2.1 Global epidemiology of PHIV+ adolescents

According to UNAIDS estimates, there were 1.7 million children living with HIV (CLHIV) in 2018, 90% of whom live in SSA.[3] Due to earlier and increased access to combination antiretroviral therapy (ART) survival of CLHIV has improved, with a growing population of PHIV+ adolescents.[4] Following the introduction of ART the number of children aging into adolescence increased from 53 000 in 2000 to 100 000 in 2005.[5] Between 2005 and 2015, 100 000 additional CLHIV reached adolescence each year, as most who acquired HIV 10 years prior (1995-2005) accessed effective ART.[5] There were an estimated 770 000 adolescents between the ages of 10-14 years in 2016.[6] These “younger” adolescents are considered to have perinatally-acquired HIV.

HIV is the eighth leading cause of death in adolescents globally and the fourth leading cause of death in Africa and low and middle income countries (LMICs).[3] Recent mortality data show that AIDS related deaths in the 10-14 year age group peaked in 2010 and has then declined. However, mortality estimates are increasing in the 15-19 year age group.[5] Deaths in older adolescents (aged 15 -19 years) are predominantly among PHIV+ adolescents and not newly infected adolescents who are yet to become ill (although acute HIV infection can result in severe morbidity).[5] A study done using data from several African countries suggested that of 5516 adolescents “transitioning” to adult services, 1463 were 20 years of age.[7]

1.2.2 South African epidemiology of PHIV+ adolescents

It is estimated that 360 000 (20% of the global estimate) of adolescents living with HIV live in South Africa.[8] This is a heterogenous group of adolescents that acquired HIV perinatally and “horizontally”. There is no estimate available for the number of adolescents with PHIV infection. The most recent

South African national survey reported an HIV prevalence of 2.5% in those between 5-14 years and 7.9% in those between 15-24 years.[9] The same survey reported a HIV prevalence of 5.8% in females and 4.7% in males between 15-19 years.[10] AIDS-related deaths in South Africa have decreased in the 10-14 age group but are increasing in the 15-19-year age group.[5] Thus, although the exact number is not certain, there is a large number of PHIV+ adolescents with an increasing mortality in the latter adolescent period. It is unclear if this mortality is due to HIV itself, or due to factors unrelated to HIV, for example trauma or violence. This increased mortality requires urgent exploration and strategies to try to prevent this need to be put in place.

In addition to this, South Africa has a rising burden of NCDs with 40% of the adult female being classified as obese.[11,12] Multi-morbidity has already been described in the adult South Africans with HIV, with the most common NCDs in HIV+ adults being hypertension and diabetes.[13] It is therefore important to look for risk factors or predictors for these conditions in the adolescent / young adult period in order to prevent or treat these conditions as PHIV+ adolescents age into adulthood.

1.3 Spectrum of morbidity (Appendix A)

There is growing recognition that CLHIV are at risk of developing chronic multisystem comorbidity and disability.[14,15] Morbidity differs to that in adults, most likely related to various factors including timing of HIV infection, absence of traditional adult risk factors, such as alcohol, smoking and ageing. Morbidity may also differ according to geographical settings due to the difference in timing of ART availability, the average age at ART initiation and the prevalence of other infectious diseases. In high income settings ART became available in 1996, whereas in SSA ART only first became available in 2004. In addition, most children in high income settings like the US started ART at an average of 0.9 years, compared to those in SSA who started later, at an average age of 7.8 years.[16] One would therefore expect PHIV+ adolescents to have had a longer period during childhood where they were vulnerable to opportunistic infections and the effects of HIV itself, resulting in increased disability and comorbidity. However, those that survived without access to ART may have been spared exposure to more toxic ART regimens that were available prior to 2004. They may also have some biological advantage to have survived to initiate ART.

1.3.1 Growth and development

Impaired growth can result from undernutrition, chronic inflammation, recurrent opportunistic infections, gastrointestinal infections or endocrine abnormalities, all of which may be caused by or associated with HIV. Stunting (low height for age), underweight (low weight for age) or wasting (low weight for height) have been described in PHIV+ adolescents, especially those in low income settings.[17] Even after starting ART, many HIV+ adolescents do not regain their height potential.[18,19]

Age of ART initiation is predictive of catch up growth, with children starting ART at > 5 years of age showing lower odds to catch up growth than those who started before 5 years of age.[20] Height for age Z- score before ART initiation also predicts failure of growth recovery.[21,22] Puberty, a critical period for growth and bone mass accrual, can be delayed in PHIV+ adolescents[21] and this may have lifelong consequences, such as increased risk of fractures.[23] In summary, impaired growth and delayed puberty may impact future health and result in increased and earlier frailty in adulthood. Those adolescents with decreased final height and delayed puberty may need further monitoring and interventions.

1.3.2 Musculoskeletal abnormalities

Factors contributing to decreased bone mineral density (BMD) in PHIV+ adolescents are stunting, low body mass, Vitamin D deficiency, previous advanced HIV disease, renal disease, drugs or delayed puberty.[24] The adolescent period is crucial for bone-mineral acquisition, and decreased bone mass in this period may result in early osteoporosis.

The gold standard for measuring bone density is dual energy x-ray absorptiometry (DXA); however, this can overestimate BMD in tall people and underestimate in those stunted, which is important to consider in PHIV+ adolescents who are often stunted with delayed puberty. Quantitative ultrasound is used in less well-resourced settings and although ultrasound is less accurate than DXA, it does correlate with DXA findings in CLHIV and PHIV+ adolescents.[25] A study from Zimbabwe showed that CLHIV initiating ART after 8 years of age had, on average, at least a 1 standard deviation lower size-adjusted lumbar spine bone density.[26] This is concerning, as this level of decreased bone density doubles the risk of fractures in adults.[27] Another study examining the lifetime fracture history in CLHIV and PHIV+ adolescents found that fracture incidence was higher in CLHIV less than 6 years of age compared to perinatally HIV-exposed uninfected children of the same age, but not among older children and adolescents.[28]

Vitamin D has been shown to improve BMD in PHIV+ adolescents.[29] In addition, a recent study that compared alendronate (a bisphosphonate), Vitamin D and calcium supplementation to Vitamin D and Calcium supplementation alone found that those receiving alendronate had improved lumbar spine and whole body BMD. [30] This study was done in a limited number of adolescents who were mostly from the US and this intervention has not yet been studied in LMICs. However, there is no reason to suspect that outcomes would differ in adolescents in less well-resourced settings, although access to DXA is limited. Fortunately, low BMD and its sequelae may be less of a concern for this population in the future as improved ART regimens become available.

1.3.3 Pulmonary abnormalities

Several studies in low income settings have reported a significant burden of chronic respiratory symptoms and signs in older children and adolescents on ART. These include breathlessness at rest, cough, reduced exercise tolerance and hypoxia.[31-33] Most of the adolescents described in these cohorts had late access to ART. They are also more likely to have experienced malnutrition and intercurrent respiratory infections, including TB, that may predispose them to chronic lung disease. The most common radiological finding on high resolution computed studies (HRCT) is mosaic decreased attenuation with or without bronchiectasis.[34,35] This finding in conjunction with hypoxia and irreversible airway obstruction is indicative of obliterative bronchiolitis (OB). In addition, many asymptomatic PHIV+ adolescents that started ART late have reduced lung function, predominantly airflow obstruction, with little response to bronchodilators which may potentially impact on lung health in adulthood.[36,37] Bronchiectasis is another well described cause of chronic lung disease in PHIV+ adolescents.[38] This can be a sequelae of recurrent lung infection, TB or lymphoid interstitial pneumonitis (LIP). The typical radiological findings of LIP have decreased in the post ART era.[35]

These findings have implications for adolescents as they reach adulthood. Even subtle changes in lung function may impact the risk for chronic lung disease in adult life. There are no specific guidelines for chronic lung disease in this population. Azithromycin use in children and adolescents (between 6-19 years of age) with HIV-associated chronic lung disease (HCLD) in Zimbabwe and Malawi had no effect on the forced expiratory volume (FEV)-Z score; however, participants on the azithromycin arm had a lower incidence of acute respiratory exacerbations and hospitalizations.[39] Many adolescents will have missed the introduction of the conjugate pneumococcal vaccine (PCV), as in South Africa the roll out of PCV only occurred in 2009. PCV vaccination, annual influenza vaccination as well as continued cotrimoxazole prophylaxis, TB preventative therapy, avoidance of tobacco smoke and indoor pollution may be strategies to optimise lung health in PHIV+ adolescents with HCLD. There are no specific estimates of pneumococcal pneumonia incidence or confirmed TB incidence in PHIV+ adolescents in LMICs and it is also uncertain whether pneumococcal pneumonia and TB occur more frequently in those with HCLD in this age group.

1.3.4 Cardiovascular abnormalities

Cardiac disease has been described in CLHIV and PHIV+ adolescents in the pre-ART era in both low and high income settings.[40,41] Abnormalities included dilated cardiomyopathy, pericardial effusion, left-ventricular diastolic dysfunction, increased left ventricular wall thickness or mass and decreased left ventricular fractional shortening.[41] More severe cardiac pathology was described in a Zimbabwean cohort of PHIV+ adolescents who initiated ART at a relatively older age.[42] In the ART era, challenges due to potential drug toxicity and ongoing inflammation still exist and together with metabolic complications, may lead to early cardiovascular disease [43]. In high income settings, the prevalence of dilated cardiomyopathy has decreased.[44,45] However, recent studies from low-income settings still report relatively high prevalence of cardiac abnormalities ranging from 14-89%, including left ventricular (LV) systolic and diastolic dysfunction, LV hypertrophy, left atrial dilatation, isolated right ventricular (RV) dilation, conduction abnormalities and pericardial thickening and effusion.[46-49] RV dysfunction was the most common form of cardiopulmonary dysfunction among PHIV+ adolescents enrolled on the CTAAC study.[50] A prospective study from Zimbabwe found that although most cardiac abnormalities persisted at 18 months, the participants were asymptomatic or did not experience worsening of their symptoms.[51]

Studies in children demonstrate that, as in adults, there is significant inflammation related to uncontrolled HIV replication.[52] This inflammation decreases but persists on effective ART.[53] Chronic inflammation may result in cardiovascular disease.[54] In adolescents enrolled in a US cohort, markers of inflammation, coagulation, endothelial and metabolic dysfunction correlated with ART and viremia.[52] Specifically, increased HIV viral load was associated with markers of inflammation and endothelial dysfunction.[55] Another study from a high income setting showed that subclinical atherosclerosis was associated with HIV and ART, particularly protease inhibitors.[56] PHIV+ adolescents enrolled in CTAAC appeared at increased risk for endothelial dysfunction compared to age- and sex-matched youth without HIV in a study that measured the reactive hyperaemic index (RHI) using endothelial peripheral arterial tonometry.[57]

In high income settings recommendations support CLHIV and PHIV+ adolescents undergoing regular screening for cardiovascular disease, including a directed history, laboratory tests and electrocardiographic monitoring. There is relatively little data informing screening for cardiovascular in PHIV+ adolescents in the ART era in resource-limited settings. Research addressing the burden of hypertension, cardiac impairment and the most relevant and cost-effective methods of screening as well as possible preventive measures once abnormalities have been detected is urgently needed.

1.3.5 Neurocognition and mental health

The most common presentation of neurological pathology in the pre-ART era was HIV encephalopathy. Early ART initiation in infancy has resulted in improved neurocognitive outcomes; however, children who start ART beyond infancy may still experience a wide range of cognitive deficits. In addition, Efavirenz, commonly used in children and adolescents, has been reported to have toxic neurological effects.[58] In a prospective study of children aged 5-11 years from four SSA countries that compared neuropsychological outcomes in CLHIV with those who were HIV-exposed but uninfected and those who were HIV-unexposed, CLHIV performed worse in all cognitive domains than the other two groups. More than 95% of the children in this study had a suppressed HIV viral load and a CD4% ≥25%, but only 1% started ART in the first six months of life.[59] The causes of neurocognitive impairment in the ART era are multifactorial and include early neurological insults prior to ART, possible ongoing inflammation and / or viral replication in the central nervous system, ART toxicity as well as socioeconomic and psychosocial factors.[60]

A meta-analysis demonstrated an association between HIV infection in children and adolescents and neurocognitive impairment, mainly in the domains of working memory, executive function and processing.[61] In addition, there was evidence of deficits in visual memory and visual-spatial ability. However, neurocognitive impairment may be subtle with deficits missed by routine testing. There is a lack of context-specific and culturally validated screening tools or standardised definitions for neurocognitive impairment in children and adolescents. One South African study has recently validated a youth-International HIV Dementia Scale.[62] Standard definitions and screening tools, like those used in adults, are urgently needed to screen children before they reach adolescence in order to support these children to achieve their optimal potential.

Several studies report a high prevalence of mental health disorders among children and adolescents with HIV. A large cross sectional study, conducted in Uganda, that assessed more than 1300 CLHIV and PHIV+ adolescents reported a prevalence of 17% of any psychiatric disorder and a 10% prevalence of any behavioural disorder - most commonly attention deficit hyperactivity disorder. These disorders were more common in adolescents than in children and behavioural and mental health disorders commonly occurred concurrently.[63] Similarly, a study from South Africa reported that adolescents with HIV had poorer functional competence, self-concept and motivation and higher levels of depression, disruptive behaviour, attention-deficit hyperactivity disorder symptoms and clinically significant anger, compared to their HIV-negative peers.[64] PHIV+ adolescents may face recurrent and cumulative stressors that differ from other chronic childhood illnesses and that may include illness and the death of their parents. This may increase their risk of development of mental health disorders.[64-66] It is possible that the neuropathological effects of HIV infection may augment risk of mental health disorders.[67] Mental health disorders impact adherence to ART and are associated with an impaired quality of life, yet often receive little attention in the face of physical health concerns. Although early ART has significantly decreased HIV encephalopathy, more subtle cognitive and mental health issues will have a major impact on PHIV+ adolescents as they reach adulthood and must negotiate their own care.

Most literature addressing neurocognitive issues does not explore the additional burden of other physical impairment and how this may affect the individual PHIV+ adolescent. Adolescents with neurocognitive and mental health issues as well as chronic lung disease and / or renal disease for example, may be particularly vulnerable, have an excessively high pill burden and may have difficulty adhering to medication. The burden of PHIV+ adolescents with neurocognitive impairment in addition to other physical impairment has not yet been quantified.

1.3.6 Metabolic abnormalities

Metabolic abnormalities due to HIV or ART are observed in PHIV+ adolescents. These include lipodystrophy, dyslipidaemia and insulin resistance.[68-70] Insulin resistance, a precursor to diabetes mellitus, has also been documented in CLHIV in South Africa and has implications for the risk of developing diabetes mellitus in adolescence.[71] A recent review reporting on metabolic complications in various cohorts worldwide found that lipodystrophy was the most commonly reported metabolic complication.[69] The Metabolic Syndrome was described in a Spanish CLHIV cohort with a reported prevalence of 3.7 % and 7.4%, depending on whether International Diabetes Federation (IDF) or National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria were used.[72] As most metabolic complications are undiagnosed in young children but can lead to cardiovascular complications early in adult life, it is important to identify and intervene in adolescents at risk.

Diabetes has also been associated with and increased risk of TB, thus preventing it may decrease TB risk, especially in settings where there is a high burden of TB-HIV coinfection such as South Africa.[73] The prevalence of these conditions and their potential risk factors need to be elucidated before optimal screening and interventions are implemented, especially in light of other competing morbidities that may be more acute and more visible.

1.3.7 Renal disease

Although widescale availability of ART has markedly decreased the incidence of HIV-associated nephropathy (HIVAN), there has been no clear benefit demonstrated in non-HIVAN kidney disease.[74-76] Drug toxicity (specifically Tenofovir disoproxil fumarate, TDF) and longer life expectancy have resulted in an overall higher incidence of kidney disease in adults living with HIV.[77]

In a large US cohort, kidney disease was one of the ten most common non-infectious conditions occurring in CLHIV and PHIV+ adolescents in the ART era, with an incidence rate of 2.6 per 100 person-years (PY).[78] In SSA, a study from Tanzania reported the prevalence of microalbuminuria (an early marker of glomerular injury) to be 20.4%.[79] It is important to note that the G1 and G2 apolipoprotein-L1 (APOL1) gene is more common in African people and is associated with increased risk of early renal disease.[80] There is little surveillance of kidney disease in HIV+ children and adolescents in SSA and not much is known about long-term progression of renal disease in adolescents on ART. There is thus a need for longitudinal follow up and monitoring of children and adolescents with microalbuminuria as well as those with established renal impairment. As with decreased bone density, access to improved ART regimens may result in decreased renal complications.

1.3.8 ENT, hearing, oral health and ophthalmological issues

The sequelae of chronic suppurative otitis media has been described in low resource countries.[81,82] A study in Ethiopia showed that tympanic membrane perforations and otorrhea were more common in CLHIV on ART than in age- matched HIV negative children.[83] A study from South Africa done in 30 CLHIV attending ART clinics showed that half the children had hearing loss.[84] The association of oral biomarkers of inflammation (performed on gingival crevicular fluid) with clinical indicators of periodontal inflammation and systemic immune activation suggests that PHIV+ youth are at higher risk for developing significant periodontal disease, associated with tooth loss and HIV progression. More frequent dental care of this group is needed to prevent potential periodontal progression.[85,86] A study from Cameroon that included CLHIV and adolescents on ART with a median age of 12.7 years found that just over half of the participants had abnormal ophthalmological findings, the majority related to the adnexa; however, 7.5% had anterior chamber issues and 2 participants (1.9%) had posterior findings indicative of CMV retinitis.[87]

Hearing and visual impairment have a profound effect on neurodevelopment and need to be detected early. Oral health, although often overlooked, also may have a great impact on adolescents over the life course. Oral health may also become less affected as many young children switch from oral solutions of ART to solid formulations due to the acidic and high sugar content of some of the syrup formulations.

1.3.9 Dermatological issues

Skin disease or scarring as a sequelae of skin manifestations may be more concerning to adolescents than impairment of other organs as it is an outward manifestation of disease and can be highly stigmatizing. Common causes of skin disease related to HIV are bacterial, viral, fungal and inflammatory. Although ART has markedly reduced skin diseases, papular pruritic eruption (PPE) may be a marker of uncontrolled viraemia.[88] In the Antiretroviral research for Watoto (ARROW) trial, children were randomized to continue cotrimoxazole or stop it and those who continued had less skin problems than those who discontinued.[89]

1.3.10 Sexual and Reproductive Health

Many PHIV+ adolescents are now reaching sexual debut. Studies in the US have found that sexual debut in PHIV+ adolescents was similar to those without HIV and was around 13 years in boys and

14 years in girls.[90] In this cohort of 330 adolescents aged between 10 and 18 years, 62% reported ever having unprotected sex and 42% had viral loads greater than 1000 copies/mL, highlighting the risk of transmission to HIV negative sexual partners. A study done in the US found that in PHIV+ youth the rate of sexually transmitted infections (STIs) doubled in those aged between 18-30 years compared to those aged between 13-17 years. STIs were more common in those with lower CD4 cell counts and non-suppressive ART.[91] In South Africa, sexual violence is common among PHIV+ youth with one study reporting that a third of PHIV+ youth experience physical and or sexual violence ever.[92] There have been few studies that report on pregnancy outcomes in PHIV + adolescents. Women with perinatal HIV transmission seem to have higher viral loads at delivery than those without perinatal infection with a higher risk of HIV transmission to the infant. [93]

1.3.11 Malignancy

Despite ART, PHIV+ adolescents are at higher risk of malignancy than adolescents without HIV.[94] This may be from long-standing immune suppression and inflammation, HIV itself and co-infection with other oncogenic viruses. PHIV+ adolescents are at risk for HIV-related malignancies, such as non-Hodgkin's lymphoma (NHL) and Kaposi Sarcoma (KS). These have been described even in youth on long-term ART.[95] A study from the US that used record linkage between HIV and cancer registries and followed participants for ten years showed that, compared to the general population, children with acquired immunodeficiency syndrome (AIDS) had an increased risk of KS, NHL, and non-AIDS-defining cancers (NADC) with leiomyosarcoma being the most frequent NADC. This study showed that the risk of developing KS and NHL had decreased in the ART era; however, there was no decrease in the incidence of NADCs.[96] Data from South Africa from linked HIV programs and oncology units showed a cancer incidence rate of 82/100000 PY with the most common cancers being KS and NHL.[97] It has also been shown that children who experienced advanced immunosuppression before ART initiation and those who started ART at a relatively older age are at increased risk of cancer than those who were moderately immunosuppressed or started ART in infancy.[98] The former is the case for the majority of PHIV+ adolescents in SSA and other resource limited settings. It is essential that they are monitored for cancers as they age into adulthood. This could be done by more frequent clinical examination and additional full blood counts in those at high risk.

The risk of acquisition of oncogenic types of human papilloma virus (HPV) may be higher in female PHIV+ adolescents than HIV negative adolescents. One study found a higher prevalence of high risk HPV and abnormal cervical cytology than in HIV negative adolescents after adjusting for age, sexual history and pregnancy.[99] However, no higher prevalence of high-risk HPV types was found in PHIV+ males compared to HIV negative males.[100] The quadrivalent HPV vaccine was safe and immunogenic in boys and girls in a study done in Kenya.[101] Current WHO guidelines recommend a 3-dose series females with HIV (rather than the standard 2-dose series for HIV negative) following studies that showed lower antibody titres post vaccination in HIV+ compared to HIV negative women.[101]

1.4 Intercurrent hospitalization in PHIV+ adolescents

There are limited disaggregated data for hospitalization rates in PHIV+ adolescents. High rates of hospitalization are reported in older HIV+ youth. One study from the US reported that the 15 to18-year age group accounted for the highest percentage of hospitalizations (632/ 1343, 52.9%) in HIV+ children and adolescents aged below 18 years in 2012. In contrast, among HIV negative children and adolescents below 18 years, the 0-4 year age group accounted for the highest percentage of hospitalizations in this time period (810 168/1921984, 42.3%).[102]

Causes of hospitalization have changed after the introduction of ART and new immunizations. A study comparing hospital admission records of HIV+ and HIV negative children and adolescents in the US from 2003 to 2012 showed that pneumonia, pneumococcal disease, varicella / herpes zoster infections decreased over time in CLHIV.[102]

In the US, a multicentre cohort study of predominantly PHIV+ adolescents with a mean age of 20 years, conducted between 2008 to 2014, showed that psychiatric, neurodevelopmental disorders, asthma, pneumonia or genital tract infections were the most common comorbid conditions.[103] An increased incidence of substance or alcohol abuse, anxiety disorders, diabetes mellitus, Vitamin D deficiency or metabolic bone disorders and fractures were also reported. The standardized mortality rate was 31.5 times higher than that of the US population.[103] The most common cause of mortality amongst PHIV+ adolescents, however, was still uncontrolled HIV.[103] This was confirmed by comparison of two long-term US cohorts showing that older youth were at higher risk for viremia, low CD4+ T-cell counts and serious clinical events including mortality.[91] Adolescents between 15-19 years of age had a mortality rate 6 times higher than that of the US general population after adjusting for age, sex and race. However, metabolic and other complications potentially related to long-term ART were infrequent probably due to using less toxic drugs over time.[91]

There are limited data from resource-limited settings including LMICs despite these areas having the predominant burden of PHIV+ adolescents. A study of PHIV+ adolescents on ART from Asian countries reported a crude hospitalization rate of 2.2 per 100 PY. There were more infectious related morbidities in early adolescence with a trend toward non-infectious and treatment related morbidity in later adolescence.[104] A systematic review and meta-analysis found that globally the leading causes of hospitalization in CLHIV and HIV+ adults were ‘AIDS-related illnesses’ and bacterial infections.[105]

The review included a study showing that in-hospital mortality in Zimbabwean adolescents living with HIV was more than three times those adolescents without HIV.[106]

1.5 Mechanisms of morbidity in PHIV+ adolescents

Successful treatment of paediatric HIV has shifted focus to management of chronic morbidity. Recently, it was reported from some settings that there is a shift from infectious to non-infectious morbidities associated with inflammation, immunodeficiency and drug toxicity in older PHIV+ adolescents.[104] Persistent immune activation, possibly driven or exacerbated by cytomegalovirus co-infection and Vitamin D deficiency, may be associated with non-AIDS defining illnesses as seen in HIV+ adults.[107,108] However, due to challenges with lifelong adherence to ART, initial suboptimal regimens and adverse effects, many PHIV+ adolescents may have high viral loads. It is therefore likely that uncontrolled HIV infection will remain a mechanism for acute morbidity in some PHIV+ adolescents.

In summary, the main mechanisms for morbidity in PHIV+ adolescents are (i) uncontrolled HIV and infectious morbidity, (ii) persistent immune activation, inflammation and premature aging (iii) drug toxicity. These mechanisms may overlap.

1.5.1 Uncontrolled HIV and infectious morbidity

As CLHIV enter adolescence, those not on effective ART may become susceptible to opportunistic infections, including cryptococcal meningitis, TB and *Pneumocystis jirovecii* pneumonia (PCP)[109,110].

Adolescence is a period during which both the risk of *Mycobacterium tuberculosis* infection and TB disease increase compared to the pre-adolescent (5-9 years of age) period.[111] HIV is a risk factor for TB and has resulted in TB becoming an epidemic in youth in high prevalence HIV countries.[112] Although access to ART has led to a decrease in TB morbidity and mortality in adults and CLHIV, they remain at increased risk of TB disease compared to uninfected.[112-115]

A study from Ethiopia showed the incidence of TB in HIV+ adolescents was 16.32/100 patient years of observation (PYO) before ART start and 2.25/100 PYO after ART initiation.[116] This rate is around ten times higher than for HIV- negative adolescents in Uganda and South Africa.[117,118] This highlights that HIV is a risk factor for TB disease even in those on ART. A systematic review and meta-analysis showed that HIV infection increases TB incidence by a factor of 8 in children less than 15 years of age, however this review was not powered to look at age stratification.[119] It also highlighted that TB incidence increased with increasing degree of immunosuppression and that ART reduced the risk of

TB by around 70%.[119] PHIV+ adolescents who are symptomatic from HCLD may be also be overtreated for TB. There is currently no data of culture confirmed TB disease in HIV+ adolescents.[120]

The current generation of PHIV+ adolescents are at risk for under-vaccination and faster waning of immunity despite being established on ART.[121-123] However, with earlier initiation of ART, prior to or during the primary vaccination schedule vaccine-induced responses to common Expanded Program on Immunization (EPI) vaccines, such as diphtheria, pertussis, tetanus and polio, are maintained.[124,125]

The incidence of invasive pneumococcal disease has decreased markedly since the introduction of PCV. Despite initial studies showing vaccine efficacy for earlier PCV vaccines[126], recent studies showed a lack of vaccine efficacy in CLHIV, when using a 2+1 schedule, highlighting the fact that an additional booster may be necessary.[127,128] The World Health Organization (WHO) recommends a 3 primary, 2+1, schedule with the recommendation of an extra booster for CLHIV.[129] Strategies informing the best schedule are still unknown and new pneumococcal vaccines in development may prove cheaper, and more effective but sustained effective ART must accompany any chosen strategy.[130]

A recent measles outbreak in Botswana highlighted the vulnerability of PHIV+ adolescent population. The median age of youth living with HIV with measles was 13 years (IQR 10-15 years).[131] In 2017, WHO vaccination guidelines were updated to include an additional dose of measles vaccine in children following ART when CD4 % is between 20-25%, or in settings where no CD4 evaluation is available, 6-12 months after ART initiation.[132] For adolescents living with HIV, only those considered ‘measles susceptible’ should receive an additional dose.[133]

1.5.2 Persistent immune activation, inflammation and premature aging

Persistent immune activation in adults , predominantly activation of monocytes and macrophages and evidenced by high levels of inflammatory biomarkers, has been linked to chronic comorbidities in adults and more recently in PHIV+ adolescents.[134,135] Pathogenetic mechanisms of premature ageing have been described in adults, and these mechanisms in addition to other factors have recently been documented in children.[136] In general, PHIV+ adolescents with increased exposure to HIV viremia and longer exposure to ART may make them even more susceptible to premature aging and immune senescence than adults.[137] These pathogenic mechanisms include altered T and B cell profiles, altered NK profiles, and elevated markers of chronic inflammation. Epigenetic age acceleration in blood has been observed in the CTAAC cohort PHIV+ adolescents in South Africa and was associated with neurocognitive impairment.[136]

1.5.3 Drug toxicity and adverse events

PHIV+ adolescents who started earlier ART regimens may have experienced toxicity and severe adverse events. Some may have permanent lipoatrophy and lipodystrophy. Drug toxicity has resulted in chronic bone, kidney and metabolic morbidities.

Various ART regimens may decrease BMD. Tenofovir disoproxil fumarate (TDF) can cause bone loss but this association may not be sustained in the long term.[138] TDF can also cause 'wasting' of low molecular weight proteins and previously caused chronic renal failure but is now being replaced by Tenofovir Alafenamide (TAF), a prodrug of TDF that has been studied in adolescents and initially showed good safety profiles.[139]

The WHO recommended continued use of stavudine in children after it was removed from adult guidelines. [140]This, along with the wider use of protease inhibitor (PI)-based therapy in children, may have contributed to higher rates of metabolic abnormalities in PHIV+ adolescents than in adults living with HIV.[141,142] Studies from Africa have shown more elevated cholesterol levels in children on PI regimens than in those on non-nucleoside reverse transcriptase inhibitors.[143] Switching to an alternative regimen (new generation PI or non-nucleoside reverse transcriptase inhibitor) improves dyslipidemia in children and adolescents and this is especially important in PHIV+ adolescents who face a lifetime of ART exposure.[144] Atazanavir (ATV), a newer generation PI, has the advantage of causing less dyslipidemia and allows for a daily regimen. In treatment-experienced children it is boosted with ritonavir (ATV/r). Switching to ATV/r may result in reduction in total cholesterol (TC) and TC: high -density lipoprotein cholesterol (HDL-C) ratio, and this may impact long-term cardiovascular disease.

While newer ART regimens may have fewer side effects, surveillance for adverse events remains crucial, especially during rapid scale up for example. Introduction of dolutegravir has been accompanied by concerns of increased weight gain compared to other ART and this may have consequences for PHIV+ adolescents with other underlying comorbidities and subclinical metabolic abnormalities.[145]

1.6 Multi-morbidity

Multi-morbidity is defined as more than one chronic disease in one person.[146] There is a paucity of studies from the ART era that address multimorbidity in PHIV+ adolescents. The adult literature describes an increase in NCD or what is termed non-AIDS defining illnesses.[1] In South Africa, an attempt to quantify multi-morbidity in an urban environment revealed that out of HIV patients attending a primary health care clinic 77% had hypertension, 24% had TB and 17% had diabetes mellitus Type

2. Of note, the presence of multimorbidity was higher in adults living with HIV on ART in the younger age groups (18-35 and 36-45 years) compared to older age groups (>45-55 years), with 26% in the 18-35 year age group and 30% in the 36-45 year age group having multi-morbidity.[13] As risk factors for multi-morbidity include time on ART, PHIV+ adolescents may be at increased risk as they progress through their life-course. In addition, those PHIV+ adolescents in SSA may be specifically vulnerable due to a lack of primary prevention of common NCD, a higher burden of inflammatory co-infections than in well- resourced settings, and the fact that although PHIV+ adolescents may have a greater duration of ART over time than adults, many of the current generation of PHIV+ adolescents will have accessed ART later than most adults.

1.7 Summary

PHIV+ adolescents may experience chronic and intercurrent morbidity from several causes.[147] [148] Strengths of existing knowledge base include a well-defined spectrum of knowledge of health related issues experienced by PHIV+ adolescents and young adults from well-resourced settings, allowing health service providers in resource-limited settings to already anticipate what PHIV+ in these settings may face in the near future.

Limitations of existing studies include cross-sectional methods, small sample sizes and absence of HIV negative adolescents for comparison, as well as a focus on a single organ system, rather than a holistic evaluation of health and multisystem involvement. Many PHIV+ adolescents studies are from high income settings where the burden of co-infections is much less than in SSA and other resource-limited settings. While risk factors for NCD may be present in this population, if the burden of acute infectious disease, specifically TB, remains high, urgent interventions to address this are needed before focusing on longer term strategies to reduce NCDs.

There is a need for a comprehensive approach to improve the long-term health of these youth.[149] The burden of multi- morbidity has not yet been studied in PHIV+ adolescents and this knowledge is crucial to inform policy makers especially in less resourced countries where there are competing demands.

Annex 1: Chronic comorbidities in children and adolescents with perinatally acquired HIV infection in sub-Saharan Africa in the era of antiretroviral therapy. *The Lancet Child & Adolescent Health* 2020

2. Study Methodology

2.1 Aim and Objectives

Overall aims:

(i) To investigate the spectrum of chronic morbidity in adolescents living with perinatally acquired HIV on ART.

(ii) To investigate the progression of disease and intercurrent morbidity over a 4 year period of follow-up in adolescents living with perinatally acquired HIV on ART.

Objective 1:

To describe the prevalence of insulin resistance and metabolic syndrome in adolescents enrolled in the CTAAC cohort at their first study visit.

Objective 2:

To describe the prevalence of risk factors for chronic kidney disease (proteinuria and microalbuminuria) in adolescents enrolled in the CTAAC cohort at their first study visit.

Objective 3:

To describe the prevalence of multisystem disease in individual PHIV+ adolescents enrolled in the CTAAC cohort at their first study visit. Systems that were explored included pulmonary, cardiac, neurological, renal and metabolic abnormalities.

Objective 4:

To describe causes of hospital admissions in in adolescents enrolled in the CTAAC cohort over a 4-year period.

Objective 5:

To describe incidence of Quanti-FERON (QFT) positivity and TB disease incidence in adolescents enrolled in the CTAAC cohort at their first study visit over a 4-year period.

2.2 Study design and population

The study was part of the Cape Town Adolescent Antiretroviral Cohort (CTAAC) that recruited adolescents living with HIV and HIV negative age-matched adolescents from 7 sites in the Western Cape in South Africa. Professor Heather Zar and Professor Landon Myer are the principal and co-principal investigators.The specific aims of the broader CTAAC study were:

1. To characterize physical and psychosocial development in a large cohort of perinatally-infected adolescents on ART and to investigate the predictors of development throughout adolescence.

2. To describe the neuropsychiatric status of children over time through neuroimaging and neurocognitive testing and associations with features of HIV disease and its management.

3. To describe the spectrum and progression of pulmonary disease, its association with OI history, and the prevalence, natural history and determinants of subclinical chronic pulmonary changes.

4. To investigate the evolution of cardiovascular dysfunction, using measures of cardiac structure/function and endothelial dysfunction, and relate these to markers of HIV disease and ART use.

5. To characterize the status of the musculoskeletal system and to describe the spectrum of rheumatic manifestations using validated screening tools.

6. To investigate patterns in the associations between abnormal neurological, pulmonary and cardiovascular structure and function in HIV-infected adolescents and to investigate the shared risk factors for multi-system pathology.

CTAAC is a prospective ongoing longitudinal cohort study. Enrollment took place from July 1, 2013 to March 31, 2015. For the longitudinal objectives (Objectives 4 and 5), data were censored on 31 October 2018.

2.2.1 Study setting

The study took place at the Research Centre and MRC unit on Child and Adolescent Health (REACH) at Red Cross War Memorial Children's Hospital, Cape Town, South Africa.

2.2.2 Participants

Five hundred and fifteen PHIV+ adolescents were enrolled from 7 different sites representing tertiary, secondary and primary level healthcare facilities in the Western Cape Province in South Africa. One hundred and ten HIV negative adolescents were recruited to provide locally-appropriate norms for several key measures. These adolescents were frequency-matched to sub-study participants on age and sex.

2.3 Inclusion Criteria

Inclusion Criteria for CTAAC were:

- PHIV+ adolescents aged 9-14 years, who were on ART for at least 6 months and resident in the Cape Town area and where informed consent and assent was provided for participation. Adolescents living with HIV had to have been disclosed to and were aware of their HIV status.
- HIV negative adolescents were healthy age-matched adolescents where informed consent and assent has been provided.

2.4 Routine Care and follow up

Enrolled adolescents received on-going clinical care at the site at which they were recruited, following standard protocols with 2-3 monthly medication refills, 1-3 monthly clinical assessments, and annual CD4 cell counts and viral loads or more frequent laboratory assessment depending on results. At each routine clinical care visit, site personnel completed the adolescents' clinical case record recording the patient visit, regimen, adherence, intercurrent medical events and most recent laboratory results.

2.5 CTAAC follow up

In addition to their routine clinical care, PHIV+ adolescents enrolled in CTAAC study had 6 monthly interviews and clinical examinations as well as annual detailed pulmonary, neurocognitive, cardiovascular and laboratory investigations. These included lung function testing, chest radiography, chest computer assisted tomography (CT), magnetic resonance imaging (MRI), a battery of neurocognitive screening tests, echocardiography and electrocardiograms. Laboratory investigations included HIV viral load, CD4 count, lipid profiles, urea, creatinine and electrolytes, urine dipstick, as well as stored urine for shipping to the US for microalbuminuria testing. Microbiological tests included sputum for TB gene Xpert and microscopy, culture and sensitivity. HIV negative participants had the same 6 monthly follow up and were retested for HIV on each study visit. Abnormal results were communicated to routine clinical care providers by study staff.

Hospitalization data were collected prospectively using participant interview from enrolment at each 6-monthly follow-up visit. Participants were often unaware of their hospitalization diagnosis; therefore, data on hospitalizations were requested from the Provincial Health Data Centre (PHDC) of the Western Cape Province, South Africa. The PHDC offers a service that provides consolidated provincial data on request.[150]

If participants missed study visits, they or their caregivers were contacted by phone. Reminders were sent to service providers and the parents of participants before study visits. A participant was considered withdrawn from the study if they had relocated to another province permanently, died, or specifically requested withdrawal from the study; however, with participants consent, we were able to track episodes of TB disease and hospitalizations from data from the PHDC until 31 October 2018.

2.6 Ethical considerations

This PhD was a sub-study of the Cape Town Adolescent Antiretroviral Cohort Study (REC Ref: 051/2013) for which all procedures and specimen collection were approved and for which consent and assent has been obtained from a parent and participant, respectively. Separate ethics permission from the Faculty of Health Sciences Human Research Ethics Committee was granted for this thesis. (REC Ref:057/2018).

2.6.1 Confidentiality and Anonymity

Strict confidentiality was maintained at all times. There were various standard operating procedures to minimize the risk of loss of confidentiality that have been included in the parent trial protocol. All participant related information is kept in locked files at the clinical research unit; all staff working on the parent study have undergone specific training in confidentiality and related patient protection issues (in addition to routine Good Clinical Practice (GCP) and Human Subjects Protection training).

Anonymous participant identification numbers were used on all study documents. Collection of participant names and other identifiers were restricted to informed consent documents, patient tracing materials and a study identification key, all of which were kept in a locked cabinet separate from other study documentation and accessible only by the study coordinator and study doctor. No study clinical record included the participant name, including records that may reflect HIV test results or information about ART use.

2.7 Data Analysis, sample size and power calculations

2.7.1 Objective 1: To describe the prevalence of insulin resistance and metabolic syndrome.

The outcomes for this analysis were:

(i) The prevalence of insulin resistance (IR) at enrolment. This was the primary outcome. This was measured using the homeostatic model assessment (HOMA). IR was defined as a HOMA >2.5 in Tanner stage 1 patients or >4.0 in Tanner stage 2-5 based on previous thresholds in the literature.

(ii) The prevalence of metabolic syndrome was a secondary outcome, defined according to the recent IDF consensus definition: abdominal obesity (WC >90th percentile) plus >2 criteria (hypertriglyceridemia, low HDL, hypertension, increased plasma glucose).

The sample size for this analysis was limited by those who had insulin and glucose results available (n=385 PHIV+ and n=63 HIV- adolescents). Variables from the study entry visit were compared between groups using t tests, Wilcoxon, and chi-square tests as appropriate.

Rates of IR between PHIV+ and HIV- participants were compared using chi-square tests in univariate analysis, and logistic regression was used to assess the association between HIV infection and IR adjusting for confounders. Among PHIV+ adolescents, multivariable linear regression was performed to evaluate factors associated with increased HOMA while adjusting for covariates.

2.7.2 Objective 2: To describe the prevalence of risk factors for renal disease.

The outcomes for this analysis were:

(i) The prevalence of proteinuria, defined as > 500 mg/dl and measured by dipstick.

(ii) The prevalence of microalbuminuria, defined as a urinary albumin/creatinine ratio of > 30 mg/g.

The sample size for this analysis was limited by the number of participants who had samples available for the assessment of microalbuminuria (n=511 PHIV+ and 109 HIV- adolescents).

Univariate analysis was performed to evaluate factors associated with microalbuminuria in PHIV+ adolescents.

2.7.3 Objective 3: To describe the prevalence of multisystem disease in individual PHIV+ and HIV- adolescents.

The primary outcomes for this analysis were the number of participants with single, dual and multisystem impairment. Single, dual and multisystem impairment were defined as having impairment of only one (single), only two (dual) or three or more (multisystem) of the following systems: cardiac, respiratory, neurological or renal. The sample size was derived from those who had completed study measures for all 4 systems (n=384 PHIV+ and 95 HIV- adolescents). Baseline variables and outcomes for PHIV+ and HIV negative adolescents were compared using t-tests, Wilcoxon and chi-square tests as appropriate. Among PHIV+ participants, logistic regression was performed to evaluate factors associated with dual or multisystem impairment.

2.7.4 Objective 4: To describe causes of hospital admissions over a 4-year period.

The primary outcome for this analysis was to describe the incidence and causes of hospitalisation over time. No sample size was calculated. Data was collected from July 2013 to October 2018 from

PHIV+ and HIV- adolescents. Participants were assessed every 6 months and data on intercurrent hospitalization were abstracted. Causes of hospitalizations were classified according to ICD-10 codes. Descriptive statistics, time-to-event analysis and Poisson regression were used to describe causes and incidence and to determine incidence rate ratios for factors associated with hospitalization.

2.7.5 Objective 5: To describe incidence of QuantiFERON (QFT) positivity and TB disease incidence over a 4 -year period.

The primary outcomes of this analysis were the incidence of a positive QFT and of TB disease over 4 years of follow-up time. TB infection was defined by a QFT of >0.35 IU/ml. TB diagnosis was defined as definite (culture-confirmed) or unconfirmed (clinical signs and symptoms and started on TB treatment). Time to event and regression analyses were used to describe the incidence and determinants of TB infection and disease.

The sample size was fixed by the parent study (515 PHIV+ 110 HIV- adolescents), a formal power calculation was only defined (retrospectively) for the TB incidence objective. The available sample of 625 (515 PHIV+ 110 HIV- adolescents) was estimated to provide 87% power to detect a relative hazard for incident TB of at least 1.4, two-sided alpha=0.05. Data was collected from July 2013 to October 2018 from PHIV+ and HIV- adolescents were analyzed.

All data was analysed using Stata version 13.1. to 15.1 StataCorpInc. College Station, Texas USA.

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Chapter 2:

Insulin Resistance in South African Youth Living with Perinatally Acquired HIV Receiving Antiretroviral Therapy.

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Abstract

Objectives: To investigate the prevalence of and risk factors for insulin resistance (IR) in a cohort of youth living with perinatally acquired HIV (YLPHIV) receiving antiretroviral treatment (ART).

Methods: A cross sectional analysis of IR in YLPHIV and age-matched HIV-uninfected youth enrolled in the Cape Town Adolescent Antiretroviral Cohort (CTAAC). South African youth aged 9-14 years with perinatally acquired HIV who were on ART for >6 months and age matched HIV-uninfected adolescents were eligible. The Homeostatic Model Assessment-Insulin Resistance (HOMA), calculated from fasting insulin and glucose measurements at enrolment, was used to assess IR. Multiple linear regression was used to examine adjusted associations between HOMA and HIV-related and traditional cardiovascular risk factors.

Results: Of 448 adolescents, 385 (85.9%) were YLPHIV; median age was 12.1 years [Interquartile Range (IQR): 10.8 – 13.5]. 50.4% were female. Median duration on ART was 7.5 (IQR:4.5-9.2) years. The prevalence of IR in YLPHIV was 18%. Among YLPHIV, waist circumference (ß=0.01, p=0.01), hypertriglyceridemia (ß=0.07, p=0.01), CD4 count >500 cells/mm^3 (ß= 0.08, p=0.02) or prior use of Abacavir (ß=0.06, p=0.04), were associated with increased HOMA, after adjusting for age, sex, body mass index and Tanner stage.

Conclusions: In a South African cohort of YLPHIV on ART, IR was not significantly different from uninfected adolescents. YLPHIV with traditional cardiovascular risk factors or Abacavir exposure may be at higher risk for IR.

Background

Metabolic complications, including insulin resistance (IR), are increasingly reported in youth living with perinatally acquired HIV (YLPHIV). Widely variable rates of insulin resistance (IR) have been reported in children and adolescents on antiretroviral therapy (ART) ranging from 0.0 to 52.0%[1-4], which may reflect variations in age, Tanner staging, ethnic and genetic differences, small sample sizes, and differing methods of measuring IR. Reports from middle income countries in Latin America and Thailand show rates of 6.8% and 6.5% in YLPHIV on ART.[5,6] There is relatively little data from Africa: two Ugandan studies; one reporting no hyperglycaemia (insulin was not measured) [4]; the other, done in younger children found an increase in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) one year after ART initiation and higher HOMA-IR in children on Abacavir.[7] Two South African studies done in younger children found an IR prevalence of 1.9 to 10%.[8-10] Both South African studies, however, had a relatively small sample size of 100 to 156 children and used different definitions of IR.

Common risk factors for IR are puberty, increased body mass index (BMI), and a family history of diabetes.[10] A study of children and YLPHIV reported an IR prevalence of 15.2% that was more closely linked to obesity than any other HIV-related variable.[1] A longitudinal study of the same cohort showed female sex, waist circumference and BMI were associated with incident or resolved insulin resistance as in HIV negative youth.[11]

Development of IR in patients with HIV is thought to be multifactorial and includes factors related to HIV and ART. Nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) can cause IR by direct inhibition of the insulin-responsive facilitative glucose transporter isoform (GLUT4).[12,13]

IR precedes the development of metabolic syndrome. Metabolic syndrome is defined as hypertension, low high-density lipoprotein-C (HDL)-cholesterol levels, hypertriglyceridemia and abdominal obesity.[14] Metabolic syndrome is an independent risk factor for cardiovascular disease. The prevalence of metabolic syndrome in a Spanish PHIV cohort of children was 1.97% using the International Diabetes Federation (IDF) criteria.[15] The prevalence of metabolic syndrome in YLPHIV in Africa is unknown.

The primary objective of this study was to investigate the prevalence of and risk factors for IR in a stable cohort of African YLPHIV on ART. A secondary objective was to investigate the prevalence of metabolic syndrome.

Methods

Study Population

The Cape Town Adolescent Antiretroviral cohort (CTAAC) is a longitudinal cohort study which enrolled 515 YLPHIV aged 9-14 years on ART for more than 6 months from 7 sites in Cape Town, South Africa and 110 age-matched HIV-uninfected youth of similar ethnicity from July 2013 to March 2015. There were no other exclusion criteria, and participants did not have to be virally suppressed at enrolment. Ethical approval was given by the Faculty of Health Sciences, University of Cape Town and Stellenbosch University, Human Research Ethics Committee (051/2013).[16] Parents gave informed consent and assent was obtained from all adolescents. All YLPHIV knew their HIV status as a pre-requisite to study enrolment.

Primary Outcome

The primary outcome was IR at enrolment visit. HOMA (defined as fasting insulin [mIU/L] x fasting glucose [mmol/L] divided by 22.5)[17] was used to assess IR. IR was defined as a HOMA >2.5 in Tanner stage 1 patients, or >4.0 in Tanner stage ≥2 based on previous thresholds in the literature.[18] Participants were fasting for approximately 12 hours. Glucose and insulin were measured on stored frozen samples.

Secondary Outcome

The prevalence of metabolic syndrome was a secondary outcome, defined according to the recent International Diabetes Federation (IDF) consensus definition: abdominal obesity (waist circumference > 90th percentile) plus >2 criteria (hypertriglyceridemia, low high density lipoprotein (HDL), hypertension, increased plasma glucose).[14]

Covariates

Routine sociodemographic data were collected at enrolment and the participant's clinical record was reviewed at their primary treatment facility. A physical examination including Tanner staging, WHO HIV staging, blood pressure (BP) and anthropometry was performed at enrolment. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). BMI was classified according to WHO reference standards.[19] BP was measured using an electronic sphygmomanometer (Spot Vital Signs, Welch Allyn). Waist circumference (WC) was measured in centimeters midway between the superior border of the iliac crest and the lower most margin of the ribs at the end of normal expiration. Median thigh circumference and median upper arm circumference were measured in centimeters using standard techniques.[20] All anthropometric measures were performed by one of two trained study nurses to ensure standardization of measures.

Laboratory measures performed at enrolment included HIV viral load (Roche COBAS Ampliprep/Taqman) and CD4 count in HIV-infected and fasting lipid sub-fractions including total cholesterol (TC), triglycerides (TG), HDL and low- density lipoprotein-C (LDL). Abnormal TC, HDL, and LDL were defined as >5.18, <1.03 and >3.37 mmol/L, respectively. Abnormal triglycerides were defined as >2.85 mmol/L if age <10 years or >3.89 mmol/L if age ≥10 years at enrolment visit.[21] Insulin was measured using an electrochemiluminescence immunoassay, Cobas 6000, Roche Diagnostics USA (Indianapolis, IN) and glucose using the enzymatic method, Cobas 6000, Roche Diagnostics USA (Indianapolis, IN).

Statistical Analysis

Baseline variables were compared between groups using t-tests, Wilcoxon, and Chi-square tests as appropriate. HOMA was log transformed to approximate a normal distribution. Rates of IR between YLPHIV and HIV-uninfected participants were compared using Chi-square tests in univariate analysis and logistic regression modelling was used to assess the association between HIV infection and IR adjusting for confounders. Amongst YLPHIV, multivariable linear regression modelling was performed to evaluate factors associated with increased HOMA while adjusting for age, gender, BMI, and Tanner stage. Covariates considered for associations with HOMA included anthropometry (BMI and waist circumference), HIV laboratory parameters, metabolic parameters, and duration and type of ART. Statistical analysis was performed using Stata version 14.1. StataCorpInc. College Station, Texas USA.

Results

Among the 625 adolescents enrolled in CTAAC, 448 (71.7 %) had samples available for HOMA (385 YLPHIV and 63 HIV-uninfected). One child with diabetes (defined as a fasting glucose of >6.9mmol/l) was excluded from this analysis. Median age, sex, and family history of diabetes were similar between YLPHIV and -uninfected adolescents (Table 1). Only 3 participants smoked tobacco at enrolment.

Median BMI was lower in YLPHIV (17.2 vs. 18.1 kg/m², p=0.01). Sixteen (4%) YLPHIV were obese vs. 7 (11%) HIV-uninfected adolescents (p=0.02). YLPHIV were more likely to be Tanner Stage 1 (pre-pubertal) than uninfected adolescents (50.1% vs. 33.9%, p= 0.02). Mean mid-thigh circumference was lower in YLPHIV than uninfected youth (39 vs. 44 cm, p=0.01). Systolic blood pressure was also lower (105 vs. 109 mm Hg, p<0.01). Median fasting glucose (86.8 vs 83.4 mg/dL, p =0.01), triglycerides (0.9mmol/L vs. 0.6mmol/L, p<0.01), total cholesterol (4.1mmol/L vs. 3.8mmol/L, p<0.01), and rates of hypercholesterolemia (11.6% vs. 3.2%, p=0.04) were higher in YLHIV (Table 1).

Amongst YLPHIV participants, median duration on ART was 7.46 (4.48-9.23) years, and median age at ART initiation was 4.6 (2.12-7.72) years. Approximately 58.7% were receiving Efavirenz-based ART with the remainder (36.9%) on Lopinavir/ritonavir-based ART. Seventy two percent received an Abacavir-containing ART. Median duration of Abacavir was 2.8 years. Eighteen percent had CD4 cell counts >1000 cells/mm^3 and 75.1% had a viral load < 50 copies/mL at enrolment. Nadir CD4 cell count was poorly documented at enrolment sites.

YLPHIV had an IR rate of 18.0% similar to uninfected youth (18% vs. 20%, p=0.17). This relationship remained unchanged after adjustment for age, sex, family history of diabetes, BMI Z-score, Tanner stage and waist circumference (adjusted Odds Ratio: 0.86, p=0.70).

In subgroup analysis of YLPHIV participants, waist circumference (ß=0.01, p=0.01), hypertriglyceridemia (ß=0.07, p=0.01), CD4 count >500 cells/mm³ (ß= 0.08, p=0.02) and 'ever' (previous or current) use of Abacavir (ß=0.06, p=0.04), were associated with increased HOMA, after adjusting for age, sex, BMI and Tanner stage (Table 2). There was no association between being on a protease inhibitor regimen and insulin resistance.

One female YLPHIV and one HIV-uninfected youth met criteria for metabolic syndrome.

Discussion

We found an 18.5% prevalence of IR in this cohort of YLPHIV, with similar rates in YLPHIV and uninfected youth. Recognition of IR is important as it represents a low level inflammatory state that can lead to early cardiovascular disease (CVD) in children.[22] Subclinical atherosclerosis has been shown in HIV-infected children on ART for more than 6 months.[23-25] YLPHIV may be at further risk of CVD due to their lifelong exposure to both HIV and ART. In children without HIV, rates of IR have been reported as 3% in young non-obese children and up to 40% in obese children and adolescents.[18] The fact that Black South Africans may have an increased genetic predisposition to Type 2 diabetes[26] may explain the finding of the high prevalence of IR in this cohort. In addition, an increasingly urban high fat diet may be contributing to high rates of IR in South Africa.[27] The median value of 1.9 was higher than in an HIV-uninfected cohort of European pre-pubertal adolescents between 10.5 years to 11 years who had a median HOMA between 1.7(girls) and 1.4 (boys).[28]

The IR prevalence of 20% in our study is similar to the 15.2 % prevalence reported in earlier cohorts of US children on ART and YLPHIV in the Paediatric HIV/AIDS Cohort Study (PHACS) Adolescent Master Protocol (AMP) study. In the PHACS cohort, HOMA was associated with higher alanine transferase (ALT), BMI, nadir CD4%, Tanner Stage 5 and ever having received amprenavir.[1] Another smaller US cohort reported a 33% prevalence, similar to that in adults living with HIV.[2] The IR rate in our cohort was above the 6.8% and 6.5% described in more recent Latin American and Thai cohorts.[5,6] Differences in rates of IR between our South African cohort and the Thai cohort are most likely explained by criteria used to define IR. The Thai study used a threshold of $\geq$3.16 which did not take into account pubertal stage. However, the PHACS and Latin American cohort studies used the same HOMA thresholds to define IR as our study (>2.5 for prepubertal and >4 for pubertal individuals). Of note, the median HOMA in our cohort of YLPHIV was higher than in the PHACS cohort (2.1 vs 1.0). In a large US study, a 1 unit rise in HOMA has been found to be associated with increased mortality in adults without diabetes.[29] The only other study done in 100 South African children living with HIV showed a prevalence of 10%, which may be explained by the lower age of this cohort of 6.5 years.[9]

Amongst YLPHIV in our cohort, traditional risk factors for IR such as waist circumference and hypertriglyceridemia were associated with increased HOMA. Waist circumference was associated with incident insulin resistance in a longitudinal study of HOMA-IR in the US. [11] In the Spanish cohort, waist circumference but not hypertriglyceridemia was associated with increased HOMA.[30] Hypertriglyceridemia is associated with IR in adults on protease inhibitors. With regards to lipid abnormalities in children and youth, other cohorts have suggested a higher rate of hypertriglyceridemia from 12%[6] to 33.6%[5] with a median age at start of ART of 10.6 and 7.1 years and a median duration of ART of 3 and 5.6 years, respectively. The rate of hypertriglyceridemia in our cohort was 8.3% in YLPHIV. This is surprising as lipid abnormalities may have been expected to be more pronounced given the longer the duration of ART use. Those adolescents on a PI regimen had a higher rate of hypertriglyceridemia than those on a NNRTI regimen (65.6% vs 34.4%, p=0.00). This rate was not significantly different to HIV-uninfected adolescents in our cohort but this may be due to a small sample size.

In addition, in subgroup analysis of YLPHIV, Abacavir usage was associated with higher HOMA. While there are no studies on Abacavir and cardiometabolic outcomes in youth, in adults living with HIV, Abacavir increases the risk of cardiovascular disease , a known potential sequela of IR.[31] In YLPHIV, a higher prevalence of risk factors for subclinical vascular disease including a higher Pathobiological Determinants of Atherosclerosis (PDAY) score have been documented.[32-34] In YLPHIV with a high PDAY score, a switch from an Abacavir-containing regimen may be warranted.

The finding that a higher CD4 count was associated with IR may reflect the fact healthier YLPHIV are likely to have more fat reserves potentially resulting in increased rates of IR. Unlike in other studies,[1,8,13] we found no association between PI usage and increased HOMA-IR in our cohort. This may be because we were unable to assess total duration on a PI regimen. Another South African study assessing HOMA- IR in a younger cohort of children also found no association between type of ART and IR.[9]

Only two participants had metabolic syndrome according to the IDF definition. Metabolic syndrome has been described in other adolescent HIV cohorts.[15] A recent Spanish study found a metabolic syndrome prevalence of 1.97% but when a different classification was used (National Cholesterol Education Program Adult treatment Panel III NCEP-ATP III) a prevalence of 5.92% was found.[15] This difference was thought to be attributable to the waist circumference that is crucial for the IDF classification. In our cohort, three adolescents had waist circumferences meeting the criteria for central obesity. IR in the Spanish cohort occurred in 17 patients (11.18%) and associated with metabolic syndrome if the NCEP-ATP III criteria were used. The low prevalence of metabolic syndrome in our cohort may be due to the low rate of hypertension and younger age (the metabolic syndrome is considered more common in late puberty- Tanner Stage 5), with half our cohort being pre-pubertal.[35]

This study was limited by the small size and lack of rigorous Tanner stage and BMI matching of the comparison group. However, it was one of the largest in sub-Saharan Africa to characterize metabolic parameters amongst YLPHIV. In addition, the cross-sectional study design precluded our ability to draw causal inferences, but future studies in our longitudinal cohort will allow us to further explore our current findings especially the association between Abacavir and IR. Glucose and Insulin were measured using previously stored frozen samples and this could have resulted in lower HOMA values as insulin may be underestimated in frozen samples.

Conclusions

In a South African cohort of YLPHIV, although a high prevalence of IR was found, it was similar to that in uninfected age-matched adolescents. In addition to traditional risk factors, such as waist circumference and hypertriglyceridemia, Abacavir exposure may be associated with increased HOMA. This finding needs further exploration. Longitudinal follow up is needed to assess which adolescents develop IR and what factors are associated with this.

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L.J.F performed data quality and statistical analyses, and wrote the manuscript; J.J. was involved in initial idea conception and wrote the manuscript; S.M. collected data, did data quality and statistical analysis, N.A.A.A collected data and data quality analysis, M.F.C was involved in overall cohort design and obtained funding, L.M. was involved in initial idea conception, overall cohort design and obtained funding. H.J.Z. was involved in oversight of the study, obtained funding, and wrote the manuscript. The authors acknowledge Ekkehard Zöllner, and CTAAC co-investigators: Helena Rabie, James Nuttall, Brian Eley, Linda Gail-Bekker, Paul Roux and the CTAAC study staff, caregivers and adolescents.

All authors declare no conflict of interest.

Table 1. Baseline Characteristics of YLPHIV and HIV-uninfected Adolescents

	YLPHIV (n=385)	HIV-uninfected (n=63)	p value
Demographics			
Age (years)	12.1 (10.8-13.5)	11.9 (10.0-13.4)	0.16
Female	194 (50.4)	29 (46.0)	0.52
Black African	354 (92.0)	63 (100)	0.02
Family history of diabetes	80 (20.89)	13 (20.63)	
Blood Pressure (mm Hg)			
Systolic (mean SD)	105.0 (11.5)	109.5 (10.5)	<0.01
Diastolic (mean SD)	67.0 (9.1)	68.2 (9.4)	0.32
Growth Measures			
BMI (kg/m^2)	17.2 (16.0-19.1)	18.1(16.5-20.4)	0.01
Waist circumference (cm)	61 (58-66)	64 (57-70)	0.21
Mid-thigh circumference (cm)	39 (37-44)	42 (38-46)	<0.01
Mid upper arm circumference (cm)	20.5 (19-22)	21 (19-23.5)	0.07
Tanner Stage			
1	190 (50.1)	21 (33.9)	0.07
2	85 (22.4)	18 (29.0)	
3	53 (14.0)	8 (12.9)	
4	30 (7.9)	10 (16.1)	
5	21 (5.5)	5 (8.1)	
Puberty			
Pre-pubertal (Tanner stage 1)	190 (50.1)	21 (33.9)	0.02
Pubertal (Tanner stages 2-5)	189 (49.9)	41 (66.1)	
Laboratory measures			
Triglycerides	0.9 (0.7-1.1)	0.6 (0.5-0.8)	0.00
Hypertriglyceridemia	32 (8.3)	1 (1.6)	0.07
Total Cholesterol	4.1 (3.6-4.6)	3.8 (3.4-4.2)	<0.01
Hypercholesterolemia	44 (11.6)	2 (3.2)	0.04
LDL	2.2 (1.8-2.6)	2 (1.5-2.4)	
HDL	1.5 (1.2-1.7)	1.5 (1.3-1.7)	0.60
Insulin (mIU/L)	9.6 (6.6-14.3)	9.7 (6.1-19.4)	0.57
Log Insulin	0.99 (0.25)	1.0 (0.38)	0.32
Glucose/Insulin Ratio	8.6 (4.2-13.3)	8.9 (6.2-12.7)	0.21
Glucose (mg/dL)	86.8 (9.0)	83.4 (9.0)	0.01
HOMA	2.1 (1.4-3.2)	1.9 (1.2-3.7)	0.98
Log HOMA	0.3 (0.3)	0.3 (0.4)	0.62
Viral Load (copies/mL)			
<50	289 (75.06)	---	----
50-1,000	44 (11.43)	---	----
1,001-10,000	28 (7.27)	---	----
>10,000	24 (6.23)	---	----
CD4 count (cells/mm³)			
<200	7 (1.83)	---	----
200-499	58 (15.14)	---	----
500-1000	248 (64.75)	---	----
>1000	70 (18.28)	---	----
WHO HIV Staging			
Stage I	24 (6.49)	---	----
Stage II	43 (11.62)	---	----
Stage III	217 (58.65)	---	----
Stage IV	86 (23.24)	---	----
Missing value	15	---	----
Age at initiation of ART (years)			
Median age	4.6 (2.12-7.72)	---	----
0-2	126 (33.4)	---	----
3-5	108 (28.65)	---	----

6-14	143 (37.93)	---	----
Missing values	8	---	----
Duration on ART prior to enrolment (years)	7.46 (4.48-9.23)		
Current ART regimen			
2 X NRTI + NNRTI	226 (58.7)	---	----
2 X NRTI + PI	142 (36.88)	---	----
Other	9 (2.34)	---	----
Unknown	8 (2.08)	---	----
Currently on D4T	35 (9.1)	---	----
Currently on DDI	7 (1.82)	---	----
Currently on ABC	280 (72.7)	---	----
Currently on AZT	60 (15.58)	---	----
Ever on D4T	261 (67.79)	---	----
Ever on DDI	34 (8.83)	---	----
Ever on ABC	305 (79.22)	---	----

All continuous variables expressed as median (interquartile range) or mean (SD) and categorical variables as number (%). YLPHIV = Youth Living with Perinatally Acquired HIV BMI = body mass index; LDL = low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol; HOMA = Homeostatic Model Assessment; WHO = World Health Organization; ART = antiretroviral treatment; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; D4T = stavudine; DDI= didanosine; ABC = abacavir; AZT = zidovudine

Table 2. Linear regression models for key predictors of HOMA amongst Youth Living with Perinatally Acquired HIV in CTAAC

Model	ß Coefficient	*p* value
Waist circumference§	0.01	0.01
Mid upper arm circumference§	0.02	0.02
Systolic Blood Pressure§	0.00	0.18
Triglycerides§	0.07	0.01
CD4 count		
≤499	Ref	
≥500	0.08	0.02
Ever on Abacavir		
Not exposed	Ref	
Exposed	0.06	0.03

All models adjusted for age, sex, BMI and Tanner stage

§ Continuous variable

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Chapter 3:

Prevalence of risk factors for chronic kidney disease in South African Youth with Perinatally-Acquired HIV

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Abstract

Background: Little is known about renal pathology among children and adolescents living with HIV in Africa. We assessed the prevalence of risk factors for chronic kidney disease in South African children and adolescents with perinatally acquired HIV-1 (HIV+) on antiretroviral therapy (ART) and HIV negative children and adolescents.

Methods: HIV+ youth aged 9-14 years, on ART for >6 months and age matched HIV negative children and adolescents were eligible for assessment via urine dipstick and microalbuminuria. Blood pressure, glomerular filtration rate, HIV-related variables and metabolic co-morbidities were assessed at enrolment.

Results: Amongst 620 children and adolescents, 511 were HIV+. The median age was 12.0 years and 50% were female. In HIV+, 425 (83.2%) had a CD4 count >500 cells/mm^3 and 391 (76.7%) had an undetectable viral load. The median duration of ART was 7.6 years (IQR: 4.6-9.3) with 7 adolescents receiving Tenofovir disoproxil fumarate. The prevalence of any proteinuria or microalbuminuria was 6.6% and 8.5% respectively, with no difference between HIV+ and negative children and adolescents. All participants had a normal glomerular filtration rate.

Conclusions: Proteinuria and microalbuminuria appeared uncommon in this population. Follow-up of those with microalbuminuria may inform long term outcomes and management of this growing population of HIV+ youth.

Keywords: HIV, perinatal, children, adolescents, microalbuminuria, South Africa

Introduction

In 2016 there were an estimated 2.1 million children younger than 15 years living with HIV, the majority of whom live in sub-Saharan Africa (SSA).[1] Prior to widespread access to antiretroviral therapy (ART), the most common cause of chronic kidney disease (CKD) in HIV-infected children was HIV-associated nephropathy (HIVAN).[2] Access to ART has markedly decreased the incidence of HIVAN, and provides renal benefits to prevent CKD in HIV+ people. The benefits of ART on non-HIVAN renal diseases are not yet clear, but there may be beneficial renal effects by decreasing the inflammatory response associated with HIV infection. ART, specifically Tenofovir disoproxil fumarate (TDF) increases the risk of proteinuria in HIV+ children and adolescents.[3,4] Despite the decrease in HIVAN, results from a large US cohort show that kidney disease is one of the ten most common non-infectious conditions occurring in children and adolescents with perinatally acquired HIV infection in the ART era with an incidence rate of 2.6 per 100 patient years.[5]

There are, however, few data from SSA on the impact of long term ART use in perinatally infected HIV+ youth on renal disease. This is compounded by little surveillance of kidney disease in HIV+ children and adolescents in SSA.[6] Some South African studies suggest resolution of proteinuria after starting ART in children with HIVAN with a study in KwaZulu Natal showing 30/40 (75%) of children had a greater than 50% reduction in proteinuria after starting ART.[7]

Proteinuria is a marker of glomerular injury in HIV-related renal disease in children. However, the time from onset of proteinuria to development of CKD can vary from 5 months to 10 years, depending on whether the children receive appropriate ART.[8,9] Microalbuminuria is a marker of endothelial and/or renal injury, associated with increased inflammatory activity.[10,11] Previous studies in HIV+ adults have shown that decreased CD4 counts, higher HIV RNA levels and non-nucleoside reverse transcriptase inhibitors are associated with increased prevalence of microalbuminuria.[10] Studies have reported that microalbuminuria is present in 11-15% of HIV-infected children on ART in the US, Spain and Brazil.[12-14] However, an Indian study reported that in a cohort of HIV+ children on ART with a mean age of 11.5 years, 20% had microalbuminuria.[15] African studies report rates of microalbuminuria in children ranging from 28.8% in Tanzania[16] to 12% in Nigeria[17], although the majority were not on ART in the Nigerian study and viral load was unavailable in the Tanzanian study.

The primary aim of this study was to investigate the prevalence of proteinuria and microalbuminuria in a cohort of perinatally infected South African HIV+ adolescents on ART, compared to HIV-negative adolescents. A secondary aim was to describe the prevalence of other potential risk factors for CKD such as hypertension and metabolic abnormalities.

Methods

Study Population

This study included all participants enrolled in the Cape Town Adolescent Antiretroviral cohort (CTAAC), a longitudinal cohort study which enrolled 515 HIV+ children and adolescents aged 9-14 years on ART for over 6 months from 7 sites in Cape Town, South Africa and 110 age-matched HIV negative youth of similar ethnicity from July 2013 to March 2015.[18]

Ethical approval was given by the Faculty of Health Sciences, University of Cape Town and Stellenbosch University, Human Research Ethics Committee (051/2013). Parents gave informed consent and assent was taken from all adolescents. All HIV+ participants knew their HIV status as a pre-requisite to study enrolment.

Primary Outcome

The primary outcome of interest was proteinuria defined as >500mg/dl and measured by dipstick or microalbuminuria defined as a urinary albumin/creatinine ratio of >30mg/g.[19,20]

Sociodemographic data were collected as part of the enrolment questionnaire and the participants' clinical records were reviewed at their primary treatment facility.

Physical examination including Tanner staging, World Health Organization (WHO) HIV staging, blood pressure (BP) and anthropometry was performed at enrolment. Weight was measured in kilograms on a Scales 2000® digital scale to the nearest 0.1kg. The standing height was measured using a stadiometer with a moveable headboard in centimetres. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). BMI was classified according to WHO reference standards.[19,21] BP was measured using an electronic sphygmomanometer (Spot Vital Signs, Welch Allyn®) validated in children.[22] Hypertension was defined as blood pressure measurements >95th percentile for height, age, and sex.[23]

All anthropometric measures were performed by one of two trained study nurses in order to ensure standardisation of measures.

Laboratory measures included viral load (Roche COBAS Ampliprep/Taqman®) and CD4 count (Beckman Coulter®) in HIV+ and urea and electrolytes. Abnormal total cholesterol (TC), high density lipoprotein (HDL), and low-density lipoprotein (LDL) were defined as >5.18, <1.03 and >3.37 mmol/L, respectively. Abnormal triglycerides were defined as >2.85 mmol/L if age <10 years or >3.89 mmol/L if age ≥10 years at the time of baseline investigations.[24] Insulin was measured using an electrochemiluminescence immunoassay (Cobas 6000, Roche USA® and glucose using the enzymatic method (Roche Cobas 6000, Roche USA®). An abnormal Highly Sensitive (hs) CRP (Ultrasensitive CRP) was defined as a hs-CRP between 1-5.0mg/L.

Serum creatinine was measured in micromoles per litre by the enzymatic method. The modified Schwarz formula was used to estimate glomerular filtration rate (GFR).[25] A GFR below 90ml/min/1.73m^2 was considered abnormal. If creatinine measures were unavailable at enrolment, GFR was calculated from clinical and laboratory measures performed at the 6 month visit.

Urine was obtained from an early morning sample and was tested with Multistix® dipstick for proteinuria. Proteinuria was defined as >500mg/dL. Laboratory analysis of microalbuminuria was done using the Siemens DCA Vantage Analyzer®. Microalbuminuria was defined as an albumin/creatinine ration of >30mg/g.

Statistical Analysis

Baseline variables were compared between groups using t-tests, Wilcoxon and Chi-square tests and odds ratios where appropriate. Univariate analysis was performed to evaluate factors associated with microalbuminuria in HIV-infected children and adolescents. Covariates considered for associations with microalbuminuria included anthropometry, HIV laboratory parameters, metabolic parameters and duration and type of ART. Statistical analysis was performed using Stata version 14.1. StataCorpInc. College Station, Texas USA.

Results

Six hundred and twenty participants (511 HIV+ and 109 HIV-uninfected adolescents) had samples available for microalbuminuria. Median age and sex were similar between those with and without HIV infection (Table 1). The median BMI and median height ,17.1 (IQR:15.9-18.9) vs. 18.7 (16.8-21.5) kg/m^2, *p*=<0.01 and 140.2 (IQR:132.5-147.6) vs. 142.8 (IQR:136.5-155) cm, *p*=<0.01, was lower in HIV+ than in HIV- adolescents.

Puberty was delayed in HIV+ youth with 49.7% being Tanner Stage 1 compared to 32.4% HIV negative adolescents (*p*=<0.01).

Systolic blood pressure was lower in HIV+ than in HIV negative (105 (IQR:98-112) vs. 108 (100-118) mm Hg, *p*<0.01). Eighty six participants had hypertension (67 HIV+ and 19 HIV-, 13.1% vs. 17.4%,p=0.24); of whom 1 had proteinuria and 7 had microalbuminuria.

Rates of hypertriglyceridaemia (OR=4.56,CI:1,08-19.16, *p*=0.04) and hypercholesterolaemia (OR=7.42, CI:1.78-30.81, *p*=<0.01) were higher in HIV+ participants than those that were HIV-. HIV+ children and adolescents had higher rates of raised hs-CRP (OR=2.23, CI:1.41-3.51, *p*=<0.01).

Over 80% percent of HIV+ participants had CD4 cell counts >500 cells/mm^3 and 76.7% had a viral load <50 copies/mL at enrolment.

The median duration of ART was 7.6. years (IQR: 4.6-9.3), and the median age at ART initiation was 4.3 years (IQR: 2.3-7.5). 305 (60.5%) were on an Efavirenz-based ART regimen with the remainder 188 (37.3%) on a Lopinavir/ritonavir-based regimen. Only 7 (1.4%) adolescents were on TDF.

There was no differences in the prevalence of proteinuria between HIV+ and HIV- participants (5.9% vs. 10.1%, *p*=0.11). Two HIV+ participants had 2+ proteinuria and 1 had 3+ proteinuria. No HIV-participant had 2+ or 3+ proteinuria. No participant had been referred for a kidney biopsy.

The overall prevalence of microalbuminuria was 8.5% with no difference between HIV+ and HIV-adolescents. Female sex was predictive of microalbuminuria regardless of HIV status, OR=3.81 (CI:2.01-7.41). There was no difference in microalbuminuria according to age, in fact younger children (9-11 years) had a higher prevalence of microalbuminuria than those that were older (12-14 years) [10.3% vs 6.4%; *p*=0.11]. No participant on TDF had microalbuminuria. All participants had a normal GFR. There were no significant associations between metabolic parameters and microalbuminuria in HIV+ youth (Table 2).

Discussion

The prevalence of proteinuria in the cohort of South African HIV+ youth on ART of 6.6% is similar to a cohort of HIV+ and negative children living in United States (9%).[26] This similar prevalence in SA adolescents is surprising, as proteinuria is more frequent in people of African ancestry[27], and the US cohort only had 50% African American children while almost all our participants were Black African. Furthermore, we found no difference in the prevalence of proteinuria between HIV+ and HIV- South African adolescents, however, only HIV+ children had ≥2+ proteinuria on urine dipstick analysis.

The prevalence of microalbuminuria (8%) in this South African cohort is also lower than the 15% prevalence reported in healthy US children and adolescents in the National Health and Nutrition Survey (NHANES) but is closer to the 7.3% prevalence in healthy adults between the ages of 20 to 39 years in the same study.[28] We found that female sex predicted microalbuminuria regardless of HIV status, as in the NHANES study where females between the ages of 6 to 19 years of age had a similar microalbuminuria prevalence to 60-79 year old women.[28] The reason for females having a higher prevalence of microalbuminuria is unknown.

Other studies reported a prevalence of microalbuminuria between 10% and 30% in African HIV+ children.[12,13,17] The lower prevalence found in this cohort could be due to children starting ART relatively earlier than in other African cohorts; or fewer children receiving a TDF based regimen. South African HIV guidelines recommend Abacavir in first line therapy and switching to TDF is only recommended for adolescents above 15 years of age and 40 kilograms as the current available formulation only allows for adult dosing.

Many adolescents switching to TDF have been on Lopinavir/ritonavir (LPV/r) since childhood. Concomitant LPV/r can increase TDF levels by more than 50%. Although only 7 participants were on TDF at the time of this analysis it will be important to monitor renal function as around 40% of HIV+ youth in our cohort were on protease inhibitors and are likely to switch to TDF in the near future as they reach guideline criteria.

Unlike a similar study in the US where HIV+ youth tended to have more microalbuminuria, we found no difference in the prevalence of microalbuminuria between HIV+ and negative children and adolescents.[28]

GFR was also normal with no difference between those with or without HIV. Other cohorts have reported similar findings.[13,14,26]

HIV+ youth had a lower BMI and were significantly shorter than HIV negative youth. In addition, HIV+ youth had higher rates of hypertriglyceridaemia, hypercholesterolaemia and hs-CRP although these comorbidities were not associated with microalbuminuria (Table 2). This is unexpected as hs-CRP may reflect chronic inflammation, a risk factor for chronic kidney disease.

The low prevalence of renal abnormalities in this cohort is reassuring and most likely reflects the relatively long use of ART. Further, long term study of this cohort and in particular those participants with microalbuminuria will be important for early identification of those who are at risk for developing renal dysfunction.

Strengths of this study are the inclusion of HIV negative adolescents as a comparison group and the large sample size. However, the study was limited by each participant only having had one measurement of proteinuria and microalbuminuria at enrolment visit. Additional limitations include lack of measurement of isotope GFR and protein/creatinine measurement. Urine samples were obtained on arrival in the morning but not all samples were 'early' morning urines if participants were delayed.

Conclusions

Proteinuria or microalbuminuria were equally uncommon in HIV+ youth and HIV negative adolescents. Assessing for microalbuminuria may allow identification of those at risk of HIVAN in order to implement more intensive follow up. Follow up of participants, especially as they are routinely switched to a TDF containing ART regimen, may inform long term outcomes and potential strategies for screening and management of this growing population of HIV-infected youth.

All authors declare no conflict of interest.

Table 1. Baseline Characteristics of Youth Living with Perinatally -Acquired HIV and HIV-uninfected Youth

	HIV-infected Adolescents (511)	HIV-uninfected Adolescents (109)	*P*-value
Demographics			
Female	249 (48.7)	60 (55.1)	0.23
Age (years)	12.0 (10.7-13.3)	11.8 (10.1-13.4)	0.40
Black African	473 (92.6)	109 (100)	0.00
BMI (kg/m^2)	17.1 (15.9-18.9)	18.7 (16.8-21.5)	<0.01
BMI- Z score	-0.19 (-0.93-0.4)	0.36 (-0.43-1.0)	<0.01
Height (cm)	140.2 (132.5-147.6)	142.8 (136.5-155)	<0.01
Height Z score	-1.27 (-2.09- -6.2)	-0.59 (-1.22- 0.03)	<0.01
Tanner stages			
Prepubertal (Tanner 1)	252 (50.3)	35 (32.41)	<0.01
Pubertal (Tanner 2-5)	249 (49.7)	73 (67.59)	
Tanner 4 and 5	62 (12.38)	21 (19.44)	0.05
Metabolic Comorbidities			
Triglycerides (mmol/L)	0.9 (0.7-1.1)	0.7 (0.5-0.85)	<0.01
Hypertriglyceridaemia	40 (7.9)	2(1.9)	0.02
Total Cholesterol (mmol/L)	4.1 (3.6-4.7)	3.8 (3.4-4.25)	<0.01
Hypercholseterolaemia	61 (12.3)	2 (1.9)	<0.01
LDL (mmol/L)	2.2 (1.8-2.6)	2 (1.6-2.4)	0.01
High LDL	28 (5.6)	2 (1.9)	0.10
HDL (mmol/L)	1.5 (1.2-1.7)	1.45 (1.2-1.7)	0.51
Low HDL	56 (11.3)	5 (4.63)	0.04
Increased HOMA-IR	78 (20.7)	14 (22.6)	0.74
Systolic BP (mmHg)	105 (98-112)	108 (100-118)	<0.01
Diastolic BP (mmHg)	67 (61-73)	69 (64-74)	0.05
Hypertension	67 (13.1)	19 (17.4)	0.24
Highly sensitive CRP	217 (42.5)	33 (30.3)	0.02
Other Laboratory Values			
Sodium	137 (136-139)	137 (136-139)	0.32
Urea	3.2(2.7-3.9)	3.4 (2.7-3.9)	0.46
Creatinine (serum)	40 (36-46)	44(39-50.5)	<0.01
Glomerular Filtration Rate	128.5 (114.6-143.7)	122.0 (109.0-138.0)	0.07
Haematuria	8 (1.57)	5 (4.63)	0.04
Any Proteinuria	30 (5.9)	11 (10.1)	0.11
Trace	24	8	
1 plus	3	3	
2 plus	2	0	
3 plus	1	0	
Microalbuminuria	43 (8)	10 (9)	0.80
Proteinuria no Microalbuminuria	13 (2.5)	4 (3.7)	0.51
Microalbuminuria no protein	26 (5.1)	3 (2.7)	0.30
Microalbuminuria 9-11 years	27 (62.8)	4 (40.0)	0.19
Microalbuminuria 12-14 years	16 (37.2)	6 (60.0)	
HIV-related Characteristics			
Viral Load			
<50 copies/mL	391 (76.7)	--	
50-1,000 copies/mL	56 (11.0)	--	
1,001-10,000 copies/mL	36 (7.1)	--	
>10,000 copies/mL	27 (5.3)	--	
CD4 count			
<200	10 (2.0)	--	
200-499	73 (14.4)	--	
500-1000	315 (62.0)	--	
>1000	110 (21.7)	--	
WHO HIV Staging			
Stage I	34 (7.0)	--	

Stage II	50 (10.3)	--	
Stage III	291 (59.8)	--	
Stage IV	112 (23.0)	--	
Age at initiation of ARVs	4.3 (2.3-7.5)		
0-2 years	188 (37.4)	--	
3-5 years	138 (27.4)	--	
6-14 years	177(35.2)	--	
Duration on ARVs	7.6 (4.6-9.3)	--	
Current ARV regimen			
2 X NRTI + NNRTI	305 (60.5)	--	
2 X NRTI + PI	188 (37.3)	--	
Others	10 (2.0)	--	
Currently on TDF	7 (1.4)		

All continuous variables expressed as median (interquartile range) or mean (SD) and categorical variables as number (%).

ABC = abacavir; AZT = zidovudine; ART = antiretroviral treatment; BMI = body mass index; D4T = stavudine; DDI= didanosine; HDL = high density lipoprotein cholesterol, HOMA = Homeostatic Model Assessment; LDL = low density lipoprotein cholesterol; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; WHO = World Health Organization YLHIV=Youth Living with Perinatally Acquired HIV

Table 2. Univariate Analysis for Microalbuminuria in HIV-infected Children and Adolescents

	Unadjusted OR (CI)	*p*-value
Age (years)	0.88 (0.72- 1.07)	0.188
Gender		
Male	Ref	
Female	4.45 (2.09-9.48)	<0.001
Blood Pressure (mm Hg)		
Systolic	1.01 (0.97-1.03)	0.903
Diastolic	0.99 (0.95-1.02)	0.528
Normal	Ref	
Hypertension	0.86 (0.33- 2.27)	0.763
Growth Measures		
BMI Z score	0.86 (0.65-1.14)	0.295
Laboratory measures		
Normal Triglycerides	Ref	
Hypertriglyceridemia	1.61 (0.59- 4.34)	0.351
Normal Total Cholesterol	Ref	
Hypercholesterolemia	0.74 (0.25-2.14)	0.572
Normal LDL	Ref	
High LDL	1.89 (0.62- 5.73)	0.261
Normal HDL	Ref	
Low HDL	0.58 (0.17-1.95)	0.382
hsCRP		
Normal (<1)	Ref	
High risk (1--5.99)	0.91 (0.44-1.84)	0.778
Active infection (≥6)	1.54 (0.67-3.51)	0.309

BMI = body mass index; HDL = high density lipoprotein cholesterol, hsCRP= highly sensitive CRP; LDL = low density lipoprotein cholesterol; OR= odds ratio, CI= confidence intervals

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Chapter 4:
Multisystem impairment in South African adolescents with Perinatally acquired HIV on antiretroviral therapy (ART)

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Abstract

Introduction: Adolescents with perinatally acquired HIV (PHIV) are at risk of chronic disease due to longstanding immune suppression, HIV disease and antiretroviral therapy (ART) exposure. However, there are few data on multisystem disease in this population. We investigated the overlapping burden of neurocognitive, cardiovascular, respiratory and/or renal impairment among PHIV positive (PHIV+) adolescents.

Methods: In this cross-sectional analysis, participants aged 9-14 years on ART for >6 months were recruited from 7 sites across Cape Town from July 2013 through March 2015, together with age-matched HIV-negative (HIV-) adolescents. Impairment at enrolment was assessed across neurocognitive functioning (using the youth-International HIV Dementia Scale); cardiac function (echocardiogram abnormality); respiratory function (abnormal spirometry) and renal function (abnormal glomerular filtration rate).

Results: Overall, 384 PHIV+ and 95 HIV- adolescents were included (mean age, 11.9 years; 49% female). Median age of ART initiation was 4.2 years (IQR: 1.7-7.6) and median CD4 count was 709 (IQR:556-944) with 302 (79%) of PHIV+ adolescents virologically suppressed. Abacavir and Zidovudine were the most commonly used nucleoside reverse transcriptase inhibitors (NRTIs) with 60% of adolescents on non-nucleoside reverse transcriptase inhibitors (NNRTI) and 38% on a protease inhibitor (PI). Amongst PHIV+ adolescents, 167 (43.5%) had single system impairment only, 110 (28.6%) had two systems involved, and 39 (10.2%) had 3 or 4 systems involved. PHIV+ participants had more 2-system and 3-system impairment than HIV-, 110 (28.6%) vs. 17 (17.9%), p=0.03 and 39 (10.2%) vs. 3(4.3%), p=0.03. PHIV+ participants who had failed a year of school (73.8% vs. 46.4%, p=0.00) and with a viral load >1000 copies/ml at enrollment (16.8% vs. 8.1%, p=0.03) were more likely to have dual or multisystem impairment. Of those with cardiac impairment, 86.7% had an additional system impaired. Similarly, in those with neurocognitive impairment, almost 60% had additional systems impaired and of those with respiratory impairment, 74% had additional systems impaired.

Conclusions: Despite relatively early ART initiation, there is a substantial burden of multisystem chronic impairment among PHIV+ adolescents. This phenomenon needs to be further explored as this population ages and begins to engage in adult lifestyle factors that may compound these impairments.

Introduction

There are 2.1 million adolescents aged 10 to 19 years living with HIV, the majority of whom live in sub-Saharan Africa and have perinatally acquired infection.[1,2] This population is growing as most children on antiretroviral therapy (ART) are surviving into adolescence, due to increased access and earlier initiation of ART.[3] Single system morbidity in perinatally HIV positive (PHIV+) adolescents is well described but there is limited data on multimorbidity in this population.

Cardiac, respiratory, neurocognitive or chronic renal impairment are common long-term sequelae of perinatal HIV infection.[4-7] PHIV+ adolescents in the United States (USA) and Europe have high rates of chronic lung disease, kidney disease and neuropsychiatric problems.[8] In sub-Saharan Africa, however, diagnosis and initiation of ART occur much later than in the USA, with the median ages at first visit and at ART initiation of 7.1 years and 7.9 years compared to 0.7 years and 0.9 years respectively.[9] HIV+ children in sub-Saharan Africa are therefore at greater risk of chronic morbidity due to untreated HIV in childhood, longstanding immune suppression and associated infection, suboptimal ART formulations and regimens or lack of access to care.[10] The interplay between these factors along with chronic inflammation result in PHIV+ adolescents being at risk for multisystem impairment.[11]

Single system morbidity has been described in resource limited settings, mostly focusing on cardiac or chronic lung disease.[12,13] A recent study from Asia showed that infectious HIV-related morbidity was more common in younger adolescence (10-14 years of age) with a trend toward non-infectious and treatment- related morbidity in later adolescence, however this study did not specifically explore chronic or multisystem morbidity.[14]

Despite the high prevalence of single system morbidity, there are surprisingly few data on prevalence of and risk factors for multisystem involvement in PHIV+ adolescents on ART, especially in Africa. Multisystem morbidity may be associated with worse clinical outcomes, increased health care utilisation and more difficulty in adhering to ART due to a high pill burden. Many studies of single organ impairment are limited by small sample sizes, with no comparison group of HIV- adolescents and many are from the pre-ART era. As growing numbers of PHIV+ adolescents present to overburdened health systems in resource limited countries, optimizing strategies to best care for them is crucial. The aim of this study was to investigate the prevalence of and risk factors for overlapping multisystem (neurocognitive, cardiovascular, respiratory and renal) impairment in PHIV+ adolescents in the Cape Town Antiretroviral Cohort (CTAAC).

Methods

Study population

This was a cross-sectional study of PHIV+ children and adolescents enrolled in CTAAC, a longitudinal cohort study in Cape Town, South Africa. Children between 9-14 years on ART for more than 6 months were enrolled from 7 sites in the Western Cape Province, South Africa with age matched HIV- youth of similar ancestry from July 2013-March 2015. Children and adolescents between the ages of 9 -14 years were considered to be perinatally infected.[15] Ethical approval was given by the Faculty of Health Sciences, University of Cape Town and Stellenbosch University, Human Research Ethics Committee (051/2013). Parents gave informed consent and assent was obtained from all adolescents. All participants knew their HIV status as a pre-requisite to study enrolment.

For this analysis, we included only those participants with complete respiratory, cardiac, neurological and renal assessments from the enrolment visit (Figure 1).

Study Measures

Sociodemographic data and other health information

Routine sociodemographic data were collected at enrolment and each participant's clinical record was reviewed at their primary treatment facility.

Participants were screened for cardiac and respiratory symptoms such as wheeze, cough, shortness of breath and a validated respiratory questionnaire derived from the International Study of Asthma and Allergies in Childhood study was performed at enrolment.[16]

A physical examination including Tanner staging, WHO HIV staging, blood pressure (BP) and anthropometry was performed at enrolment. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). BMI was classified according to WHO reference standards.[17] BP was measured by electronic sphygmomanometer (Spot Vital Signs, Welch Allyn, USA). All anthropometric measures were performed by one of two trained study nurses to ensure standardization of measures.

Laboratory measures performed at enrolment included viral load (COBAS Ampliprep system, Roche Molecular Systems, Branchburg, NJ, USA) and CD4 count (Beckman Coulter®, USA) in HIV+ participants. Abnormal total cholesterol (TC), high density lipoprotein (HDL), and low-density lipoprotein (LDL) were defined as >5.18, <1.03 and >3.37 mmol/L, respectively. Abnormal triglycerides were defined as >2.85 mmol/L if age <10 years or >3.89 mmol/L if age ≥10 years at the time of baseline investigations.[18] The Homeostatic Model Assessment (HOMA) (fasting insulin [mIU/L] x fasting glucose [mmol/L] divided by 22.5) was used to assess insulin resistance (IR).[19]

Cardiac measures

Echocardiograms were performed by two trained research echocardiographers using either a Philips iE33 or CX50 (Phillips, Netherlands) using standardized techniques. All echocardiograms were interpreted by a single paediatric cardiologist and a random subset was read by a second blinded paediatric cardiologist. Left ventricular shortening fraction was measured by M-mode and ejection fraction was derived using standard methods. The ejection fraction was also measured using the modified Simpson's method.[20] Left ventricular diastolic function was measured using Doppler assessment of mitral inflow. Tissue Doppler techniques were used to measure mitral annular velocity. Tricuspid annular plane excursion was measured using M-mode.[20] Pulmonary artery pressures (systolic and diastolic) were estimated using standard continuous and pulse wave Doppler methods. Cardiac dimensions were assessed either using direct measurement of 2-D images or M-mode recordings.

Body surface area (BSA) was estimated using the Mosteller formula.[21] Echocardiographic structural parameters were expressed as raw means as well as a deviation from the BSA–corrected mean (z-scores), based on normal values.[22]

Cardiac impairment was defined as any one or more of the following six findings: (i) Left ventricular (LV) hypertrophy defined as a LV mass > 88 g/m^2 for females and >102 g/m^2 for males[23] (ii) LV systolic dysfunction defined by LV shortening fraction (LVSF) ≤25%[20], (iii) LV diastolic dysfunction defined by the early to late ventricular filling ratio (E/A ratio)[24], (vi) right ventricular (RV) systolic dysfunction determined using a tricuspid annular plane systolic excursion (TAPSE) z-score <2[25], (v) fractional RV area change (FAC) ≤34%[26] and (vi) mean pulmonary arterial pressure (mPAP) > 25mmHg.[27]

Respiratory measures

Spirometry was done using the NDD EasyOne Pro LAB® (NDD, Switzerland). Testing adhered to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines.[28-30] Lower limit of normal (LLN) for spirometry outcome variables was calculated using the African-American reference cohort in global lung initiative (GLI) software, -1.64 standard deviations (SD) below the mean.[31] Lung function testing was deferred if the participant had an acute respiratory illness. **Respiratory impairment** was defined as a forced expiratory volume (FEV_1) below the lower limit of normal (FEV_1) <LLN) or FEV_1/ forced vital capacity (FEV_1/FVC<LLN).[32]

Neurocognitive measures

The youth-International HIV Dementia Scale (y-IHDS), a sensitive screening test for neurocognitive disorders, was used to screen for cognitive impairment.[33] The y-IHDS is a 3-part test that includes timed finger tapping, a time alternating hand sequence test and a two-minute delayed recall of 4 words.[34] Each participant is asked about a history of repeating a grade/grades at school, with one point subtracted from the total score for positive response. The test was conducted in the participant's home language by trained study doctors. **Neurocognitive impairment** was defined as a y-IHDS less than or equal to 10.[33]

Renal Measures

Enrolment blood was taken to assess creatinine and standing height was measured using a stadiometer with a moveable headboard in centimetres. Serum creatinine was measured in micromoles per litre by the enzymatic method. The modified Schwarz formula was used to estimate glomerular filtration rate (GFR).[35] **Renal impairment** was defined as glomerular filtration rate (GFR) below 90ml/min/1.73m^2.

Statistical Analysis

Primary outcomes

The primary outcomes were the number of participants with single, dual and multisystem impairment.

Single, dual and multisystem impairment were defined as having impairment of only one (single), only two (dual) or three or more (multisystem) of the following systems: cardiac, respiratory, neurological, or renal.

Baseline variables and outcomes for PHIV + and HIV- adolescents were compared using t-tests, Wilcoxon, and Chi-square tests as appropriate. Amongst PHIV+ participants, logistic regression was performed to evaluate factors associated with having dual or multisystem impairment. Covariates considered for associations with multisystem impairment included anthropometry, a history of having had Tuberculosis (TB), HIV laboratory parameters, and duration and type of ART.

Statistical analysis was performed using Stata version 14.1. StataCorpInc. College Station, Texas USA.

Results

Four hundred and seventy-nine participants (384 PHIV+ and 95 HIV-) had complete respiratory, cardiac, neurological and renal assessments from the enrolment visit.

Characteristics of participants

Of 479 participants, 384 (80%) were PHIV+ and 95 (20%) were HIV-. The mean age was 11.9 years (SD 1.6); 234 (49%) were female and 456 (95.2%) were Black African. PHIV+ participants were more likely to have a history of TB treatment (p=<0.01), a history of asthma (p=0.03), or to have failed a grade at school (p=<0.01) compared to HIV-. Respiratory rate and blood pressure were significantly different although these differences were not clinically important. PHIV+ participants also had a lower BMI, height and were more stunted than HIV- participants (p=<0.01 for all). Almost half (46.4%) PHIV+ participants were prepubertal (Tanner Stage 1) vs. 32.6% of HIV- participants, *p*=<0.01. Lipid abnormalities were more frequent in PHIV+ participants with 56 (14.6%) vs 3 (3.2%) having hypercholesterolemia, *p*=<0.01 (Table 1).

PHIV+ participants initiated ART at a median age of 4.2 years (IQR: 1.7-7.6) with a median duration of ART of 7.9 years (IQR:4.7-9.5). The median CD4 count was 709 (IQR: 556-944) with 79% of HIV+ participants having a viral load of less than 50 copies/mL.

Two hundred and twenty-five (60%) participants were on a non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimen and 144 (38%) were on a protease inhibitor (PI) - based regimen, with only 8 (2.1%) on an alternate regimen (either lamivudine monotherapy or a combination of darunavir /ritonavir and raltegravir).

PHIV+ participants had more cardiac (46.1% vs. 33.7%, *p*=0.03), respiratory (27.1% vs. 14.7%, *p*=0.01) or neurocognitive impairment (56.3% vs. 45.3%, *p*=0.05) than HIV- participants. There were few participants with renal impairment (2.3% vs. 2.1%, *p*=0.89) in both groups (Table 2).

Two hundred and fifteen (44.9%) of all adolescents had single system impairment only. One hundred and twenty-seven (26.5%) and 42 (8.8%) had dual and multisystem (3 or 4 systems) involved, respectively. PHIV+ participants had more ‘dual system’ (28.6% vs. 17.9%, *p*=0.03) and ‘multisystem’ impairment (10.2% vs. 4, *p*=0.03) than HIV- (Table and Figure 2).

PHIV+ participants

Neurocognitive impairment was the most common type of single system impairment.

Overall, a low TAPSE and FAC, indicative of right ventricular dysfunction, accounted for the majority of echocardiogram abnormalities with 104 (27.1%) and 42 (10.9%) of those PHIV+ adolescents that had cardiac impairment having an abnormal TAPSE and FAC, respectively. Left heart dysfunction was rare, with only one PHIV+ adolescent having a decreased LVSF and 32 (8.3%) having evidence of left ventricular diastolic dysfunction. No participant had dilated cardiomyopathy and 2 (0.5%) PHIV+ adolescents had raised pulmonary artery pressure. Ninety -seven (25.3%) PHIV+ participants had an abnormal FEV1 and 35 (9.1%) had a FEV 1/FVC< LLN.

The most common patterns of dual and multisystem impairment were 'cardiac and neurocognitive' and 'cardiac, neurocognitive and respiratory' impairment. Only 2 (0.5%) PHIV+ participants had impairment of all 4 systems. In participants with any impairment, the majority had additional system impairment. Of those with cardiac impairment, 126/177 (86.7%) had an additional system impaired. Similarly, in those with neurocognitive impairment almost 60% had additional systems impaired and of those with respiratory impairment, 74% had additional systems impaired.

In univariate analysis, having failed a grade or having a viral load >1000 copies/mL at enrollment were more common in those with dual or multisystem impairment compared to those with no impairment or single system impairment, 73.8% vs. 46.4%, p=0.00 and 16.8% vs. 8.1%, p=0.03 (Table 3). Participants with dual and multisystem disease were also older (12.2 years vs. 11.8 years, p=0.02).

In multivariable analysis failing a grade was associated with dual or multisystem disease (OR=3.2, CI 2.1-5.1, p=<0.01)

Discussion

This is the first study to report on multisystem impairment in African PHIV+ children and young adolescents on ART. The study found the prevalence of **any** cardiac, respiratory or neurocognitive impairment in PHIV+ participants was significantly higher than in HIV- participants. Similar findings of individual system involvement have been reported in other studies of adolescents with perinatally acquired HIV with a high prevalence of cardiac, respiratory, neurocognitive or less commonly renal impairment.[5,36,37]

Neurocognitive impairment occurring most commonly is perhaps the most concerning morbidity, impacting on PHIV+ adolescents as it influences all spheres of health including treatment adherence and school performance. The measure used, the y-IHDS, is a screening test and cannot definitively diagnose the type or extent of neurocognitive impairment. The score has been validated previously in a subset of this cohort and shown to be sensitive for neurocognitive disorder screening.[33] In addition, it is quick and easily performed in busy clinic settings. We found a prevalence of neurocognitive impairment of 56% using y-IHDS, similar to the 45% of HIV+ youth that met criteria for neurocognitive disorder diagnosis through an extensive battery of neurocognitive tests in the same setting.[38] However, in other African settings, the adult IHDS score overestimated the burden of neurocognitive impairment.[39] Follow-up data from our adolescent cohort will be valuable to assess whether the y-IHDS screening tool correlates with confirmed impairment.

Cardiac impairment was the second most commonly affected system. This was based on echocardiogram parameters reflecting subtle right ventricular dysfunction. The majority of these participants were asymptomatic with no difference between PHIV+ and HIV- participants. These results are consistent with a Spanish study showing subtle cardiac abnormalities found on echocardiogram and no difference in the control population[4], but the authors did not report on right heart dysfunction. Lower TAPSE has been reported in HIV+ young adults but not in HIV- controls. [40] Follow up of all participants in our study may indicate if these findings are clinically relevant as both TAPSE and FAC are more useful in longitudinal studies.[41,42]

Respiratory single system impairment was reflected by subtle reduction in lung function that may impact on adult lung health. Our findings are consistent with the prevalence of abnormal spirometry, reported as between 24 and 38% in various African adolescent cohorts from Zimbabwe, Malawi or South Africa.[32,43,44]

Renal impairment was surprisingly rare given the genetic predisposition to HIV nephropathy in Black Africans but may reflect survivor bias as those with more severe kidney disease may have died or already being followed at specialized renal clinics. The prevalence of proteinuria and microalbuminuria, risk factors for chronic kidney disease has been shown to be high in HIV+ adolescents however, we previously reported no difference in these measurements between PHIV+ and HIV- adolescents in this cohort.[45]

Dual system impairment affected about a quarter of participants, but multisystem involvement was relatively uncommon. This may reflect that the cohort were ambulatory and relatively well, were on ART for several years and were adherent to therapy. Despite this, there remains a small but clinically significant proportion of adolescents that will need complex clinical services to ensure optimum care. In addition, a significant proportion of those with impairment in one system had another system involved.

Multivariable analysis found that failing a grade at school and age at enrollment was significantly associated with dual or multisystem disease. School failure may be due to neurocognitive impairment or chronic or prolonged illness and hospitalization resulting in missing significant periods of the school year.[46] As this was a cross sectional study we were unable to infer that multisystem disease had a causal relationship with school failure. Checking for school failure may be helpful in deciding whom to screen for multisystem impairment.

There was no evidence for the association of duration or for the relatively later start of ART and dual or multisystem impairment. A possible explanation for later start of ART not being associated with multisystem impairment is that historically children that were started on early ART prior to the guidelines recommending early start for all were severely ill and this illness may have resulted in multisystem impairment despite early access to ART. In addition, treatment histories were often not available or difficult to interpret. Reasons for switching ART regimens were poorly documented and it was not possible to accurately assess viral suppression prior to study enrolment.

The study is limited by the cross- sectional analysis that limited the ability to detect longitudinal changes. Only four systems were included, and a more comprehensive assessment of system involvement including hearing, dermatological complications of HIV and musculoskeletal abnormalities may be useful.

An additional limitation is that the measures of impairment that we chose for each system differ in their ability to assess severity of impairment with the y-IHDS score being a relatively crude estimate of neurocognitive impairment compared to detailed assessment of lung function that is obtained with spirometry. Severity of impairment across different systems thus cannot be directly compared.

Conclusions

In those PHIV+ adolescents that had one system impaired a significant proportion had another system involved. Although multisystem impairment is relatively rare, a small minority of youth will require clinical attention for complex multisystem issues. Young adolescents with system impairment will need close observation as they transition to adulthood and are increasingly at risk for engaging in adult lifestyle factors such as smoking or recreational drug use. Adult onset diabetes and hypertension may also compound these impairments. Longitudinal follow up is needed to ascertain whether system impairment may impact long term morbidity.

Authors contributions

LJF contributed to the initial concept of the paper, did the statistical analysis and wrote the manuscript. KB did statistical analysis. SM contributed towards data management and did statistical analysis. LG, DG performed and interpreted spirometry and were involved in the initial pulmonology concept of CTAAC, LZ read echocardiograms and contributed to the initial cardiology concept of CTAAC, PN gave input on renal measures, DJS and JH gave input on neurocognitive concepts, MFC, LM, HJZ were involved in the initial concept of the paper and obtained funding for CTAAC. All authors have read and approved the final manuscript.

Author information

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All authors declare no conflict of interest.

Table 1. Clinical and Laboratory Characteristics of study participants

	PHIV+ (n=384)*	HIV- (n=95)	*P*-value
Clinical Characteristics			
Age (years)	12.0(1.7)	11.8(1.6)	0.28
Female	183(47.7)	51(53.7)	0.29
Black African	361(94.0)	95(100)	**0.01**
Previous TB treatment	234(61.6)	2(2.2)	**0.00**
Hospital admissions in past 12 months	8(2.1)	1(1.1)	1.00
History of wheeze	43(11.2)	6(6.3)	0.19
History of asthma diagnosis	49(12.8)	4(4.4)	**0.03**
History of dyspnoea	12(3.1)	2(2.1)	1.00
History of cough	52(13.7)	8(8.6)	0.23
History of failing a grade at school	219(57.0)	33(34.7)	**0.00**
BMI (kg/m^2)	17.1(15.9-18.8)	18.8(16.6-21.5)	**0.00**
Height	140.6(10.4)	144.6(11.1)	**0.00**
Stunting	101(26.3)	4(4.2)	**0.00**
Puberty			
Pre-pubertal (Tanner stage 1)	175(46.4)	31(32.6)	**0.02**
Pubertal (Tanner stage 2-5)	202(53.6)	64(67.4)	
Laboratory measures			
Triglycerides	0.9(0.7-1.1)	0.7(0.5-0.8)	**0.00**
Hypertriglyceridemia	12(3.1)	1(1.1)	0.48
Total Cholesterol	4.1(0.8)	3.8(0.7)	**0.00**
Hypercholesterolemia	56(14.6)	3(3.2)	**0.00**
LDL	2.2(0.7)	2.0(0.6)	**0.02**
HDL	1.5(1.3-1.7)	1.40(1.2-1.7)	0.28
HOMA	2.0(1.3-3.0)	1.9(1.2-3.6)	0.97
Log HOMA	0.3(0.3)	0.3(0.4)	0.46
Viral Load (copies/mL)			
<50	302(79.0)	---	----
50-1,000	37 (9.7)	---	----
>1,000	44 (11.3)	---	----
CD4 count (cells/mm³)			
<200	8(2.1)	---	----
200-499	54(14.1)	---	----
≥500	320(83.8)	---	----
WHO HIV Staging			
Stage I	28 (7.7)	---	----
Stage II	38(10.4)	---	----
Stage III	217(59.4)	---	----
Stage IV	82(22.5)	---	----
Age at initiation of ART (years)			
Median age	4.2(1.7-7.6)	---	----
0-2	151(40.1)	---	----
3-5	95(25.3)	---	----
6-14	136(34.6)	---	----
Current ART regimen			
2 X NRTI + NNRTI	225(60.0)	---	---
2 X NRTI + PI	144(38.0)	---	---
Other	8(2.1)	---	---

All continuous variables expressed as median (interquartile range) or mean (SD) and categorical variables as number (%)
BMI = body mass index; LDL = low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol, HOMA = Homeostatic Model Assessment; WHO = World Health Organization; ART = antiretroviral treatment NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor* Total number may vary according to missing data for each variable.

Table 2. Types of impairment (N=479)

	PHIV+ (N=384)	HIV- (N=95)	*P*-value
Any Impairment **Cardiac, renal, respiratory or neurocognitive**	**316(82.3%)**	**68(72.6)**	**0.02**
Cardiac	177(46.1)	32(33.7)	**0.03**
Renal	9(2.3)	2(2.1)	0.89
Respiratory	104(27.1)	14(14.7)	**0.01**
Neurocognitive	216(56.3)	43(45.3)	**0.05**
Single system impairment	**167(43.5)**	**48(50.5)**	
Cardiac	51(13.3)	14(14.7)	0.71
Renal	1(0.3)	1(1.1)	0.36
Respiratory	27(7.0)	8(8.4)	0.66
Neurocognitive	88(22.9)	25(26.3)	0.50
Dual system impairment	**110(28.6)**	**17(17.9)**	**0.03**
Renal Cardiac	0(0)	0(0)	----
Renal Respiratory	0(0)	0(0)	----
Renal Neurocognitive	0(0)	0(0)	----
Cardiac Respiratory	20(5.2)	2(2.1)	0.20
Cardiac Neurocognitive	67(17.5)	13(13.7)	0.44
Respiratory Neurocognitive	23(6.0)	2(2.1)	0.20
3 system impairment	**39(10.2)**	**3(4.3)**	**0.03**
Renal Cardiac Respiratory	1(0.3)	0(0)	----
Renal Neurocognitive Respiratory	0(0)	0(0)	----
Renal Neurocognitive Cardiac	5(1.3)	1(1.1)	1.00
Cardiac Neurocognitive Respiratory	31(8.1)	2(2.1)	**0.04**
4 system impairment **Renal Respiratory Neurocognitive Cardiac**	2(0.5)	0(0)	1.000

All categorical variables are expressed as number (%).

Table 3. No impairment or single system impairment versus dual or multisystem impairment in HIV-infected participants

	None/Single System (n=235)	Dual/Multisystem (n=149)	*P*-value
Demographics			
Age (years)	11.8(1.6)	12.2(1.6)	**0.02**
Female	121(51.5)	62(41.6)	0.06
History and symptoms			
Wheeze	21(8.9)	22(14.8)	0.08
Shortness of breath	7.(3.0)	5(3.4)	1.00
Cough	26(11.1)	26(17.8)	0.07
Repeated a year at school	109 (46.4)	110(73.8)	**0.00**
Previous TB	135(58.2)	99(66.9)	0.09
Age at ART initiation			
Median Age	4.0(1.7-7.7)	4.4(2.1-7.0)	0.60
0-2	97(42.4)	54(36.7)	0.16
3-5	50(21.8)	45(30.6)	
6-14	82(35.8)	48(32.7)	
Current ART regimen			
2 X NRTI + NNRTI	134(58.3)	91(61.9)	0.09
2 X NRTI + PI	94(40.9)	50(34.0)	
Other	2(0.9)	6(4.1)	
Growth Measures			
BMI (kg/m^2)	17.2(16.0-19.0)	16.9(15.7-18.6)	0.12
Height (cm)	140.3(10.0)	141.2(10.9)	0.41
Stunted	54(23.0)	47(31.5)	0.06
Puberty			
Pre-pubertal (Tanner stage 1)	109(47.4)	66(44.9)	0.64
Pubertal (Tanner stage 2-5)	121(52.6)	81(55.1)	
WHO stage			
Stage I	18(8.0)	10(7.2)	0.90
Stage II	25(11.1)	13(9.4)	
Stage III	131(58.0)	86(61.9)	
Stage IV	52(23.6)	30(21.6)	
Laboratory measures			
Viral Load (copies/mL)			
<50	192(82.1)	110(73.8)	**0.03**
50-1,000	23(9.8)	14(9.4)	
>1,000	19(8.1)	25(16.8)	
CD4 count <200 (cells/mm³)	5(2.2)	3(2.0)	0.50

All continuous variables expressed as median (interquartile range) or mean (SD) and categorical variables as number (%).

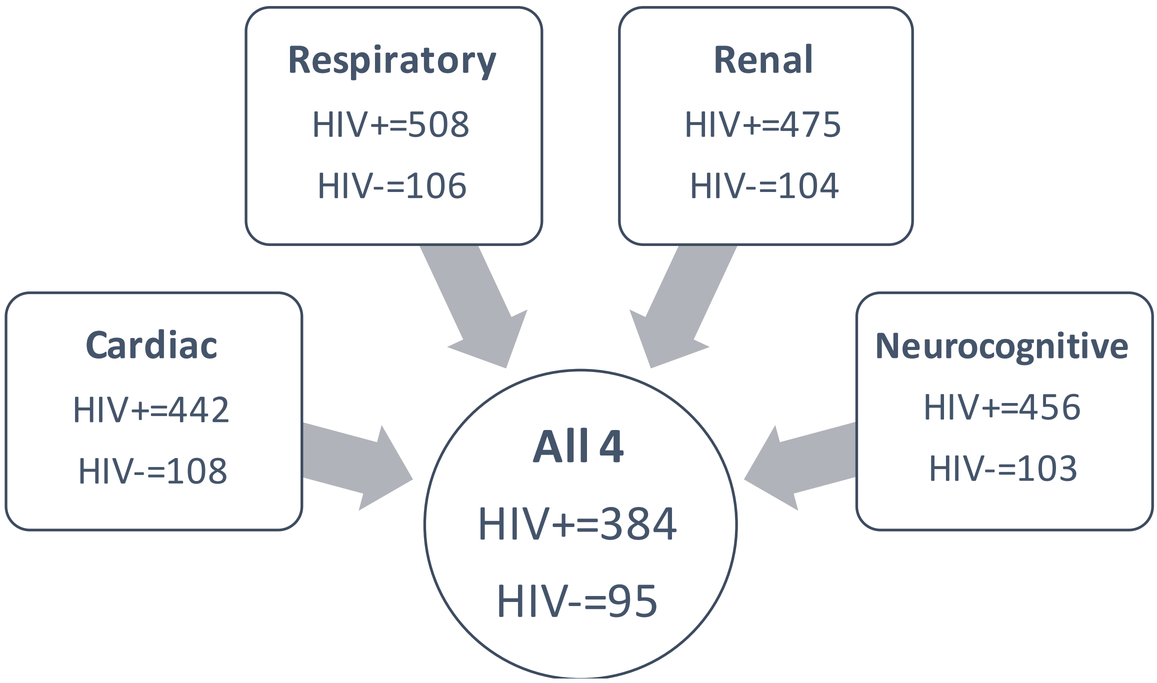

Figure 1. Participants with completed study measures from a total of 625 participants.

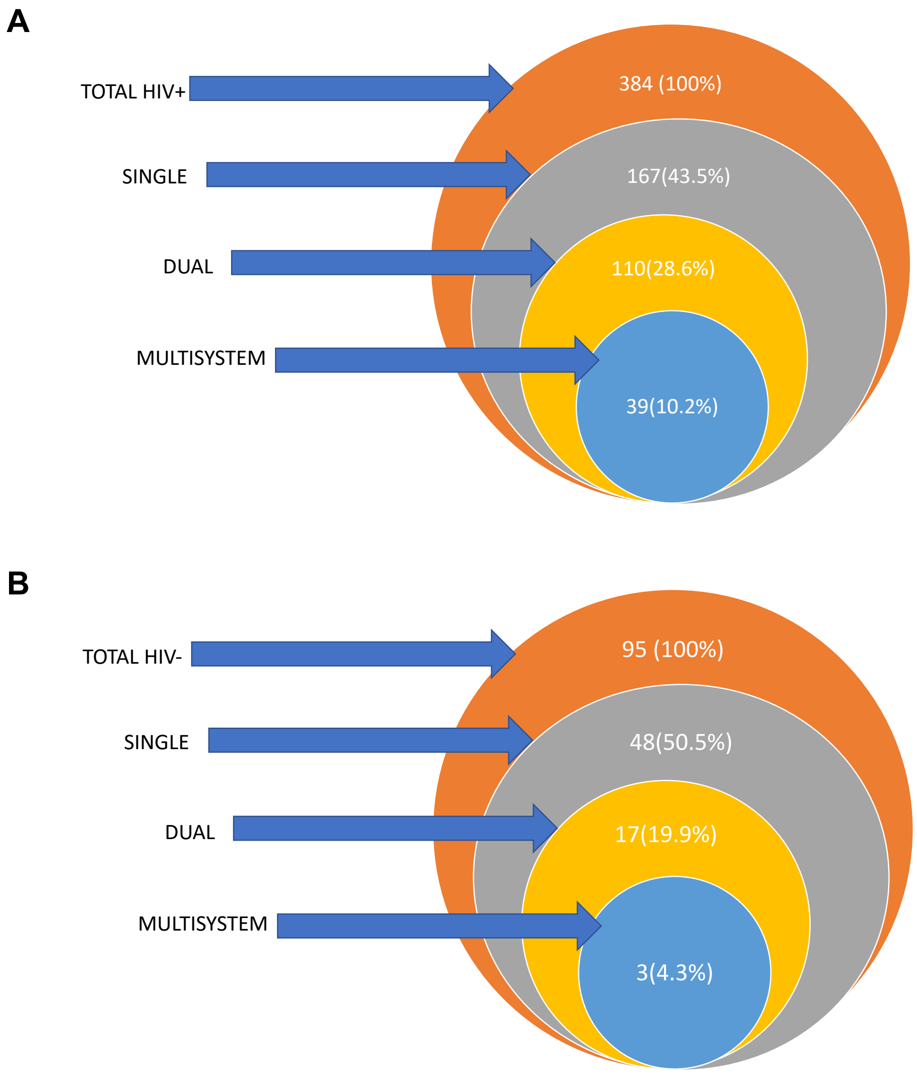

Figure 2. Burden of system impairment in HIV+ (a) and HIV- (b) adolescents.

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Chapter 5:
Hospitalization in South African Adolescents with Perinatally - acquired HIV on Antiretroviral Therapy

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Abbreviated title: Hospitalization of Adolescents with Perinatally-acquired HIV

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Abstract

Background: Little is known about hospitalization in African adolescents with perinatally acquired HIV (PHIV+ adolescents). We described the incidence and causes of hospitalization in participants enrolled in the Cape Town Adolescent Antiretroviral Cohort in South Africa.

Methods: Data collected from July 2013 through October 2018 from PHIV+ and HIV- adolescents were analyzed. Participants were assessed every six months and data on intercurrent hospitalization were abstracted. Causes of hospitalizations were classified according to ICD-10 codes. Descriptive statistics, time-to-event analysis and Poisson regression were used to describe causes and incidence and to determine incidence rate ratios for factors associated with hospitalization.

Results: 515 PHIV+ and 109 HIV- participants had a median follow up of 4.1 years (IQR: 3.7-4.6). At enrollment HIV+ participants had a median duration of ART of 7.6 years (IQR: 4.6-9.2), median CD4 count of 713 cells/mm^3 (IQR: 561.0-957.5) and 387 (75%) had a viral load <50 copies/mL. There were 149 hospitalizations over 64-months. Crude incidence rates for hospitalization were 6.6 (95%CI: 5.7-7.8) and 2.2 (95%CI:1.2-4.3) per 100- person years (*p*=<0.01) in HIV+ and HIV- participants, respectively.

Ninety of 149 (60%) admissions in HIV+ participants were classified as non-infectious, 36/149 (24%) were infectious, and 23/149 (16%) were ‘other HIV-related’ or ‘unknown’. Older age (15-19 years) and maintaining a CD4>500 cells/cm^3 were associated with decreased risk of hospitalization: adjusted incidence rate ratios of 0.61 (CI: 0.44-0.86, *p*=<0.01) and 0.68 (CI: 0.49-0.94, *p*=0.02), respectively.

Conclusions: PHIV+ adolescents had a high incidence of hospitalization despite ART. Strategies addressing infectious and non- infectious morbidity must be strengthened.

Introduction

In 2018 there were 1.7 million children less than 15 years of age living with HIV, most of whom live in sub-Saharan Africa.[1,2] Of these, 770 000 are estimated to have perinatally-acquired infection. This population is growing as most children on antiretroviral therapy (ART) are surviving into adolescence.[3] Adolescents living with perinatally acquired HIV (PHIV+) are at risk of co-morbid disease resulting from immunodeficiency, ART related toxicity, chronic inflammation, premature aging and mental health issues.[4-6] PHIV+ adolescents have a higher rates of loss to follow up, poorer virologic outcomes than younger children, with those in the 15-19 year-old age category being the only group where mortality incidence is not decreasing.[7,8]

There are limited data about hospitalization in PHIV+ adolescents from resource limited settings, including SSA, despite these areas having the greatest burden of PHIV+ adolescents. A study of PHIV+ adolescents on ART from Asian countries reported a crude hospitalization rate of 2.2 per 100-person years.[9] There were more infectious related morbidities in early adolescence (10-14 years) with a trend toward non-infectious and treatment related morbidity in later adolescence (15-19 years). In-hospital mortality in Zimbabwean HIV+ adolescents was more than three times that of HIV- adolescents.[10]

We recently reported a high prevalence of multisystem comorbidity in PHIV+ adolescents in the Cape Town Adolescent Antiretroviral Cohort (CTAAC). At least one third of the cohort had morbidity of more than one organ system with ten percent having 3 or 4 systems involved.[5] The aim of this study was to describe the incidence and causes of hospitalization and factors associated with hospitalization in adolescents from CTAAC.

Methods

Study population

PHIV+ children and adolescents were enrolled in CTAAC, a longitudinal cohort study, if they were 9-14 years old, on ART for more than 6 months and knew their HIV status. Enrolment occurred across 7 sites in the Western Cape Province, South Africa. Age, sex and race matched HIV- youth from the same community were enrolled concurrently. HIV- adolescents were recruited from the respective primary care facilities where they sought care. HIV- youth with chronic/ systemic inflammatory conditions or known neurological, pulmonary and or cardiovascular disease (such as congenital anomalies) were excluded They were confirmed to be HIV- prior to enrolment, and were retested annually with consent. Enrolment took place from 01 July 2013 through 31 March 2015, and participants were followed for up to 5 years. For this analysis, data was censored on 31 October 2018.

Study and routine care procedures

At enrolment, routine sociodemographic data were collected and each participant's clinical record was reviewed at his or her primary treatment facility. A composite poverty score was calculated based on housing type, access to household assets and caregiver employment status, with participants categorized into tertiles from most to least disadvantaged.[11] Participants were followed every six months at the CTAAC study site but received routine clinical care at their enrolment sites that included ART, cotrimoxazole and isoniazid preventive therapy (IPT) according to National guidelines.[12] The most common adolescent ART regimens consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) - most commonly abacavir and lamivudine at the time of enrolment in 2013 - and a non-nucleoside reverse transcriptase inhibitor (efavirenz) or a protease inhibitor (most commonly lopinavir/ritonavir). IPT was based on tuberculin skin testing (TST) and whether or not an individual had started ART. Older adolescents (aged 15-19 years) with a positive TST should have received 36 months of IPT and those ages 10-14 years with a positive TST should have received 6 months IPT; however, these guidelines were not widely implemented. All HIV+ adolescents with a known TB contact would be eligible for 6 months IPT as per national guidelines.[12] This was also not widely implemented.

Participants, according to the country's standard Expanded Program on Immunization (EPI) schedule, would have received BCG, diphtheria-whole cell pertussis-tetanus (DwPT), *Haemophilus influenzae* type b (Hib), hepatitis B (HBV), measles and polio immunizations (oral and inactivated).[13] Notably, as acellular pertussis, pneumococcal conjugate and rotavirus vaccines were only introduced into the public immunization program of South Africa in 2009, none of the participants would have received these vaccines. Annual influenza vaccine is recommended to all people including children older than 6 months and adolescents living with HIV, but this is also poorly implemented.

A physical examination including Tanner staging, World Health Organization (WHO) HIV staging and anthropometry was performed at enrolment and annually thereafter. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).[14] Laboratory measures performed at enrolment included viral load (COBAS Ampliprep system, Roche Molecular Systems, Branchburg, NJ, USA) and CD4 count (Beckman Coulter®, USA) in HIV+ participants.

Hospitalization data were collected prospectively (using participant interview) from enrolment until 31 October 2018 at each 6-monthly follow-up visit. Participants were often unaware of their hospitalization diagnoses; therefore, data on hospitalizations were requested from the Provincial Health Data Centre (PHDC) of the Western Cape Province, South Africa. The PHDC offers a service that provides consolidated provincial data on request.[15] The database included date of hospitalization, date of discharge, primary diagnosis and subsidiary diagnosis (classified according to the International Classification of Diseases, 10th Revision (ICD-10)), and department and hospital where the participant was hospitalized. It included "day cases" in which participants were electively admitted for surgical/ENT procedures. Where there was no ICD-10 diagnosis recorded, the investigator contacted the hospital and asked to review the participants record. The first diagnosis that was not 'HIV' was used as the primary admission diagnosis. Diagnoses were subsequently classified as infectious and non-infectious by the investigators. Where there were two recorded diagnoses, the investigator made a judgment about the primary admission diagnosis; for example, for primary cause "bacterial pneumonia" and subsidiary cause "bronchiectasis", bacterial pneumonia was taken as the primary admission diagnosis and as 'infectious'.

Deaths were communicated to the investigators by staff from the participant's primary care facility or by the family of the participant.

Ethical approval was given by the Faculty of Health Sciences, University of Cape Town and Stellenbosch University, Human Research Ethics Committee (051/2013). Parents gave informed consent and assent was obtained from all adolescents; this was renewed annually. Once participants reached 18 years of age they completed adult consent forms. All participants knew their HIV status as a pre-requisite to study enrolment. All participants gave permission for their medical records to be reviewed.

Statistical Analysis

Participant characteristics, including the number and type of hospitalizations were described using means (SD), medians (IQR) and frequencies (%) as appropriate. Crude incidence rates with 95% confidence intervals (CI) were calculated for hospitalizations, with person-year observations calculated from enrollment dates until 31 October 2018, death or relocation to another province. Time-to-event analyses were used to determine the cumulative incidence of first hospitalization or multiple hospitalizations. Loss to follow-up was not a competing event as we could still access recorded hospitalizations from the Western Cape provincial database.

Poisson regression was used to determine incidence rate ratios for factors associated with hospitalization for HIV + adolescents. Covariates included socioeconomic status, not having mother as primary caregiver, age, sex, CD4 count, HIV viral load and age at ART initiation. Covariates with a p-value <0.1 on univariate analysis were included in multivariate analyses. Logistic regression was used to determine associations between covariates and multiple admissions.

All statistical analyses were performed using Stata version 15.1 (SataCorp LP, College Station, Texas, US).

Results

Five hundred and fifteen HIV+ and 109 HIV- participants were included in the analysis. One HIV- participant who acquired HIV during the follow up period was not excluded from the analysis. Two HIV+ and no HIV-participants died. One participant committed suicide and one died of ‘presumed AIDS’ and was certified dead on arrival at hospital. Eleven HIV+ and 7 HIV- participants relocated to other provinces without further access to their clinical information.

Characteristics of participants

Two hundred and sixty- three (51.1%) HIV+ and 49 (44.9%) HIV- participants were male, p=0.13. There was no difference in the mean age at enrollment between HIV+ and HIV- participants, (12.0 vs. 11.8 years, p=0.31). HIV+ participants had a median follow up time of 4.1 years (IQR: 3.7-4.7) with 2241.9 total person-years. HIV- adolescents had a similar median follow up time (4.3 years) with total person years of 440.5 (Table 1).

The mothers of 24.4% (116/476) of HIV+ and of 4.0% (4/100) HIV- participants had died (p=<0.01). Only 2.9% (15/515) HIV+ and 1.8% (2/109) HIV- participants had written documentation of vaccinations; 41.9% (257/614) gave a verbal report of vaccinations. 115 (22.7%) and 117 (22.7%) of HIV+ participants were taking isoniazid or cotrimoxazole prophylaxis, respectively, at enrolment.

HIV+ participants had a median duration on ART of 7.6 years (IQR:4.6-9.2), with a median CD4 count of 715 cells/mm^3 (IQR:564-959); 387 (75%) had a viral load <50 copies/mL. The average age at ART initiation was 4.3 years (IQR: 2.2-7.3).

Hospitalizations

Over the study period, 149 hospitalization episodes (910 hospitalization days) occurred in 91 (17.7%) HIV+ participants compared to 10 hospitalizations in 7 (6.4%) HIV-participants (p<0.01).

The crude incidence of a hospitalization event was 6.6 (95%CI: 5.7-7.8) in HIV+ adolescents compared to 2.2 (95%CI: 1.2-4.3) events per 100-person years in HIV- adolescents (p<0.01). The cumulative incidence of hospitalization at 4 years of follow-up was 23.9% (95%CI: 20.6- 27.6) in HIV+ participants compared to 8.4% (95% CI:4.5-15.6) in HIV- participants (p<0.01; Table 2).

There were no differences in age, sex, BMI and CD4 count at enrollment between HIV+ participants subsequently hospitalized or not; however, the number of participants with a viral load <50 copies/mL was lower in hospitalized versus not hospitalized (60 (65.9%) vs. 333 (78.7%), p=<0.01; Table 3).

Causes of hospitalization in HIV+ participants

In HIV+ participants, 90/149 (60%) of admissions were for non-infectious causes, 36/149 (24%) were due to infectious causes, 16/149 (11%) were classified only as 'HIV-related' and 7/149 (5%) had no diagnosis documented.

The most common causes of the 90 non-infectious hospitalizations were hearing loss and ear related (28%) and surgical causes (19%). Other non-infectious causes included obstetric/gynecologic (5/90, 5.6%), seizures (5/90, 5.6%), psychiatric hospitalizations (3/90,3.3%), oncological hospitalizations (2/90,2.2%), cardiovascular (2/90,2.2%), gastrointestinal (7/90,7.8%), hepatic (2/90, 2.2%), respiratory (2/90,2.2%), renal (1/90,1.1%), musculoskeletal (1/90,1.1%), 1/90 (1.1%) metabolic/endocrine (1/90,1.1%), dermatological (2/90,2.2%), trauma-related to assault with sharp object (1/90,1.1%) and social protection (1/90,1.1%). There were 13 (14.4%) other causes.

Causes of the 36 infectious hospitalizations included pulmonary tuberculosis (TB) (41.7%), pneumonia (not caused by TB) (22%), abscesses (17%), herpes (2.8%), varicella (2.8%), viral meningitis (2.8%), scabies (2.8%), mastoiditis (2.8%) and unspecified bacterial infections (5.6%). There were 2 vaccine preventable infections: *Haemophilus influenzae* type b pneumonia and varicella. There were no cases of Pneumocystis pneumonia (PCP) or cryptococcal meningitis. TB related hospital admissions contributed 304/910 days, 33.4% of total hospital duration.

Causes of hospitalization in HIV- participants

In HIV- participants, the causes of the 10 hospitalizations were pregnancy related (40%), TB meningitis (20%), and unspecified chronic bronchitis (10%); three had missing data.

Univariate and multivariate analysis of factors associated with hospitalization in HIV+ participants.

In univariate analysis, participants aged 15-19 years had a lower incidence rate ratio (IRR) of hospitalization than those aged 10-14 years [IRR=0.6 (CI:0.5-0.9, *p*=0.01)]. Those that maintained a CD4 count >500 cells/mm^3 throughout the study period, compared to those who did not, had a lower IRR of 0.7 (0.5-0.99, *p*=0.04).

In multivariable analysis, older age (15-19 years) was associated with a lower adjusted incidence rate ratio (aIRR) of 0.6 (CI: 0.4-0.9, *p*=<0.01) than age below 15 years. Maintaining a CD4 count above 500 cells/mm^3 during the study period was associated with a significantly lower aIRR of 0.7 (CI: 0.5-0.9, *p*=0.02) (Table 4).

Thirty-five (38.5%) HIV+ participants, compared to only three 3 HIV- (42.9%, p=0.67), had more than one hospitalization episode. Fifteen HIV+ adolescents had more than three hospitalizations while none of the HIV- had more than 3 hospitalizations. Amongst HIV+ participants,12 readmissions (34.2%) were within 30 days of discharge. Those moderately or least disadvantaged (according to the predefined composite poverty score) had decreased odds of hospitalization more than once compared to those who were most disadvantaged: OR 0.6 (CI: 0.4-0.8, p=<0.01) and OR 0.7 (CI: 0.5-0.9, p=<0.01), respectively. Those aged 3-5 years at ART initiation had decreased odds of multiple admissions compared to those started by age 2 years: OR=0.3 (CI: 0.2-0.5, p<0.01).

Discussion

This is the first study reporting on incidence rates of hospitalization in PHIV+ adolescents on ART in sub-Saharan Africa. The crude rate of hospitalization (6.6/100-person years) was higher than in HIV- adolescents in this study and higher than in HIV+ adolescents from other contexts (1.8 per 100-person years in a study that represented 6 countries in Asia).[9] Despite most adolescents having well controlled HIV disease, this rate is similar to the pre-ART rate reported in the US (6.5 per 100-person years) in 1994.[16] The high rate of hospitalization may partly be explained by higher exposure to infectious comorbidities such as TB and that ART was started at a later age than those in the USA.

TB was the most common cause of infectious related morbidity and contributed the most days to hospital stay. TB was also responsible for 20% of the hospitalizations in HIV- adolescents. In another study of PHIV+ adolescents on ART for more than ten years from a single clinic in Cape Town, TB comprised 20% of admissions and occurred in 5/9 (56.6%) adolescents with CD4 reconstitution and viral suppression.[17] This highlights the high burden of TB despite well controlled HIV and suggests that strategies such as TB preventive therapy (TPT) must be strengthened.

Although less than a quarter of the hospitalization episodes were classified as infectious, many of the non-infectious causes represent sequelae of an infection such as chronic suppurative otitis media resulting in hospitalizations for tympanoplasty, or meningitis leading to hospitalization for seizures.

Ear related hospitalizations (hearing assessment and tympanoplasty) were the most common causes of non-infectious admissions. The burden of the sequelae of chronic suppurative otitis media has been described in low resource countries.[18,19] A study in Ethiopia showed that tympanic membrane perforations and otorrhea were more common in HIV+ children on ART than in HIV- age-matched children.[20] There were no admissions for ART toxicity. This may be an underestimation due to lack of recording of diagnoses can also reflect that most ART toxicity is managed on an outpatient basis.

Causes of hospitalization are likely to change as children have earlier access to ART. Many of the causes of hospitalization in these adolescents can be attributed to untreated HIV that resulted in repeated infections. Introduction of new vaccines such as the pneumococcal vaccine and initiation of ART in the neonatal period will hopefully result in a decrease in traditional causes of hospitalization.

Factors associated with admission included younger age and CD4 less than during the study period. Children starting ART at ages 3-5 years had decreased odds of multiple admissions than those who started by the age of 2 years. This probably reflects a survivor effect as those under the age of 2 years were likely sicker and would not have survived without ART.

Limitations

The study is limited by almost twenty percent of ‘missing’ causes of hospitalization (unknown and classified only as ‘HIV’). In addition, adolescents enrolled in CTAAC may have been hospitalized elsewhere in South Africa beyond the Western Cape where we could not access information; however, this is unlikely as children were followed every six months with high cohort retention. Another limitation is that investigations and diagnoses at time of hospitalization were dependent on treating clinician and the resources available to them. In particular, adolescents may have been under-investigated for TB. The provincial database is dependent on accurate record keeping and many discharge diagnoses were incomplete.

Findings may not be generalizable to other settings where there is a low TB or high malaria prevalence. There was limited data on TPT and cotrimoxazole prophylaxis, but the majority of adolescents had CD4 counts >500 when they entered the study so would not have received cotrimoxazole. Less than half the adolescents gave a verbal report that they had completed their vaccination schedule.

Conclusions

PHIV+ adolescents on ART have a higher incidence of hospitalization than those HIV-. Strategies to address infectious and non- infectious morbidity must be strengthened, specifically Isoniazid prophylaxis. Hearing assessment and follow up of ear related pathology is essential as such pathology comprises a large burden of hospitalizations. Treatment related morbidity and metabolic complications are still uncommon but continued surveillance is necessary as treatment regimens change and children are started on ART at an earlier age.

Table 1. Characteristics HIV+ and HIV- participants at enrolment

Characteristic	HIV+ (N=515) *	HIV- (N=109) *	p-value
Median age at enrollment	12 (1.6)	11.8 (1.8)	0.31
Sex (male)	263 (51.1)	49 (44.9)	0.13
Socioeconomic status**			
Least disadvantaged	195(37.9)	20(18.3)	<0.01
Moderately disadvantaged	186(36.1)	26(23.9)	
Most disadvantaged	134(26.0)	63(57.8)	
Maternal orphan at enrolment	116 (24.4)	4(4)	<0.01
BMI at enrollment	17.1 (16.0-18.9)	18.6 (16.7-21.5)	<0.01
Median CD4 count (cells/mm^3)	713 (561-957.5)	NA	
Number of participants with viral load <50 copies/ml	387 (75)	NA	
Median duration of ART at enrolment (years)	7.6 (4.6-9.2)	NA	
Median age at ART initiation (years)	4.3 (2.2-7.3)		

Mean (S.D.), median (IQR) and frequency (%) reported as appropriate.
*Total is not 515 and 109 for all characteristics due to missing data
** This was according to a composite score

Table 2. Hospitalizations	HIV+	HIV-	*P*-value
Median follow up time (years)	4.1 (3.7-4.6)	4.1(2.8-4.4)	-
Total person years	2241.9	440.5	-
Hospitalizations (number)	149	10	-
Number of participants hospitalized	91	7	-
Sex (male)	46 (50.5)	2 (28.6%)	0.11
Median age at hospitalization	14.2 (12.8-15.9)	15.4(15-16.5)	0.11
Crude incidence of hospitalization (per 100 person- years)	6.6 (5.7-7.8)	2.2 (1.2-4.3)	<0.01
Cumulative incidence of hospitalization	23.9 (20.6-27.6)	8.4 (4.5-15.6)	<0.01
Number of admissions			
1	56	4	
2	20	3	
3	9	-	
4	5	-	
5	0	-	
6	1	-	
Median CD4 count (cells/mm^3)	713 (561.0-957.5)	NA	
Number of participants with viral load <50 copies/ml	387 (75)	NA	
Median duration of ART at enrolment	7.6 (4.6-9.2)	NA	

Mean (S.D.), median (IQR) and frequency (%) reported as appropriate.

Table 3. Characteristics of HIV+ participants by hospitalization status

Characteristic at study entry	Admitted (n=91)	Never admitted (n=424)	*P*-value
Age (years)	12.2(1.6)	11.9(1.6)	0.11
BMI (kg/m^2)	17.6(16.1-19.4)	17.0 (15.9-18.8)	0.23
CD4 count (cells/mm^3)			
Median	666(511-918)	731(570-972)	0.12
<200	4(4.4)	6(1.4)	0.10
200-499	16(17.6)	58(13.8)	
≥500	71(78.0)	357(84.8)	
Viral load (copies/mL)			
<50	60(65.9)	333(78.7)	**0.03**
50-1000	15(16.5)	41(9.7)	
>1000	16(17.6)	49(11.6)	
Age at ART initiation (years)	4.2(2.3-6.8)	4.4(2.0-7.7)	0.89
0-2	34(37.8)	155(37.2)	
3-5	28(31.1)	111(26.6)	0.58
6-14	28(31.1)	151(36.2)	

Mean (S.D.), median (IQR) and frequency (%) reported as appropriate.

Table 4. Univariate and multivariable analysis of factors associated with hospital admission in HIV+ participants

Characteristic	Events/person years	IRR [95%CI]	p-value	aIRR [95%CI] *	*P*-value
Age (years)					
10-14	94/1241.8				
15-19	55/1000.1	0.64(0.5-0.9)	**0.01**	0.6 (0.4-0.9)	**<0.01**
Sex					
Male	68/1147.4				
Female	81/1094.5	1.3 (0.9-1.7)	0.20		
Socioeconomic status**					
Least	53/856.6				
Moderately	50/813.4	1.0 (0.7-1.5)	0.96		
Most	46/571.9	1.3(0.9-2.00)	0.15		
Maternal orphan at study entry					
Yes	34/509.5				
No	97/1565.9	0.9(0.6-1.4)	0.76		
CD4 count (cells/mm^3)					
> 500	79/1395.7				
< 500	70/846.2	0.7 (0.5-1.00)	**0.04**	0.68 (0.5-0.9)	**0.02**
Viral Load (< 40 copies/ml)					
Not suppressed throughout**	96/1430				
Suppressed throughout	53/811.9	1.00 (0.7-1.4)	0.93		
Age at ART initiation (years)					
0-2	59/829.4				
3-5	35/599.1	0.8(0.5-1.3)	0.37		
6-14	53/783.3	1.00(0.7-1.4)	0.97		

*Adjusted for age and CD4 count

** "Throughout" refers to throughout the entire study period i.e. If the participant had 3 viral loads during the study period, they were all lower than detectable limit.

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Chapter 6:

Tuberculosis infection and disease in South African perinatally HIV-infected adolescents on antiretroviral therapy: a cohort study

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Abstract

Introduction: There are limited data on Tuberculosis (TB) in adolescents with perinatal HIV (PHIV+). We examined the incidence and determinants of TB infection and disease in the Cape Town Adolescent Antiretroviral Cohort (CTAAC).

Methods: PHIV+ adolescents between 9-14 years on antiretroviral therapy (ART) for more than 6 months in public sector care, and age-matched HIV-negative adolescents, were enrolled between 2013-2015 and followed 6-monthly. Symptom screening, chest radiograph, viral load, CD4 count, QuantiFERON (QFT) and sputum for Xpert MTB/RIF, microscopy, culture and sensitivity were performed at enrolment and annually. TB infection was defined by a QFT of >0.35 IU/mL. TB diagnosis was defined as confirmed (culture or Xpert MTB/RIF positive) or unconfirmed (clinical diagnosis and started on TB treatment). Analyses examined the incidence and determinants of TB infection and disease.

Results: Overall 496 PHIV+ and 103 HIV- participants (median age at enrolment 12 (interquartile range, IQR 10.6-13.3) years were followed for a median of 3.1 years (IQR 3.0-3.4); 50% (298/599) were male. PHIV+ participants initiated ART at median age 4.4 (IQR 2.1-7.6) years. At enrolment, 376/496 (76%) had a HIV viral load of <40 copies/mL, median CD4 count was 713 cells/mm^3, and 179/559 (30%) were QFT+, with no difference by HIV status (PHIV+ 154/468, 30%; HIV- 25/91, 27%; *p*=0.31). The cumulative QFT+ prevalence was similar (PHIV+ 225/492, 46%; 95%CI 41% to 50%; HIV- 44/98, 45%; 95% CI 35% to 55%; *p*=0.88). PHIV+ adolescents had a higher incidence of all TB disease than HIV- adolescents (2.2/100PY, 95% CI 1.6-3.1 vs. 0.3/100PY, 95% CI 0.04-2.2; IRR 7.36, 95% CI 1.01-53.55). The rate of bacteriologically confirmed TB in PHIV+ adolescents was 1.3/100 PY and 0.3/100PY for HIV- adolescents, suggesting a 4-fold increased risk of developing TB disease in PHIV+ adolescents despite ART. In addition, a positive QFT at enrolment was not predictive of TB in this population.

Conclusions: High incidence rates of TB disease occur in PHIV+ adolescents despite similar QFT conversion rates to HIV-negative adolescents. Strategies to prevent TB in this vulnerable group must be strengthened.

Introduction

Adolescence is a period of increased risk for both *Mycobacterium tuberculosis (Mtb)* infection and tuberculosis disease (TB), compared to the pre-adolescent period.[1,2] Globally, an estimated 1.8 million people between the ages of 10 -24 years developed TB in 2012, with 534 000 of these living in Africa.[3,4] Adolescents are also more likely to be infectious (smear positive) than younger children.[5] HIV is a risk factor for *Mtb* infection and TB. Countries with a high HIV prevalence have a substantive TB disease burden including in adolescents.[6] Although access to antiretroviral therapy (ART) has led to a decrease in TB incidence in adolescents and children with HIV, they remain at increased risk of TB than those who are HIV-negative.[7-10] Indeed, TB remains a major cause of hospitalization and mortality in adolescents living with HIV.[11] Further, TB may have long term consequences on lung health with subsequent impairment of lung function.[12]

Previous estimates of *Mtb* infection prevalence among adolescents in sub-Saharan Africa have indicated high annual risks among the general population but often lack data on HIV status.[13,14] For example, a recent review that focused on bacteriologically confirmed TB in adolescents and youth found no studies among those with HIV.[15] This may be due to challenges in confirming TB in high burden settings. Adolescents living with perinatally acquired HIV (PHIV+) are likely to be a specifically vulnerable group given risks of disengagement from care, treatment fatigue and increased exposure to adults with TB, in turn placing them at higher risk for *Mtb* infection and TB disease. There is therefore a clear and urgent need to understand the burden of HIV-associated TB in adolescents in high HIV and TB prevalence settings, utilizing robust data collection for TB diagnosis and measures of HIV severity. To address this critical knowledge gap, we investigated the incidence and determinants of *Mtb* infection and TB disease in a South African cohort of PHIV+ adolescents on ART compared to a matched HIV-negative group.

Methods

Study Population and Design

This analysis draws on data from the Cape Town Adolescent Antiretroviral Cohort (CTAAC), a prospective study that enrolled PHIV+ participants between 01 July 2013 to 31 March 2015. Youth aged 9-14 years already accessing HIV care at one of seven public service sites in the Western Cape Province South Africa were eligible for enrolment, provided they had been on antiretroviral therapy (ART) for at least 6 months and were aware of their HIV status. Concurrently, a comparison group of HIV-negative (HIV-) adolescents from primary care facilities in the same communities were enrolled, frequency-matched on sex and age. We excluded HIV- youth with chronic/ systemic inflammatory conditions or known chronic neurological, pulmonary or cardiovascular disease. HIV status was confirmed at enrolment, with annual retesting thereafter, following informed consent.

Sociodemographic data were collected at enrolment and the participant's clinical record was reviewed. Thereafter, participants were seen biannually at the study site. A structured questionnaire and physical examination including Tanner staging, World Health Organisation (WHO) HIV staging and anthropometry was performed at enrolment and annually. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in metres squared (kg/m^2) and classified by WHO reference standards.[16] At each study visit, screening for signs and symptoms of TB was performed; if symptomatic, participants had a chest radiograph (CXR). In addition, two sputum specimens (induced /expectorated) were collected at enrolment, annually and if an intercurrent pneumonia was suspected, and sent for Xpert MTB/RIF (Cepheid) and microscopy, liquid culture using semi-automated 7H9 broth-based Mycobacterial Growth Indicator Tubes (MGIT960; Becton Dickinson, Sparks, MD). Any participant diagnosed with TB at a study visit was referred for treatment at their site of routine clinical care.

At enrolment, all participants received Quanti-FERONTB Gold In-Tube® (QFT; Qiagen, Hilden, Germany) testing. For participants with a positive QFT at enrolment or subsequently, the test was not repeated. For prior negative or indeterminate QFT tests, the assay was repeated annually for 3 years. QFT tests were not done real time, but were stored and batched tested, so results could not inform participant care. Additional laboratory measures performed at enrolment and annually included HIV viral load (Roche COBAS Ampliprep/Taqman) and CD4 cell count (Beckman Coulter®, Brea, CA, USA) in HIV+ participants; HIV- participants were retested every 6 months for HIV infection, using Alere Determine™ (Abbott, Chiba, Japan).

Throughout study follow-up, participants continued to receive routine care at their primary care sites, including ART and prophylaxis against opportunistic infections (OI). The most commonly used ART regimens, according to national guidelines, were Abacavir, Lamivudine and either Efavirenz or a protease inhibitor. According to national guidelines, isoniazid (INH) prophylaxis is given to children living with HIV between the ages of 5-14 years for 6 months, while those >15 years are recommended to receive INH for 12 months. Cotrimoxazole is given to adolescents with a CD4 count below 200 cells/mm^3 and is discontinued once CD4 is sustained above 200 cells/mm^3.[17] Routine care includes TB screening if symptomatic with a CXR, sputum for Xpert MTB/RIF and culture, at the discretion of the primary physician. All participants received the South African standard Expanded Program on Immunization (EPI), including BCG at birth, diphtheria-pertussis-tetanus (DwPT), *Haemophilus influenzae* type b (HIB), hepatitis B (HBV), measles and polio immunizations.[18] Notably, as acellular pertussis and pneumococcal conjugate vaccines were only introduced into the public immunization program in 2009, no participant would have received these vaccines.

Laboratory investigations

QFT tests results were expressed as international units (IU/mL) and considered positive if Nil value was ≤0.8 UL/mL, the TB Ag was ≥0.35 IU/mL and <25 % of Nil value after subtraction of the negative control value (Ag-Nil) and the Mitogen-Nil value was ≥0.5 IU/m; Negative if the Nil value was ≤0.8 IU/mL, the TB Ag was <0.35 IU/mL or ≤0.35 and <25 % of Nil value after subtraction of the negative control value (Ag-Nil) and the Mitogen-Nil value was ≥0.5 IU/mL. For indeterminate status, the Nil value was ≤0.8 UL/mL, the TB Ag was <0.35 IU/mL or ≤0.35 and <25 % of Nil value after subtraction of the negative control value (Ag-Nil) and the Mitogen-Nil value was <0.5 IU/mL or if the Nil value was <0.8 UL/mL.

Diagnosis of TB

Diagnoses were obtained from study laboratory results as well as data abstracted from a provincial database that recorded initiation of TB treatment.[19] We included all TB diagnoses between study enrolment and follow-up to 31st October 2018. A pediatric infectious disease specialist reviewed all records and diagnoses in each participant's clinical record. TB disease was defined as "confirmed" (culture-confirmed or Xpert MTB/RIF positive) or "unconfirmed" (participants who presented with clinical signs and symptoms suggestive of TB and documented as initiating TB treatment at their primary care facilities, but without microbiological confirmation).[20]

Statistical methods

Data analysis used Stata version 14.1 (StataCorp, College Station, Texas); all statistical tests were 2-sided at α=0.05. The primary outcomes were the incidence of a positive QFT and of TB disease over time. Following standard data exploration, regression analyses were structured as follows: (i) Prevalence of QFT positivity (QFT+) at enrolment (cross-sectional, logistic regression), (ii) QFT conversion rates during first 3 years (Cox proportional hazards, PH; and negative binomial regression models with generalized estimating equations, GEE); restricted to those testing QFT negative at baseline, person-time censored at first QFT+ test or last known time alive.

TB incidence rates over full follow-up period were calculated using Cox PH for time-to-first TB event; person-time staggered by 6 months for prevalent TB cases, censored at first TB event or last known time alive up to 31st October 2018 and negative binomial regression with GEE for overall incidence of all TB events; person-time staggered for prevalent cases, censored at last known time alive up to 31st October 2018. Loss to follow-up was not a competing event as we could still access recorded TB episodes from the database covering all public health services in the province.[19] CD4 count, viral load, Tanner stage, BMI and age were time-varying (annual changes). Known category boundaries were assigned *a priori* based on clinical relevance. We explored the impact of HIV viral suppression on differential risks of TB infection (QFT +) and TB disease between HIV+ and HIV- youth by creating four dummy variables to compare each category of HIV viral load (VL) suppression (at 40 and 1000 copies/mL) separately to the reference group of HIV- adolescents. Sensitivity analyses evaluated differences by confirmed TB diagnosis, and variation in person-time definition.

Ethical approval was given by the University of Cape Town and Stellenbosch University (051/2013). A parent or legal guardian provided informed consent and assent was obtained from all adolescents; this was renewed annually. All participants knew their HIV status as a pre-requisite to study enrolment and gave permission for medical record reviews.

Role of the funding source

The funders were not involved in the collection, analysis, and interpretation of the data; in the writing of the report; or in the decision to submit for publication. The corresponding author confirms that she had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 795 eligible adolescents screened, 625 (515 HIV+, 110 HIV-) were enrolled (Figure 1). Most study participants attended at least 7 study visits over the follow-up period (383 [74%] of 515 HIV+; and 85 [77%] of 110 HIV-). Data collected at two or more visits were available for 599 adolescents (496 [96%] of HIV+, 103 [94%] of HIV-; *p*=0.20), who were followed for a median of 3.1 years (interquartile range, IQR 3.0-3.4; HIV+ for 3.1 years and HIV- for 3.4 years). There were 2 deaths (both HIV+, one suicide and one at home after disengaging from clinical care). Enrolment characteristics are shown in Table 1; minor differences between those included in the analysis versus those excluded are shown in Supplemental Table 1.

Overall, 50% (298/599) were male, with median enrolment age of 12 (IQR 10.6-13.3) years, similar for HIV+ and HIV- participants (Table 1). At enrolment, HIV+ (vs. HIV-) adolescents were more likely to be pre-pubertal (Tanner Stage 1: 231/496 [47%] vs. 34/103 [33%] and underweight (BMI<18.5 in 347/496 [70%] vs. 50/103 [48%]). They were also more likely to have a prior history of TB disease (298/496 [61%] vs. 3 [0.3%]). HIV+ participants had initiated ART at median age 4.4 (IQR 2.1-7.6) years, with 376/496 (76%) having an HIV-VL of <40 copies HIV RNA copies/mm^3, and the median CD4 count was 713 (IQR: 564-954) cells/mm ^{3} at enrolment (Table 1). Median ART duration was 7.6 years (IQR:4.6-9.2). Two hundred and seventy (54%) adolescents were on an efavirenz-based regimen and 143 (29%) were on a lopinavir/ritonavir-based regimen at enrolment, while 83 (17%) were on other regimens. One hundred and thirty-one (27%) HIV+ adolescents and 3 (3%) HIV- adolescents were receiving INH prophylaxis at enrolment and 9/459, 2% of HIV+ and 4/96 (4%) HIV- adolescents had a household TB contact at enrolment.

QFT positivity

At enrolment, 179 (32%) of 559 adolescents were QFT+, with no difference by HIV status (HIV+ 154/468, 33%; HIV- 25/91, 27%; *p*=0.31; Table 1). There was also no difference in the number of indeterminate tests by HIV status. Among HIV+ youth, concurrent diagnosis of TB disease was associated with increased odds of QFT+ (vs. those without TB at enrolment, OR 5.23, 95% CI 1.00-27.30; *p*=0.049). No other enrolment characteristics predicted baseline prevalence of QFT+ among either HIV- or HIV+ youth (Supplemental Table 2), after excluding those with prevalent TB disease. By 36 months of follow-up, the cumulative prevalence of QFT+ was 46% (95% CI 42-50%) among 590 participants with known QFT results; prevalence was similar for HIV+ (225/492, 46%; 95%CI 41% to 50%) and HIV- adolescents (44/98, 45%; 95% CI 35% to 55%), *p*=0.88.

Among 380 adolescents initially QFT-, 82 subsequently tested QFT+ (63/314 HIV+ and 19/66 HIV-), Supplemental Table 3. The overall QFT conversion incidence was 28.8/person-year (PY); 26.7/PY

(95% CI 20.8-34.1) among HIV+ and 39.1/PY (95% CI 24.9-61.2) among HIV- adolescents; rates by subgroups of HIV+ youth are shown in Supplemental Table 3. Using a time-to-event approach, HIV+ adolescents had marginally lower hazard of QFT conversion than HIV- (HR 0.65, 95% CI 0.39-1.09), Table 2a. Other factors associated with QFT conversion overall are shown in Table 2a; and restricted to HIV+ adolescents, in Table 2b. There was a notable gradient in hazard of QFT conversion by CD4 count and VL (Table 2a, Table 2b; Figures 2A, 2B and 3A), in both crude and adjusted analyses. Compared with all HIV- youth, HIV+ youth with VL ≤ 40 copies/mL had 25% lower hazard of QFT conversion (HR 0.75, 95% CI 0.44-1.27), although precision was limited. Those with HIV-VL >40 but <1000 copies/mL had an even lower relative hazard of QFT conversion (HR 0.59, 95% CI 0.29-1.23) while the lowest relative hazard of QFT conversion was seen among HIV+ youth with HIV-VL ≥1000 copies/mL (vs. all HIV- youth, HR 0.23; 95% CI 0.08-1.00, p=0.05; Figure 3A). A similar relationship was seen in analysis limited to HIV+ youth, where those with CD4< 500 cells/μL had a 62% decreased hazard of QFT conversion (vs CD4≥500 cells/μL: aHR 0.38, 95% CI 0.15-0.97), after adjusting for age, Tanner staging and HIV-VL. Age at ART initiation was not associated with QFT conversion rates (Table 2b).

TB disease

At enrolment, 7 (HIV+) participants had TB disease (5 cases were confirmed TB). During follow-up, an additional 35 episodes of incident TB (20 confirmed (19 HIV+, 1 HIV-), 15 unconfirmed) occurred in 30 participants (two HIV+ participants had two episodes, and one had three). During follow up, there were 30 cases of pulmonary TB (PTB), two cases of TB meningitis (one HIV+ and one HIV- participant) and 3 cases of unspecified TB. A total of 14 (3 %) HIV+ and 1(1%) HIV- participants were hospitalized for TB. In total, 11 CXR results were reported by radiologists of which 7 were reported as suggestive of PTB. All *Mtb* isolates were sensitive to INH and rifampicin, except for 1 INH mono-resistant isolate.

The overall incidence of TB disease was 1.9/100PY (95% CI 1.4-2.6) for the full cohort (Table 3a, Supplemental table 4). HIV+ participants had a markedly higher incidence than HIV- participants in both crude and adjusted analysis (2.2/100PY, 95% CI 1.6-3.1 vs. 0.3/100PY, 95% CI 0.04-2.2; IRR 7.36, 95% CI 1.01-53.55; Table 3a). When restricting TB diagnoses to those with confirmed TB disease, TB incidence rate for the full cohort was 1.1/100PY (Supplemental Table 4) with a consistently higher incidence in HIV+ (1.3/100PY) than HIV- (0.3/100PY), although the association was attenuated (IRR 4.12, 95% CI 0.65-272.3) with less precision (Supplemental Table 4). In sensitivity analyses (Supplemental Table 4), inferences were unchanged using different person-time calculations. Incidence and IRR of confirmed TB among HIV+ adolescents stratified by enrolment and time-varying characteristics are shown in Supplemental Table 5.

CD4 count and HIV-VL were strong predictors of overall incidence of TB disease (confirmed and unconfirmed) in HIV+ participants, using both analytic approaches (IRR, shown in Tables 3a and 3b; and HR, hazard ratios, shown in Tables 4a and 4b). HIV+ youth with HIV-VL >1000 copies/mL had 12-fold higher hazard of TB compared to all HIV- youth (HR 12.21, 95% CI 1.46-102.36, Table 4a; in contrast, those with HIV-VL <40 copies/mL had only a slightly higher hazard relative to HIV- youth (HR 6.22, 95% CI 0.82-47.35). The hazard for HIV+ youth with HIV-VL 40-1000 copies/mL (vs. HIV-youth) fell in between the former two categories (HR 8.84, 95% CI 1.08-72.38); inferences were unchanged after adjustment (Table 4a and Figure 3A). A similar gradient was observed comparing strata of CD4 cell counts (Tables 4a and 4b). Although these associations were attenuated when restricting events to only confirmed TB diagnoses, the pattern of a risk gradient by disease severity remained (Supplemental Tables 5 and 6). Increasing HIV-VL or decreasing CD4 cell count was therefore associated with increased likelihood of TB disease but a decreased likelihood of QFT+ conversion. Figures 3A and 3B provide a direct visual comparison of changing relative hazard by HIV disease severity.

Of 27 HIV+ participants with a single incident episode of TB, 16 (59%) completed treatment and 8 (30%) had weight gain and resolution of symptoms but we could not confirm treatment completion. One (3.7%) participant had treatment stopped after the TB diagnosis was changed after extensive workup, while a further two (7.3%) participants were lost to follow-up after TB diagnosis and no further information could be found. One HIV+ participant had three episodes of TB and did not complete treatment during the first episode. This participant completed treatment for the subsequent two episodes. Of the two participants with two incident TB episodes, one completed treatment for both episodes (with a negative culture between episodes) and the other completed treatment for the first episode but was lost to follow up after diagnosis of the second episode. The HIV- participant completed treatment and had resolution of symptoms.

Discussion

These data demonstrate that, in the context of high HIV and TB burden, PHIV+ adolescents have a much higher rate of TB disease than HIV- adolescents despite ART and despite similar rates of QFT conversion. These findings appeared driven mainly by incomplete CD4 immune reconstitution or non-suppressive ART, as evidenced by higher rates of TB disease in those with lower CD4 counts or higher HIV-VL. In addition, the prevalence of *M.tb* infection was high in both HIV- and PHIV+ participants. These findings highlight that *M.tb* infection is common and that TB remains an important cause of morbidity among PHIV+ adolescents.

The prevalence of positive QFT at enrolment for the entire cohort was 32% with a cumulative prevalence of 46% after 3 years of follow up at a median age of 15 years. This is similar to the prevalence reported in Western Kenya, where at 14.4 years the prevalence of *M.tb* infection (using Tuberculin skin testing) was also 32%; however, only 0.5% adolescents were HIV+ in that study and 41% had unknown HIV status.[14] The prevalence in our study is similar to the 53% prevalence reported in Cape Town in 2011 in adolescents between 14-17 years.[13] However, in our study, a positive QFT was not predictive of developing TB. In addition, low CD4 count or high VL were associated with decreased risk of QFT conversion. This is consistent with an adult study that reported a moderate predictive effect of QFT to diagnose incident TB, with a negative effect of CD4 count on sensitivity.[21]

The incidence of TB in PHIV+ adolescents in our study was very high, at 2.2/100 PY. This is higher than the rate of 0.7/100 PY reported from the same setting in 2011 (in both HIV+ and HIV- adolescents) and higher than in Ethiopian adolescents (1.6/100 PY) on ART for more than 5 years.[22] This may be due to the extremely high prevalence of TB in Western Cape communities where most of these adolescents live, or high exposure in HIV households.[23] To our knowledge, this is the first study from sub-Saharan Africa to report on the incidence of confirmed TB in PHIV+ adolescents. The rate of confirmed TB was 1.3 (CI:0.80-1.97)/100 PY, which is very high, especially given that adolescents were on ART for several years. There are no comparable studies of PHIV+ adolescents, but between 2005 and 2007 an adolescent cohort in the Western Cape, South Africa, reported TB disease incidence of 0.45 per 100 PY; however, this study did not include routine HIV testing. Low CD4 count and high VL were associated with increased risk of TB. This highlights a vulnerable group of PHIV adolescents who are at increased risk of TB and who may paradoxically have false negative QFT results. The results also emphasise the need to integrate HIV and TB adolescent programs.

Limitations and strengths

Limitations include that history of prior TB and TB contact could not always be verified. Data on INH prophylaxis were limited. Serial QFT testing was not done on QFT positive participants, so QFT reversion could not be reported. There was limited availability of CXRs from the time of TB diagnosis. Strengths of our study include long-term follow-up of a large cohort of PHIV adolescents in a resource-limited setting with high cohort retention; microbiologic confirmation of TB and longitudinal QFT testing over an extended period of time. In addition a comparison group of matched HIV- participants strengthens inferences by providing insights into the background risks of TB infection and disease in this setting.

Conclusions

In summary, we found that despite similar rates of *M.tb* infection there is a higher rate of TB in PHIV+ adolescents on ART compared to HIV- adolescents. This increased rate of TB was driven by those adolescents with high VL and decreased CD4 counts, highlighting the importance of screening for incident TB disease in PHIV+ adolescents failing ART. These adolescents should have access to clinical assessment including CXR and rapid molecular diagnostics and culture as IGRA assays may not be sensitive or predictive of TB disease, especially if HIV poorly controlled. In addition, strategies to prevent TB such as TB preventive therapy should be strengthened.

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Contributors

LJF (corresponding author) was the study doctor, assisted with collection of data; helped in planning the analyses; wrote the first draft of the manuscript and confirms that she had full access to all the data and takes final responsibility for the decision to submit for publication. KB was responsible for data management and oversight. SLR did the analysis with input from LJF. LM and HJZ conceived the CTAAC study, and were responsible for study design, funding, implementation and overall leadership. KW, WP and SR were responsible for QFT testing on the study. LG was a study doctor and contributed to the initial study design. All authors contributed to and approved the final manuscript.

Table 1. Characteristics of study participants at enrolment

Characteristic	Total (N=599)	HIV+ (N=496)	HIV- (N=103)	*p*-value
Age (years)	12.0 (10.6-13.3)	12.0 (10.7-13.3)	11.6 (10.0-13.4)	0.199
Male sex	298 (50%)	251 (51%)	47 (46%)	0.358
Relative poverty categories[1]				<0.0001
Least disadvantaged	209 (35%)	191 (39%)	18 (18%)	
Moderate disadvantage	203 (34%)	179 (36%)	24 (24%)	
Most disadvantaged	185 (31%)	125 (25%)	60 (59%)	
Previous TB disease	301 (51%)	298 (61%)	3 (3%)	<0.0001
On treatment for any TB disease at enrolment	7 (1%)	7 (1%)	0	0.225
Previous isoniazid preventive therapy	134 (22%)	131 (27%)	3 (3%)	<0.001
Current known TB contact	13/555 (2%)	9/459 (2%)	4/96 (4%)	0.194
Tanner stage				0.041
Prepubertal (Stage I)	265 (44%)	231 (47%)	34 (33%)	
Adolescent (Stages II-IV)	303 (51%)	240 (48%)	63 (61%)	
Mature (Stage V)	31 (5%)	25 (5%)	6 (6%)	
Body mass index (BMI, (kg/m^2)	17.3 (16.1-19.2)	17.1 (16.0-18.9)	18.7 (16.6-21.5)	0.0001
BMI categories				<0.0001
Underweight, BMI<18.5	397 (66%)	347 (70%)	50 (48%)	
Normal, BMI ≥ 18.5 <25	179 (30%)	134 (27%)	45 (44%)	
Overweight/obese, BMI ≥ 25	23 (4%)	15 (3%)	8 (8%)	
Age at ART initiation (years)	n/a	4.4 (2.0-7.6)	n/a	n/a
Categories of age at ART initiation				
≤ 2 years	n/a	120 (25%)	n/a	n/a
>2, < 6 years	n/a	196 (40%)	n/a	n/a
≥ 6 years	n/a	172 (35%)	n/a	n/a
HIV viral load (log_{10} copies/mL)	n/a	1.59 (1.59-1.60)	n/a	n/a
Categories of HIV viral load				
<40 copies/mL	n/a	376 (76%)	n/a	n/a
40-1000 copies/mL	n/a	56 (11%)	n/a	n/a
>=1000 copies/mL	n/a	64 (13%)	n/a	n/a
CD4 cell count (cells/µL)	n/a	713 (564-954)	n/a	n/a
CD4 cell count categories				
>500 cells/µL	n/a	414 (83%)	n/a	n/a
>350, ≤500 cells/µL	n/a	39 (8%)	n/a	n/a
≤350 cells/µL	n/a	43 (9%)	n/a	n/a
QFT result at enrolment[2]				0.052
QFT positive	179 (30%)	154 (31%)	25 (24%)	
QFT negative	362 (60%)	299 (60%)	63 (61%)	
QFT indeterminate	18 (3%)	15 (3%)	3 (3%)	
QFT results not available	40 (7%)	28 (6%)	12 (12%)	

Abbreviations: QFT - interferon gamma release assay (QuantiFERON-TB); mL, milliliter; µL, microliter;
Numbers are median (interquartile range) or n (column percentage); *p*-values from Chi2 or Kruskal-Wallis testing, not corrected for multiplicity.

[1]Tertiles of a continuous score incorporating a standardized asset score (including type of housing, access to running water and flush toilet), employment and education; missing (n=2).

Table 2a. Time to QFT conversion among previously QFT negative study participants, comparing HIV+ to HIV- participants (N=380): crude and adjusted hazard ratios from Cox proportional hazards regression

	Crude HR (95% CI)	**aHR (95% CI)[1]**
HIV infection: HIV-positive vs HIV-negative	0.65 (0.39-1.09)	-
Effects of time-varying HIV viral suppression on relative Hazard of QFT conversion among HIV+ vs HIV- youth		
HIV-negative (reference)	1.00	1.00
HIV-positive, viral load ≤ 40 copies/mL	0.75 (0.44-1.27)	0.92 (0.52-1.62)
HIV-positive, viral load >40, <1000 copies/mL	0.59 (0.29-1.23)	0.75 (0.35-1.62)
HIV-positive, viral load ≥ 1000 copies/mL	**0.23 (0.07-0.79)**	**0.29 (0.08-1.00)**
Male vs female sex	0.71 (0.46-1.11)	-
Age at study visit (time-varying, per year increase)	0.92 (0.81-1.05)	**0.86 (0.73-1.00)**
Categories of age at study visit (time-varying, in years)		
<12 years (reference)	1.00	-
≥ 12, <14 years	1.55 (0.73-3.31)	-
≥ 14, <16 years	1.14 (0.52-2.52)	-
≥ 16 years	1.00 (0.39-2.56)	-
BMI categories (time-varying)		
Normal weight, ≥ 18, <25 kg/m^2 (reference)	1.00	-
Underweight, <18 kg/m^2	0.67 (0.41-1.10)	-
Overweight/obese, ≥ 25 kg/m^2	0.87 (0.44-1.72)	-
Tanner staging at study visit (time-varying)		
Stage I (pre-adolescent), reference	1.00	1.00
Stage II to IV (adolescent)	1.02 (0.56-1.84)	1.34 (0.71-2.52)
Stage V (mature)	1.46 (0.73-2.90)	**2.40 (1.06-5.42)**
Known TB contact at enrolment (yes vs no)	1.78 (0.33-5.65)	-

Abbreviations: QFT - interferon gamma release assay (QuantiFERON-TB); HIV, human immunodeficiency virus; CD, cluster of differentiation; ml, milliliter; uL, microliter; HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence intervals; [1]multivariable model adjusted for all variables with adjusted estimates shown as well as baseline measures of relative poverty.

Table 2b. Time to QFT conversion among previously QFT negative study participants, restricted HIV+ participants (N=314): crude and adjusted hazard ratios from Cox proportional hazards regression

	Crude HR (95% CI)	aHR (95% CI)[1]
HIV viral load $\log_{10}$ copies/mL (per $\log_{10}$ increase)	**0.58 (0.36-0.95)**	0.67 (0.40-1.10)
CD4 cell count categories		
HIV-positive, CD4 ≥500 (reference)	1.00	1.00
HIV-positive, CD4 <500	**0.28 (0.11-0.71)**	**0.38 (0.15-0.97)**
CD4 cell count cells/μL (per 100 increase)	**1.08 (1.00-1.17)**	
Male vs female sex	0.74 (0.45-1.23)	-
Age at study visit (time-varying, per year increase)	0.90 (0.77-1.05)	0.84 (0.70-1.01)
Categories of age at study visit (time-varying, in years)		
<12 years (reference)	1.00	-
≥ 12, <14 years	1.13 (0.52-2.48)	-
≥ 14, <16 years	0.93 (0.41-2.10)	-
≥ 16 years	0.76 (0.27-2.14)	-
BMI categories (time-varying)		
Normal weight, ≥ 18, <25 kg/m^2(reference)	1.00	-
Underweight, <18 kg/m^2	0.65 (0.38-1.11)	-
Overweight/obese, ≥ 25 kg/m^2	0.68 (0.26-1.73)	-
Tanner staging at study visit (time-varying)		
Stage I (pre-adolescent), reference	1.00	1.00
Stage II to IV (adolescent)	0.99 (0.51-1.89)	1.39 (0.69-2.79)
Stage V (mature)	1.51 (0.70-3.24)	**2.51 (1.01-6.26)**
Known TB contact at enrolment (yes vs no)	0.76 (0.10-5.50)	-
Age at ART initiation		
≤ 2 years (reference)	1.00	-
>2, <6 years	1.08 (0.55-2.11)	-
≥6 years	1.10 (0.56-2.17)	-

Abbreviations: QFT - interferon gamma release assay (QuantiFERON-TB); HIV, human immunodeficiency virus; CD, cluster of differentiation; ml, milliliter; uL, microliter; HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence intervals; [1]multivariable model adjusted for all variables with adjusted estimates shown as well as baseline measures of relative poverty.

Table 3a. Incidence rates of all tuberculosis disease events over follow-up, comparing HIV- to HIV+ participants: crude and adjusted incidence rate ratios from negative binomial regression with generalized estimating equations

	TB Events	Person-years	Rate/100PY (95% CI)	Crude IRR (95% CI)	Adjusted IRR (95% CI)[1]
Full cohort (both HIV+ and HIV- youth)	35	1840.6	1.9 (1.4-2.6)	-	-
HIV status					
HIV-negative (reference)	1	328.2	0.3 (0.04-2.2)	1.00	-
HIV-positive	34	1512.3	2.2 (1.6-3.1)	**7.36 (1.01-53.55)**	-
HIV status and/or HIV viral suppression categories					
HIV-negative (reference)	1	328.2	0.3 (0.04-2.2)	1.00	1.00
HIV-positive, viral load ≤ 40 copies/mL	17	1036.8	1.6 (1.0-2.6)	5.39 (0.72-40.02)	4.92 (0.60-40.22)
HIV-positive, viral load >40, <1000 copies/mL	8	285.5	2.8 (1.4-5.6)	**9.23 (1.17-72.72)**	7.94 (0.93-67.78)
HIV-positive, viral load ≥ 1000 copies/mL	9	190.1	4.7 (2.5-9.1)	**15.64 (1.94-125.92)**	**12.99 (1.46-115.68)**
HIV status and/or CD4 cell count categories					
HIV-negative (reference)	1	328.2	0.3 (0.04-2.2)	1.00	-
HIV-positive, CD4 ≥500 cells/μ*L*	21	1192.3	1.8 (1.1-2.7)	5.76 (0.78-42.67)	-
HIV-positive, CD4 <500 cells/μ*L*	13	320.0	4.1 (2.4-7.0)	**13.36 (1.76-101.5)**	-
Sex					
Female (reference)	19	925.0	2.0 (1.3-3.2)	1.00	-
Male	16	915.6	1.7 (1.1-2.9)	0.85 (0.41-1.78)	-
Age at study visit (time-varying, per year increase)	-	-	-	**1.40 (1.17-1.68)**	**1.37 (1.12-1.68)**
Categories of age at study visit (time-varying, in years)					
<12 years (reference)	2	338.3	0.6 (0.1-2.4)	1.00	-
≥ 12, <14 years	10	646.6	1.5 (0.8-2.9)	2.62 (0.58-11.9)	-
≥ 14, <16 years	14	604.4	2.3 (1.4-3.9)	3.92 (0.90-17.14)	-
≥ 16 years	9	251.2	3.6 (1.9-6.9)	**6.06 (1.22-30.15)**	-
BMI categories (time-varying)					
Normal weight, ≥ 18, <25 kg/m^2 (reference)	15	803.3	1.9 (1.1-3.1)	1.00	-
Underweight, <18 kg/m^2	18	867.1	2.1 (1.3-3.3)	1.11 (0.54-2.30)	
Overweight/obese, ≥ 25 kg/m^2	2	170.1	1.2 (0.3-4.7)	0.63 (0.16-2.42)	-
Tanner staging at study visit (time-varying)					
Stage I (pre-adolescent), reference	5	445.3	1.1 (0.5-2.7)	1.00	1.00
Stage II to IV (adolescent)	22	1090.1	2.0 (1.3-3.1)	1.78 (0.73-4.34)	0.92 (0.31-2.72)
Stage V (mature)	8	305.2	2.6 (1.3-5.2)	2.32 (0.66-8.10)	0.90 (0.24-3.31)
Known TB contact, household or otherwise					

	TB Events	Person-years	Rate/100PY (95% CI)	Crude IRR (95% CI)	Adjusted IRR (95% CI)[1]
No (reference)	34	1798.8	1.9 (1.4-2.6)	1.00	-
Yes	1	41.8	2.4 (0.3-17.0)	1.26 (0.18-8.82)	-
QFT result at study enrolment					
QFT-negative (reference)	26	1176.9	2.2 (1.5-3.2)	1.00	-
QFT-positive	6	548.7	1.1 (0.5-2.4)	0.49 (0.20-1.20)	-
QFT test results unknown	3	115.0	2.6 (0.8-8.1)	1.18 (0.27-5.23)	-

Abbreviations: TB, tuberculosis disease; PY, person-years; IRR, incidence rate ratio from population-averaged negative binomial regression with generalized estimating equations and robust variance; QFT - interferon gamma release assay (QuantiFERON-TB); [1]multivariable model adjusted for all variables with adjusted estimates shown as well as baseline measures of relative poverty

Table 3b. Incidence of all tuberculosis disease events among HIV+ participants, stratified by participant characteristics: crude and adjusted incidence rate ratios from negative binomial regression with generalized estimating equations

	TB Events	Person-years	Rate/100PY (95% CI)	Crude IRR (95% CI)	Adjusted IRR (95% CI)[1]
All HIV+ youth	34	1512.3	2.2 (1.6-3.1)	**-**	-
HIV viral load (log_{10} copies/mL)	-	-	-	**1.53 (1.14-2.05)**	
HIV viral suppression categories (time-varying)					
HIV-viral load ≤ 40 copies/mL (reference)	17	1036.8	1.6 (1.0-2.6)	1.00	1.00
HIV-viral load >40, <1000 copies/mL	8	285.5	2.8 (1.4-5.6)	1.71 (0.77-3.79)	1.58 (0.71-3.52)
HIV-viral load ≥ 1000 copies/mL	9	190.1	4.7 (2.5-9.1)	**2.90 (1.28-6.60)**	**2.45 (1.08-5.55)**
CD4 cell count (per 100 cells/μL)	-	-	-	0.97 (0.87-1.07)	0.97 (0.86-1.10)
CD4 cell count categories					
CD4 ≥ 500 cells/μL (reference)	21	1192.3	1.8 (1.1-2.7)	1.00	-
CD4 <500 cells/μL	13	320.0	4.1 (2.4-7.0)	**2.32 (1.17-4.60)**	-
Age at ART initiation					
≤ 2 years (references)	2	377.4	0.5 (0.1-2.1)	1.00	-
>2, <6 years	12	597.0	2.0 (1.1-3.5)	3.80 (0.85-17.02)	-
≥6 years	18	512.2	3.5 (2.2-5.6)	**6.67 (1.54-28.97)**	-
Sex					
Female (reference)	18	741.6	2.4 (1.5-3.8)	1.00	-
Male	16	770.7	2.1 (1.3-3.4)	0.85 (0.41-1.80)	-
Age at study visit (time-varying, per year increase)	-	-	-	**1.37 (1.15-1.67)**	**1.32 (1.06-1.65)**
Categories of age at study visit (time-varying, in years)					
<12 years (reference)	2	262.2	0.8 (0.2-3.0)	1.00	-
≥ 12, <14 years	10	543.7	1.8 (1.0-3.4)	2.42 (0.53-11.00)	-
≥ 14, <16 years	14	501.9	2.8 (1.6-4.7)	3.67 (0.84-16.03)	-
≥ 16 years	8	204.4	3.9 (2.0 -7.8)	5.15 (1.00-26.47)	-
BMI categories (time-varying)					
Normal weight, ≥ 18, <25 kg/m^2 (reference)	14	650.6	2.2 (1.3-3.6)	1.00	-
Underweight, <18 kg/m^2	18	747.9	2.4 (1.5-3.8)	1.12 (0.53-2.34)	-
Overweight/obese, ≥ 25 kg/m^2	2	113.7	1.8 (0.4-7.0)	0.82 (0.21-3.14)	-
Tanner staging at study visit (time-varying)					
Stage I (pre-adolescent, reference)	5	380.5	1.3 (0.5-3.2)	1.00	1.00
Stage II to IV (adolescent)	21	888.7	2.4 (1.5-3.6)	1.79 (0.73-4.36)	0.93 (0.31-2.78)
Stage V (mature)	8	243.1	3.3 (1.6-6.6)	2.49 (0.72-8.67)	1.00 (0.27-3.64)
Known TB contact, household or otherwise					
No (reference)	33	1485.1	2.2 (1.6-3.1)	1.00	-
Yes	1	27.2	3.7 (0.5-26.1)	1.65 (0.25-11.00)	-
QFT result at study enrolment					
QFT-negative (reference)	25	967.0	2.6 (1.7-3.8)	1.00	-

	TB Events	Person-years	Rate/100PY (95% CI)	Crude IRR (95% CI)	Adjusted IRR (95% CI)[1]
QFT-positive	6	467.4	1.3 (0.6-2.8)	0.49 (0.20-1.21)	-
QFT test results unknown	3	77.9	3.8 (1.2-11.9)	1.49 (0.34-6.49)	-

Abbreviations: TB, tuberculosis disease; PY, person-years; IRR, incidence rate ratio from population-averaged negative binomial regression with generalized estimating equations and robust variance; QFT - interferon gamma release assay (QuantiFERON-TB); [1]multivariable model adjusted for all variables with adjusted estimates shown as well as baseline measures of relative poverty

Table 4a. Time to first TB disease event comparing HIV+ to HIV- participants (N=599): crude and adjusted hazard ratios from Cox proportional hazards regression

	Crude HR (95% CI)	**aHR (95% CI)[1]**
HIV infection: HIV+ vs. HIV-	**7.56 (1.02-56.04)**	-
Effects of time-varying HIV viral suppression on relative hazard of incident TB among HIV+ vs HIV- youth		
HIV- (reference)	1.00	1.00
HIV+, viral load ≤ 40 copies/mL	6.22 (0.82-47.35)	5.39 (0.69-42.17)
HIV+, viral load >40, <1000 copies/mL	**8.84 (1.08-72.38)**	7.69 (0.92-64.14)
HIV+, viral load ≥ 1000 copies/mL	**12.21 (1.46-102.26)**	**9.65 (1.12-82.92)**
Male vs. female sex	0.84 (0.41-1.70)	-
Age at study visit (time-varying, per year older)	**1.35 (1.08-1.68)**	**1.44 (1.12-1.86)**
Categories of age at study visit (time-varying)		
<12 years (reference)	1.00	-
≥ 12, <14 years	3.96 (0.48-32.42)	-
≥ 14, <16 years	5.26 (0.65-42.88)	-
≥ 16 years	6.94 (0.77-62.74)	-
BMI categories (time-varying)		
Normal weight, ≥ 18, <25 kg/m^2 (reference)	1.00	-
Underweight, <18 kg/m^2	1.29 (0.61-2.72)	-
Overweight/obese, ≥ 25 kg/m^2	0.33 (0.04-2.52)	-
Tanner staging at study visit (time-varying)		
Stage I (reference)	1.00	1.00
Stage II to IV	1.20 (0.43-3.33)	0.70 (0.23-2.09)
Stage V	1.15 (0.33-4.02)	0.48 (0.12-1.95)
Known TB contact at enrolment (yes vs no)	1.45 (0.28-7.43)	-
QFT result at study enrolment		
QFT-negative (reference)	1.00	-
QFT-positive	0.64 (0.26-1.59)	-
QFT test results unknown	1.20 (0.28-5.18)	-

Abbreviations: QFT - interferon gamma release assay (QuantiFERON-TB); HIV, human immunodeficiency virus; CD, cluster of differentiation; ml, milliliter; µL, microliter; HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence intervals; [1]multivariable model adjusted for all variables with adjusted estimates shown as well as baseline measures of relative poverty.

Table 4b. Factors associated with time to first TB disease event among HIV+ participants (N=496): crude and adjusted hazard ratios from Cox proportional hazards regression

	Crude HR (95% CI)	**aHR (95% CI)[1]**
HIV viral load log_{10} copies/mL (per log_{10} increase)	**1.41 (1.00-1.98)**	1.25 (0.86-1.82)
HIV viral suppression categories (time-varying)		
HIV viral load ≤ 40 copies/mL (reference)	1.00	-
HIV viral load >40, <1000 copies/mL	1.41 (0.59-3.40)	-
HIV viral load ≥ 1000 copies/mL	1.96 (0.76-5.04)	-
CD4 cell count cells/µL (per 100 increase)	0.90 (0.79-1.03)	-
CD4 cell count categories (time-varying)		
CD4 ≥500 cells/µL (reference)	1.00	1.00
CD4 <500 cells/µL	**2.35 (1.10-5.03)**	1.74 (0.75-4.03)
Male vs. female sex	0.82 (0.40-1.69)	-
Age at study visit (time-varying, per year increase)	**1.33 (1.06-1.68)**	**1.37 (1.06-1.78)**
BMI categories (time-varying)		
Normal weight, ≥ 18, <25 kg/m^2 (reference)	1.00	-
Underweight, <18 kg/m^2	1.35 (0.63-2.91)	-
Overweight/obese, ≥ 25 kg/m^2	0.44 (0.06-3.42)	-
Tanner staging at study visit (time-varying)		
Stage I (reference)	1.00	1.00
Stage II to IV	1.20 (0.43-3.36)	0.74 (0.25-2.21)
Stage V	1.35 (0.39-4.72)	0.59 (0.14-2.44)
Known TB contact at enrolment (yes vs no)	1.93 (0.34-10.85)	-
QFT result at study enrolment		
QFT-negative (reference)	1.00	-
QFT-positive	0.64 (0.26-1.60)	-
QFT test results unknown	1.66 (0.38-7.22)	-
Age at ART initiation		
≤ 2 years (reference)	1.00	-
>2, <6 years	3.50 (0.77-15.80)	-
≥6 years	**5.68 (1.30-24.87)**	-

Abbreviations: QFT - interferon gamma release assay (QuantiFERON-TB); HIV, human immunodeficiency virus; CD, cluster of differentiation; ml, milliliter; µL, microliter; HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence intervals; [1]multivariable model adjusted for all variables with adjusted estimates shown as well as baseline measures of relative pover

Supplemental Table 1. Characteristics of HIV+ and HIV- participants at study enrolment, comparing those included vs excluded from analysis

Characteristic	HIV-positive (N=515)			HIV-negative (N=110)		
	In analysis (n=496)	Not in analysis (n=19)	*p*-value	In analysis (n=103)	Not in analysis (n=7)	*p*-value
Age (years)	12.0 (10.7-13.3)	11.2 (10.3-12.3)	0.18	11.6 (10.0-13.4)	13.7 (10.5-14.0)	0.30
Relative poverty categories[1]			0.17			0.29
Most disadvantaged	125 (25%)	8 (42%)		60 (59%)	2 (29%)	
Moderate disadvantage	179 (36%)	7 (37%)		24 (24%)	3 (43%)	
Least disadvantaged	191 (39%)	4 (21%)		18 (18%)	2 (28%)	
Male sex	251 (51%)	12 (63%)	0.28	47 (46%)	2 (29%)	0.38
Tanner stage			**0.007**			0.46
Prepubertal (Stage I)	231 (47%)	15 (83%)		34 (33%)	1 (14%)	
Adolescent (Stages II-IV)	240 (48%)	2 (11%)		63 (61%)	5 (71%)	
Mature (Stage V)	25 (5%)	1 (6%)		6 (6%)	1 (14%)	
Body mass index (BMI, (kg/m^2)	17.1 (16.0-18.9)	16.7 (15.7-18.7)	0.56	18.6 (16.6-21.5)	18.8 (17.8-23.5)	0.24
Age at ART initiation (years)	4.4 (2.1-7.6)	4.1 (1.4-6.4)	0.65	-	-	-
ART regimen at enrolment			0.64			
ABC-3TC-EFV	239 (48%)	10 (53%)		-	-	-
ABC-3TC-LPV(r)	106 (21%)	4 (21%)		-	-	-
AZT-3TC-LPV(r)	37 (7%)	3 (16%)		-	-	-
d4T-3TC-EFV	31 (6%)	0		-	-	-
Other	78 (16%)	2 (10%)		-	-	-
Unknown	5 (1%)	0		-	-	-
HIV viral load (log_{10} copies/mL)	1.59 (1.5901.60)	1.59 (1.59-1.60)	0.98	-	-	-
CD4 cell count (cells/µL)	713 (564-953)	780 (536-1222)	0.33	-	-	-
Previous TB disease	298 (61%)	11 (61%)	0.91	3 (3%)	0	0.69
On treatment for TB disease at enrolment	7 (1%)	0	0.60	0	0	-
Previous isoniazid preventive therapy	131 (27%)	6 (33%)	0.63	3 (3%)	1 (20%)	0.06
Current known TB contact	9 (2%)	1 (6%)	0.30	4 (4%)	1 (20%)	0.11
QFT result at enrolment			0.33			**0.05**
QFT positive	154 (31%)	9 (47%)		25 (24%)	2 (29%)	
QFT negative	314 (63%)	8 (42%)		66 (64%)	2 (29%)	
QFT results unknown	28 (6%)	2 (11%)		12 (12%)	3 (42%)	

Abbreviations: QFT, interferon gamma release assay (QuantiFERON-TB®); mL, milliliter; uL, microliter; Numbers are median (interquartile range) or n (column percentage); p-values from Chi2 or Kruskal-Wallis testing, not corrected for multiplicity

[1]Tertiles of a continuous score incorporating a standardized asset score (including type of housing, access to running water and flush toilet), employment and education

Supplemental Table 2. Factors associated with QFT positivity at enrolment, among HIV+ participants who did not have TB disease at baseline (N=461): odds ratios from logistic regression analysis

	OR (95% CI)
Age (per year increase)	1.08 (0.96-1.22)
Male vs female sex	1.03 (0.69-1.52)
Known previous (vs. no previous) TB disease	0.93 (0.62-1.38)
Current known TB contact (vs no current contact)	0.59 (0.12-2.89)
Tanner stage	
Prepubertal (Stage I)	1.00
Adolescent (Stages II-IV)	1.18 (0.79-1.77)
Mature (Stage V)	0.75 (0.28-1.97)
BMI categories	
Normal, BMI ≥ 18.5 <25	1.00
Underweight, BMI<18.5	1.19 (0.76-1.87)
Overweight/obese, BMI ≥ 25	1.33(0.42-4.23)
Categories of age at ART initiation	
≤ 2 years	1.00
>2, < 6 years	0.78 (0.47-1.29)
≥ 6 years	0.79 (0.47-1.32)
Categories of HIV viral load	
<40 copies/mL	1.00
40-1000 copies/mL	0.49 (0.24-1.02)
≥1000 copies/mL	1.31 (0.73-2.34)
CD4 cell count categories	
<250 cells/µL	1.00
≥250, <350 cells/µL	6.53 (0.72-60.05)
≥350, <500 cells/µL	5.38 (0.62-46.50)
≥500, <800 cells/µL	7.39 (0.95-57.32)
≥800 cells/µL	6.82 (0.87-53.16)

Analysis restricted to HIV+ youth who received QFT testing at baseline without prevalent TB at enrolment Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; QFT, interferon gamma release assay (QuantiFERON-TB®); TB, tuberculosis disease; CD, cluster of differentiation; mL, milliliter; µL, microliter; ART, triple agent antiretroviral therapy

Supplemental Table 3. Incidence rates of QFT conversion over the first 3 years of follow-up (among study participants who tested QFT negative at enrolment)

	Number of QFT conversions	Person-years	Rate/PY (95% CI)
All study participants (HIV+ and HIV-), N=380	82	2.85	28.8 (23.2-35.7)
By HIV status			
HIV-, N=66	19	0.49	39.1 (24.9-61.2)
HIV+, N=314	63	2.36	26.7 (20.8-34.1)
Restricted to HIV+ participants (N=66)			
HIV viral suppression categories (time-varying)			
HIV-viral load ≤ 40 copies/mL	48	1.66	28.9 (21.8- 38.3)
HIV-viral load >40, <1000 copies/mL	12	0.42	28.8 (16.3-50.7)
HIV-viral load ≥ 1000 copies/mL	3	0.28	10.6 (3.4-37.7)
CD4 cell count (per 100 cells/µL)			
CD4 cell count categories			
CD4 ≥ 500 cells/µL	58	1.84	31.4 (24.3-40.7)
CD4 <500 cells/µL	5	0.52	9.6 (4.0-23.1)
Age at ART initiation			
≤ 2 years	13	0.54	24.3 (14.1-41.8)
>2, <6 years	25	0.97	25.8 (17.4- 38.1)
≥6 years	23	0.83	27.7 (18.4-41.7)
Sex			
Female	36	1.18	30.54 (22.03-42.34)
Male	27	1.18	22.8 (15.6-33.26)
Categories of age at study visit (time-varying, in years)			
<12 years	9	0.45	19.7 (10.4-37.8)
≥ 12, <14 years	26	0.88	29.5 (20.1-43.3)
≥ 14, <16 years	20	0.74	26.9 (17.3-41.6)
≥ 16 years	8	0.28	28.7 (14.4-57.5)
BMI categories (time-varying)			
Normal weight, ≥ 18, <25 kg/m^2	36	1.03	34.9 (25.2-48.4)
Underweight, <18 kg/m^2	22	1.15	19.2 (12.6-29.2)
Overweight/obese, ≥ 25 kg/m^2	5	0.18	27.0 (11.2-64.9)
Tanner staging at study visit (time-varying)			
Stage I (pre-adolescent)	13	0.64	20.3 (11.8-34.9)
Stage II to IV (adolescent)	34	1.35	25.1 (17.9-35.1)
Stage V (mature)	16	0.37	43.6 (26.7-71.2)
Known TB contact, household or otherwise			
No	62	2.30	27.0 (21.0-34.5)
Yes	1	0.06	17.3 (2.4-123.3)

Abbreviations: QFT, interferon gamma release assay (QuantiFERON-TB®); TB, tuberculosis disease; PY, person-years; person-years calculated from date of study enrolment (staggered entry by 6 months for those with prevalent TB); censored at the last known date alive prior to 31st October 2018.

Supplemental Table 4. Sensitivity analysis I: variation in incidence of tuberculosis disease (TB) overall and by HIV status, using varying definitions of TB events and duration of person-time

	Total (N=599)	HIV+ youth (N=496)	HIV- youth (N=103)	Absolute difference in IR (95% CI), HIV+ vs HIV-	IRR (95% CI) comparing HIV+ to HIV-
Incidence A					
Events (all TB diagnoses, including recurrent events)	35	34	1	-	-
Person-years (censored at last known time alive)[1]	1840.6	1512.3	328.2	-	-
Incidence of TB per 100 person-years (PY)	1.9/100PY	2.2/100PY	0.3/100PY	1.9 (1.0-2.9)/100PY	7.38 (1.24-299.9)
Incidence B					
Events (only confirmed TB diagnoses, including recurrent events)	20	19	1	-	-
Person-years (censored at last known time alive)[1]	1840.6	1512.3	328.2	-	-
Incidence of TB per 100 person-years (PY)	1.1/100PY	1.3/100PY	0.3/100PY	0.9 (0.1-1.8)/100PY	4.12 (0.65-171.3)
Incidence C					
Events (all TB diagnoses, including recurrent events)	35	34	1	-	-
Person-years at risk (censored at last known time alive, with 6 months subtracted per incident TB event)[1]	1823.1	1495.3	327.7	-	-
Incidence of TB per 100 person-years (PY)	1.9/100PY	2.3/100PY	0.3/100PY	2.0 (1.0-2.9)/100PY	7.45 (1.25-302.9)
Incidence D					
Events (only confirmed TB diagnoses, including recurrent events)	20	19	1	-	-
Person-years at risk (censored at last known time alive, 6 months subtracted for each incident TB event)[1]	1830.6	1502.8	327.7	-	-
Incidence of TB per 100 person-years (PY)	1.1/100PY	1.3/100PY	0.3/100PY	1.0 (0.1-1.8)/100PY	4.14 (0.66-172.2)

[1]For all person-time, entry into risk-set is staggered by 6 months for those who presented with prevalent TB (i.e. with TB diagnosis and on TB treatment at enrolment visit)

Supplemental Table 5. Sensitivity analysis II: TB occurrence and predictors among HIV+ participants: all TB diagnoses versus bacteriologically confirmed TB diagnoses only, using negative binomial regression with GEE to estimate overall TB incidence, including recurrent events [incidence rate ratios]

Characteristic	All TB diagnoses (confirmed and probable)		Confirmed TB disease only (positive culture)	
	Incidence rate/100PY (95% CI)	IRR (95% CI)	Incidence rate/100PY (95% CI)	IRR (95% CI)
HIV+ (all)	2.2 (1.6-3.1)	**-**	1.3 (0.80-1.97)	-
HIV viral load log_{10} copies/mL (per log_{10} increase)	-	**1.53 (1.14-2.05)**	-	1.38 (0.93-2.05)
HIV viral suppression categories				
HIV-viral load ≤ 40 copies/mL (reference)	1.6 (1.0-2.6)	1.00	1.0 (0.5-1.8)	1.00
HIV-viral load >40, <1000 copies/mL	2.8 (1.4-5.6)	1.71 (0.77-3.79)	1.8 (0.7-4.2)	1.83 (0.67-7.04)
HIV-viral load ≥ 1000 copies/mL	4.7 (2.5-9.1)	**2.90 (1.28-6.60)**	2.1 (0.8-5.6)	2.18 (0.68-7.04)
CD4 cell count categories				
CD4 <500 cells/µL (reference)	1.8 (1.1-2.7)	1.00	1.3 (0.8-2.1)	1.00
CD4 ≥500 cells/µL	4.1 (2.4-7.0)	**2.32 (1.17-4.60)**	1.2 (0.5-3.3)	1.00 (0.37-2.73)
Age at ART initiation				
≤ 2 years (reference)	0.5 (0.1-2.1)	1.00	0.3 (0.03-1.9)	1.00
>2, <6 years	2.0 (1.1-3.5)	3.80 (0.85-17.02)	0.7 (0.2-1.8)	2.52 (0.26-24.92)
≥6 years	3.5 (2.2-5.6)	**6.67 (1.54-28.97)**	2.5 (1.5-4.4)	**9.30 (1.19-72.84)**
Age at study visit (per year increase)	-	**1.37 (1.15-1.67)**	-	**1.37 (1.05-1.78)**
Tanner staging at study visit (time-varying)				
Stage I (pre-adolescent), reference	1.3 (0.5-3.2)	1.00	0.5 (0.1-2.1)	1.00
Stage II to IV (adolescent)	2.4 (1.5-3.6)	1.79 (0.73-4.36)	1.4 (0.8-2.4)	2.52 (0.62-10.17)
Stage V (mature)	3.3 (1.6-6.6)	2.49 (0.72-8.67)	2.1 (0.9-4.9)	3.85 (0.60-25.54)
QFT test result at enrolment				
QFT- (reference)	2.6 (1.7-3.8)	1.00	1.3 (0.8-2.3)	1.00
QFT+	1.3 (0.6-2.8)	0.49 (0.20-1.21)	0.9 (0.3-2.3)	0.62 (0.19-1.97)
QFT results not available	3.8 (1.2-11.9)	1.49 (0.34-6.49)	2.6 (0.6-10.3)	1.91 (0.25-14.42)

Abbreviations: Cox PH, proportional hazards regression model; TB, tuberculosis disease; PY, person-years; IRR, incidence rate ratio; CI, confidence interval; QFT, interferon gamma release assay (QuantiFERON-TB®); CD, cluster of differentiation; mL, milliliter; µL, microliter; ART, triple agent antiretroviral therapy

Supplemental Table 6. Sensitivity analysis III: Predictors of time to first TB event among HIV+ participants: all TB diagnoses versus bacteriologically confirmed TB diagnoses only, using Cox proportional hazards regression

	All TB diagnoses (confirmed and unconfirmed) Crude HR (95% CI)	Bacteriologically confirmed TB diagnoses only Crude HR (95% CI)
HIV viral load log_{10} copies/mL (per log_{10} increase)	**1.41 (1.00-1.98)**	1.25 (0.73-2.12)
HIV viral suppression categories		
HIV+, viral load ≤ 40 copies/mL	1.00	1.00
HIV+, viral load >40, <1000 copies/mL	1.41 (0.59-3.40)	1.46 (0.46-4.61)
HIV+, viral load ≥ 1000 copies/mL	1.96 (0.76-5.04)	1.35 (0.29-6.29)
CD4 cell count categories		
CD4 ≥500 cells/μL	1.00	1.00
CD4 <500 cells/μL	**2.35 (1.10-5.03)**	1.00 (0.28-3.60)
Age at study visit (per year increase)	**1.33 (1.06-1.68)**	**1.43 (1.03-2.01)**
QFT test result at enrolment		
QFT-	1.00	1.00
QFT+	0.64 (0.26-1.60)	0.92 (0.28-2.98)
QFT results not available	1.66 (0.38-7.22)	1.42 (0.18-11.26)
Age at ART initiation		
≤ 2 years	1.00	1.00
>2, <6 years	3.50 (0.78-15.79)	1.89 (0.20-18.16)
≥6 years	**5.68 (1.30-24.87)**	7.60 (0.97-59.27)

Abbreviations: Cox PH, proportional hazards regression model; TB, tuberculosis disease; PY, person-years; HR, hazard ratio; IRR, incidence rate ratio; CI, confidence interval; HR, hazard ratio; IRR, incidence rate ratio; QFT, interferon gamma release assay (QuantiFERON-TB®); CD, cluster of differentiation; mL, milliliter; uL, microliter; ART, triple agent antiretroviral therapy

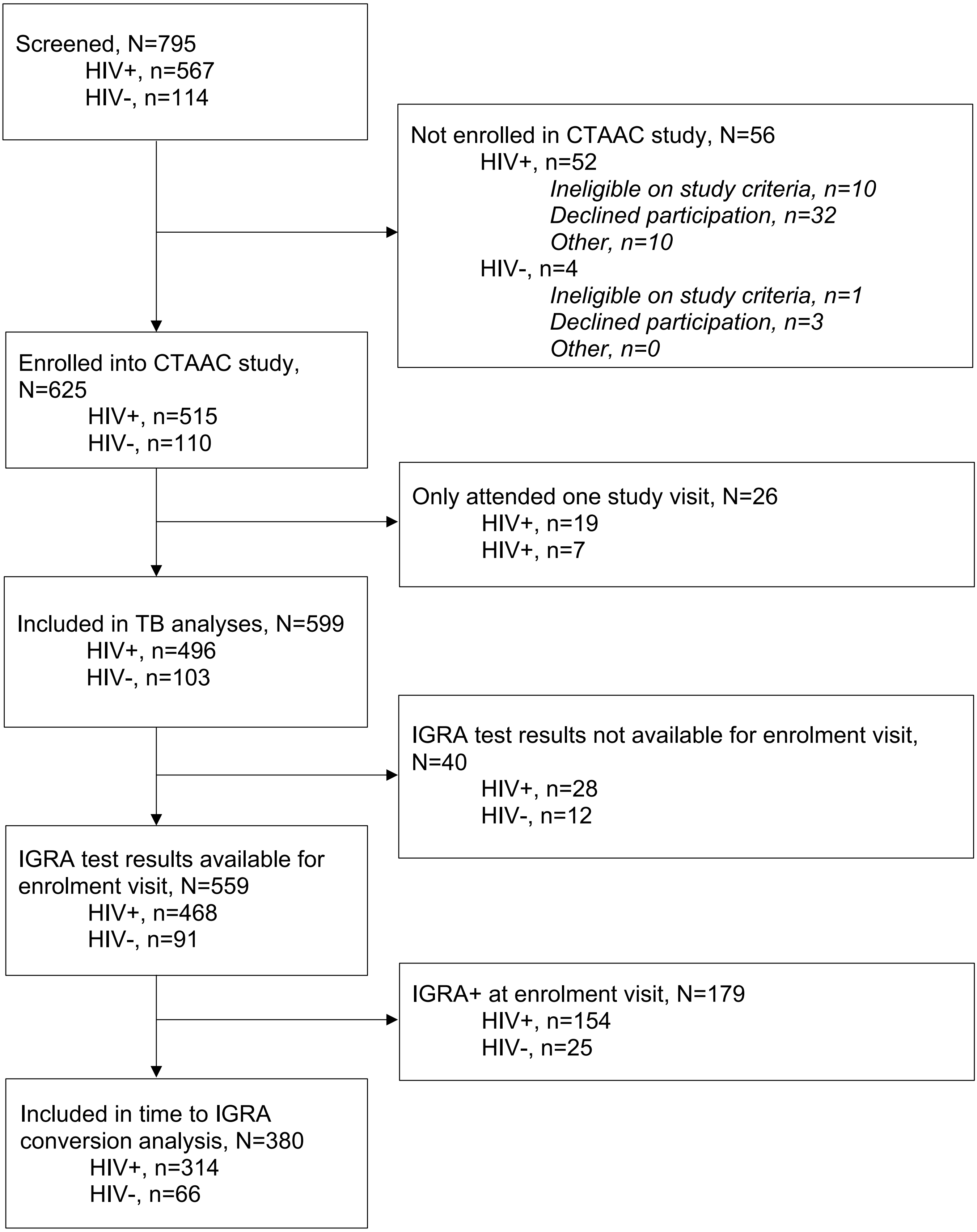

Figure 1. Study flow diagram

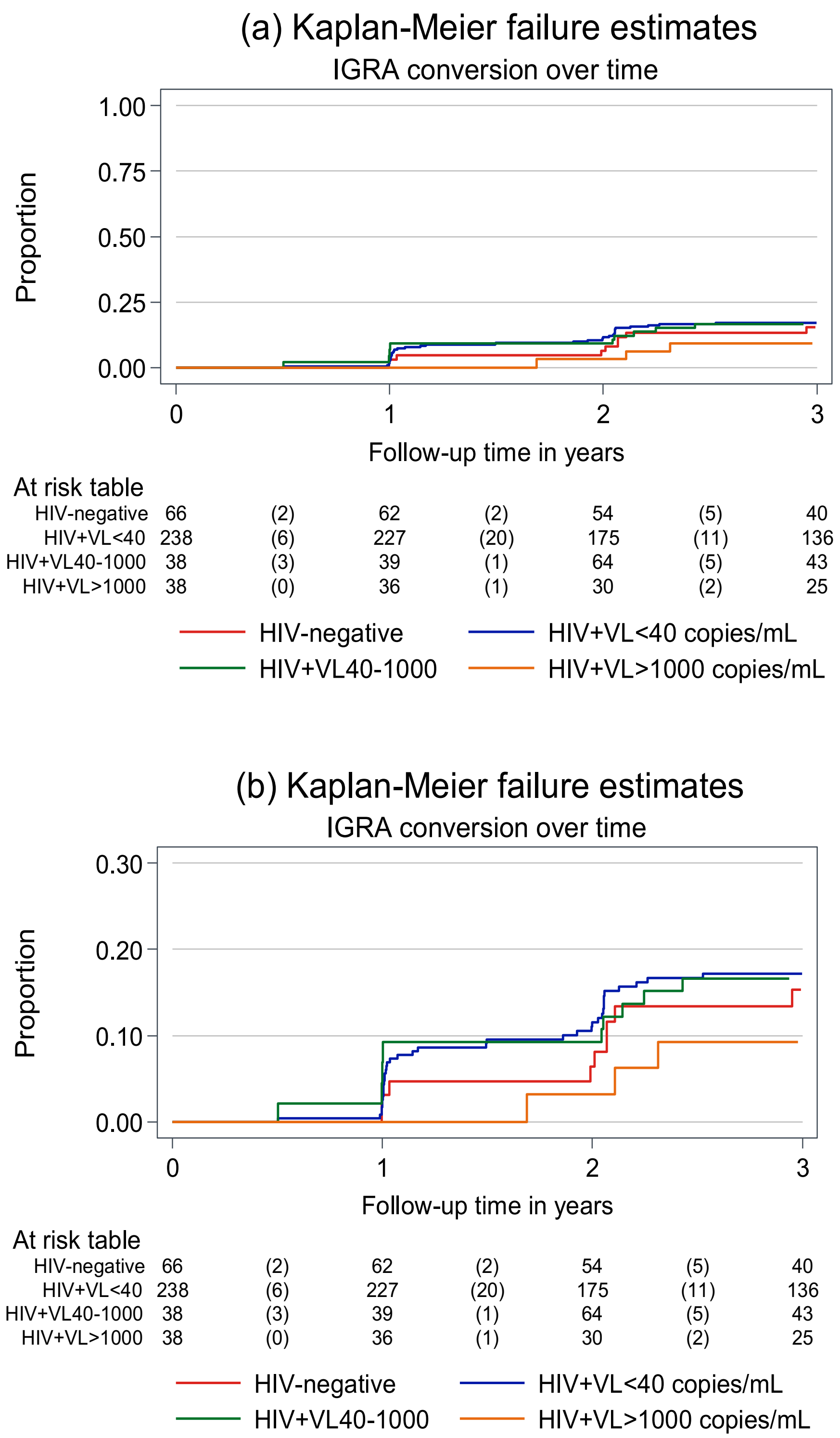

Figure 2. Kaplan-Meier estimates for QFT conversion among HIV+ and HIV- participants over the first 36 months of follow-up, stratified by time-varying HIV viral load: (a) overall and (b) with restricted Y-axis.

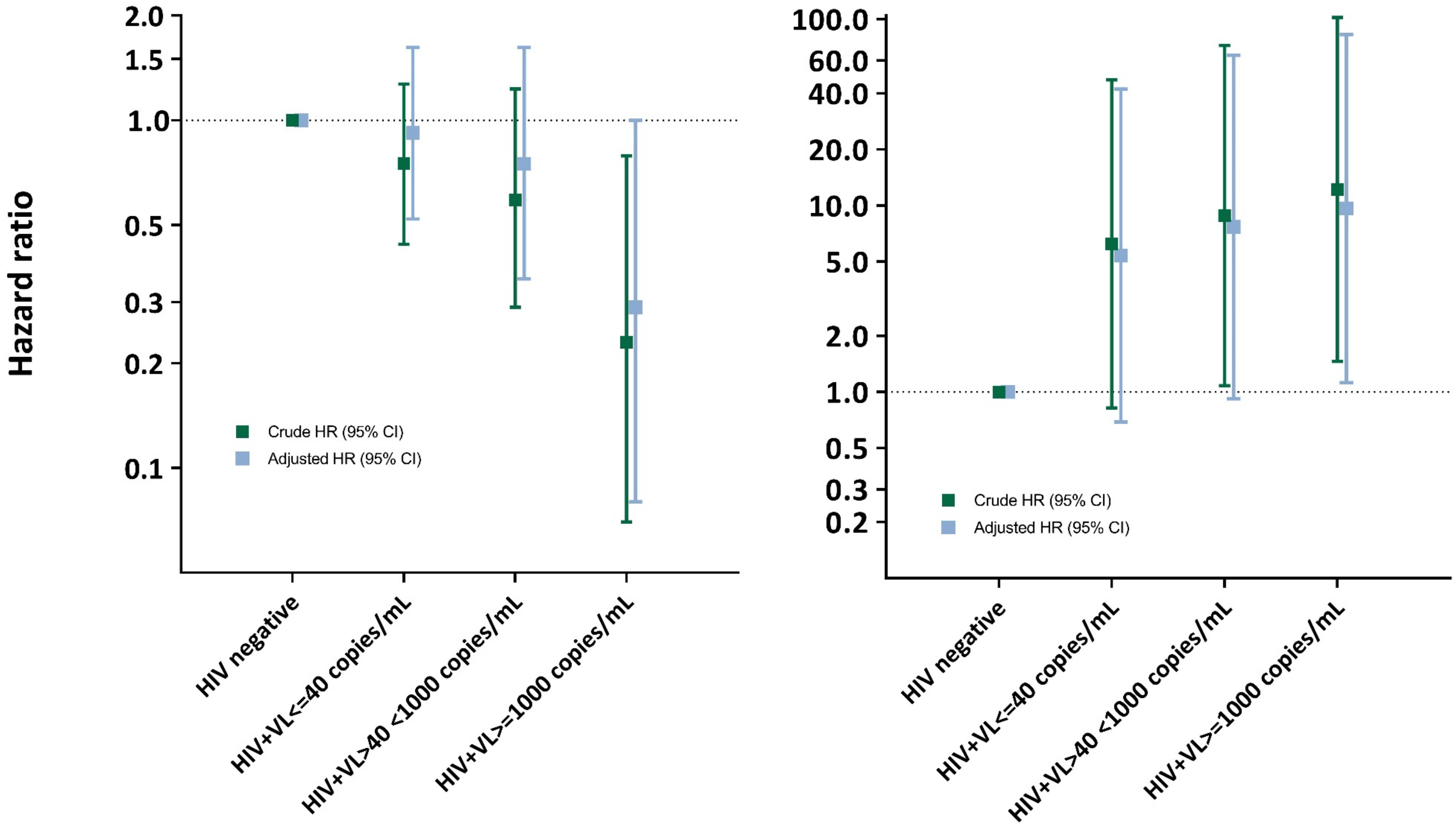

Figure 3. Relative hazard of (a) QFT conversion and (b) incident tuberculosis disease comparing HIV+ to HIV- participants in strata of HIV viral control during follow-up.

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Chapter 7:
Summary and Recommendations

This study investigating the spectrum, progression and predictors of morbidity in PHIV+ adolescents on ART, had the following key findings:

1.0 Major findings

1.1 Metabolic and renal abnormalities including risk factors for cardiovascular disease

1.1.1 At enrolment, the prevalence of insulin resistance in PHIV+ adolescents was similar to that in HIV- (18% vs. 20%) adolescents. The rate of insulin resistance was similar to that of PHIV+ adolescents in the US in the PHACS cohort, but higher than the rates described in cohorts from Latin America and Thailand.[1-3] Insulin resistance is important as it may predispose to diabetes and early cardiovascular disease.[4] However, in longitudinal studies of the PHACS cohort, insulin resistance resolved for some participants. Resolving insulin resistance was associated with male sex and low-BMI-scores. In this cohort, insulin resistance in PHIV+ adolescents was associated with obesity as in HIV- youth.[5]

1.1.2 The prevalence of proteinuria and microalbuminuria in PHIV+ adolescents were 5.9% and 8.0%, respectively, and were similar to that of HIV- adolescents (10.1% and 9%, respectively). This was lower than the prevalence of microalbuminuria found in children and adolescents from other African cohorts of children and adolescents living with HIV.[6] This may be because of the longer duration of ART in our cohort compared to cohorts in other African settings.

1.1.3 The prevalence of hypertension (defined as BP > 95 percentile for height, age and sex) was 13.1% in PHIV+ participants and 17.4% in HIV- participants. Although blood pressure was higher in those that were HIV-, this was not statistically significant. This is similar to rates seen in adolescents with HIV (both PHIV+ and horizontally-infected) from the Western Cape, South Africa.[7]

1.1.4 The prevalence of hypercholesterolemia was 12.3% and this was significantly higher in PHIV+ compared to HIV- adolescents (12.3% vs. 1.9%, p=<0.01). This probably reflects long term use of ART, especially lopinavir/ritonavir. These lipid abnormalities may decrease in the future as HIV+ adolescents switch from protease inhibitor to integrase inhibitor regimens.

1.1.5 Only 1 (0.3%) PHIV+ and 1 (1.6%) HIV- adolescent met the criteria for metabolic syndrome, defined as hypertension, and raised low high-density lipoprotein (HDL) cholesterol, hypertriglyceridaemia and abdominal obesity. This may be because the median age of this cohort was 12 years and metabolic syndrome occurs more commonly in older HIV-infected adolescence (15-19 years).[8]

1.2 Multisystem impairment

1.2.1 At enrolment, neurocognitive impairment (as defined by the youth-International HIV Dementia Scale, y-IHDS) was the most commonly involved system in PHIV+ and HIV- adolescents (56.3% vs. 45.3%, *p*=0.05). Lung or cardiac impairment were also relatively common in PHIV+ compared to HIV-participants (27.1% vs. 14.7%, *p*=0.01 and 46.1% vs. 33.7%, *p*=0.03, respectively). Renal impairment was rare and similar in both groups (2.3% vs. 2.1%, *p*=0.89). One hundred and ten (28.6%) PHIV+ adolescents had two system impairment. Only 39 (10.2%) of PHIV+ adolescents had 3 or 4 -system impairment. This supports the need to thoroughly examine PHIV+ adolescents in whom one system is found to be affected.

1.3 Hospitalization

1.3.1 Incidence rates for hospitalization were 6.6 per 100 PY in PHIV+ adolescents; three times that of HIV- adolescents. This rate is higher than described in other cohorts on ART.[9] This high rate is most likely due to the high prevalence of TB in the setting and the inclusion of day admissions for elective ENT procedures.

1.3.2 Sixty percent of hospitalization episodes were due to non- infectious causes. These non-infectious causes were often related to sequelae of infections such a seizures post meningitis or assessment for tympanoplasty post chronic suppurative otitis media. Infectious causes of hospitalization included TB disease, pneumonia (not caused by TB) and abscesses.

1.4 Tuberculosis infection and disease

1.4.1 At enrolment , PHIV+ and HIV- adolescents had a similar prevalence of TB infection as measured by QuantiFERON. The cumulative QFT+ prevalence was similar over 3 years (PHIV+ 46% vs. 45%; *p*=0.88). PHIV+ adolescents had a markedly higher incidence of TB disease than HIV-adolescents, 2.2/100 PY vs. 0.3/100 PY, despite a similar rate of TB infection as measured by QuantiFERON testing, 26.7/100 PY among PHIV+ and 39.1/100 PY among HIV- adolescents.

1.4.2 The rate of bacteriologically confirmed TB was 1.3/100 PY for PHIV+ adolescents compared to 0.3/100 PY for HIV- adolescents. This is the first measure of confirmed TB in PHIV+ adolescents.

1.4.3 Increasing HIV viral load or decreasing CD4 cell count were associated with increased likelihood of TB disease but a decreased likelihood of QuantiFERON positivity in PHIV+ adolescents.

1.4.4. A positive QuantiFERON test at enrolment was not associated with development of TB disease for PHIV+ adolescents.

2.0 Strengths and Limitations

The strengths of this research include that the cohort of PHIV+ adolescents was large and that a cohort of age-matched HIV- adolescents from the same communities was included as a comparator. All adolescents were followed longitudinally, and cohort retention was high. In addition, for the objectives that required longitudinal follow-up, routine data could be accessed from a provincial database to strengthen the study data. Diagnostic tests were detailed, longitudinal and frequent.

Limitations include the lack of data on HIV-exposure in the HIV- participants. It would have been useful to compare PHIV+ adolescents to those who were HIV-exposed but uninfected allowing exploration of the adolescents' psychosocial environments effect versus the biological effect of HIV itself.

Another limitation was the poor documentation of TB preventive therapy and cotrimoxazole prophylaxis throughout the study period. It was also difficult to reliably establish the presence of a household TB contact. CD4 and viral load tests were often not available at the time of hospitalization or TB diagnosis. When this occurred, the CD4 count and viral load prior to the hospitalization or TB episode was used.

An additional limitation was that the measures of impairment that were chosen for each system differ in their ability to assess severity of impairment. The y-IHDS score is a relatively crude estimate of neurocognitive impairment and may have overestimated neurocognitive impairment, and this may have resulted in the high prevalence of neurocognitive impairment found in PHIV+ and HIV- adolescents. Overestimation of neurocognitive impairment using the IHDS score has been described in HIV+ adults.[10] However, the y-IHDS is widely used in LMICs. For cardiology, echocardiogram is an insensitive measure, and is unable to detect subtle changes in cardiac function. Cardiac magnetic resonance imaging is more sensitive and may provide more detailed information.[11]

3.0 Generalizability

Our findings may not be generalizable to settings where ART was started at a later age, where there is low ART coverage, or a lower burden of TB. In settings where access to ART and ability to screen for subclinical disease are limited the priorities may differ. For example, in more resource limited settings in Africa the first priority may be earlier diagnosis of HIV and scale up of ART rather than screening for chronic impairment.

The cohort was also relatively young (3 of the analyses included data that was extracted from the baseline visit when the median age was around 12 years) and findings may not be applicable or may change in later adolescence or early adulthood. Older adolescents may develop further risk factors for NCDs, be more prone to disengagement from care or non-adherence and have additional causes for morbidity related to sexual health, smoking, alcohol and drug abuse.[12]

In addition, as ART regimens change, the burden and spectrum of metabolic abnormalities will change. For example, integrase inhibitors are less likely to cause hyperlipidaemia than protease inhibitors; however, complications of rapid weight gain are starting to be documented in youth on integrase inhibitors.[13]

4.0 Recommendations

PHIV+ adolescents experience chronic morbidities although these may not be clinically overt. Screening for chronic morbidities must be included into routine HIV services for adolescents.

4.1 Specific screening recommendations

Specific recommendations might include:

4.1.1 Screening for metabolic derangements, such as insulin resistance/diabetes (and hyperlipidaemia in those on lopinavir/ritonavir), in PHIV+ adolescents at least annually in early adolescence. Further follow up will be needed to inform screening of metabolic comorbidity as ART regimens change and adolescents achieve puberty. Regular checking of weight as well as dietary and physical exercise interventions should be integrated into health care visits to prevent obesity.

4.1.2 Screening of other systems if one system is found to be involved should be undertaken at least once for PHIV+ adolescents accessing clinical services. For example, if a PHIV+ adolescent is neurocognitively impaired, one should do a thorough cardiac examination and lung examination.

4.1.3 Routine screening for neurocognitive impairment in all PHIV+ adolescents. This should ideally be initiated in early childhood but should also be done in the adolescent period in case it has been missed in childhood. Detection of neurocognitive impairment in childhood would allow interventions to be put in place to allow CLHIV and PHIV+ adolescents to optimize their potential and also support optimal adherence as adolescents take ART independently.

4.1.4 Screening neurocognitive impairment using the y-IHDS score as well as screening for other potential system involvement in those that have failed a grade at school should also be implemented.

4.2. General recommendations

4.2.1 A minority of PHIV+ adolescents have multisystem impairment that will require long term care. It is recommended that this is documented, managed and referral plans are put in place before adolescents are transitioned to adult care services.

4.2.2 Better documentation of causes of hospitalization in PHIV+ adolescents is needed. This would allow increased understanding of why the hospitalization rate remains so high.

4.2.3 PHIV+ adolescents admitted to hospital with pneumonia should have microbiological testing for *M.tb* infection. Strategies to prevent TB and other infectious causes of hospitalization need strengthening.

4.2.4 Research to determine the need for catch up vaccination is this population is needed as evidenced by some hospital admissions being caused by vaccine preventable illnesses.

4.2.5 Strategies to screen and prevent TB in PHIV+ adolescents are urgently needed. TB preventive therapy and ensuring optimal ART regimens and adherence must be strengthened.

4.2.6 Guidelines that rely on positive tests of *M.tb* infection may miss PHIV+ adolescents most in need of TB preventive therapy, and these may need to be revised. Adolescents with low CD4 counts and high viral loads should be prioritized for TB screening (assessing symptoms) and if symptomatic, relevant microbiological diagnostics should be sent to the laboratory.

5.0 Implications

The above recommendations have clinical and service delivery implications. If screening is implemented, there needs to be available interventions or treatments. It is still unclear what many of the optimal interventions in this population are; however, there are many interventions that could already be implemented, such as TB preventive therapy.

South Africa already has implemented 'chronic disease clinics' for adults.[14] At these clinics, adults with one comorbidity are offered screening for other commonly prevalent NCDs. This could be offered at clinics where adolescents access ART. If screening and interventions are to be implemented, health care workers will need upskilling, as the majority of health- care workers that treat PHIV+ at community level are only trained in HIV care. Care should be taken not to overwhelm health-care workers as they already have a heavy workload. Health records and program monitoring should prompt providers to document anthropometry, blood pressure, blood glucose and urine analysis at a minimum. These are not routinely documented in busy clinics where populations are perceived as young and 'healthy'. Lastly, infection prevention control is vital to consider in these settings, especially as there is a high incidence of TB in this population.

Although there are many challenges to implementing screening and prevention of multimorbidity, opportunities to integrate this kind of care into already existing adolescent activities are numerous. Some clinics have already established 'adolescent groups' where various health issues are addressed. HIV is one of the only conditions that result in an adolescent population interacting regularly with the health system, so we should leverage this and integrate a more comprehensive approach to health.

6.0 Implications for HIV- adolescents

Although the focus was on PHIV+ adolescents, the findings also have implications for HIV- adolescents from this setting. HIV- adolescents had a similar rate of insulin resistance and may merit further screening for diabetes, especially in light of the high prevalence of diabetes mellitus in South Africa.[15] This, in addition with the high burden of hypertension described, has relevance for the development of future cardiovascular disease. Similarly, 45% had neurocognitive impairment as defined by the y-IHDS. Cardiac impairment was also relatively common, but this was defined by subtle changes in echocardiogram parameters and was not clinically significant. In addition, there was a high prevalence of mild respiratory impairment as measured by lung function. These subclinical impairments may have implications over the life course and result in earlier lung and cardiovascular disease. It is concerning that the prevalence of many of the cardiovascular and other risk factors documented in the PHIV+ participants were similar to those that were HIV- in this setting, and this has implications for screening and interventions.

All these findings are relevant for planning for adolescent health and may suggest the screening and interventions are required more broadly and not just within the HIV adolescent population. It may be necessary to screen for some of the abnormalities in adolescence or early adulthood rather than in late adulthood, as is the standard of care in South Africa currently.

7.0 Future Research

Further exploration and monitoring of this population are essential; however, research in this particular population can be challenging. The current generation of PHIV+ adolescents have often been attending research facilities for most of their childhood and many adolescents may experience 'research fatigue'.[16] New and innovative ways of engaging this population are necessary in order to continue to follow them as they transition to adult care. This can be done using technology to communicate as well as by accessing electronic records and thereby decreasing the need for face to face visits. Future research planning should include adolescents in the prioritizing the research agenda and respect the 'nothing about us, without us' principle.

Adolescents may begin to engage in high risk behaviors, such as smoking, alcohol and drug use. These 'adult' lifestyle risk factors may compound the underlying chronic morbidities and result in increased cardiovascular or metabolic health issues. Other research priorities that need to be addressed as adolescents age are sexual and reproductive health, educational achievement and employment and transition to adult care.

Infectious morbidity needs to be addressed urgently in resource limited settings. Research to elucidate optimal TB screening and preventive therapy strategies should be prioritized. The need for catch up vaccination is this population and how this should be implemented is another area that needs investigation. The interaction between infectious and non-infectious morbidity and how this influences health outcomes is of specific relevance in resource limited settings and should include studying drug-drug interactions, pill burden and cost-effective modes of service delivery implementation.

8.0 Conclusions

The above findings highlight that there is considerable multi-morbidity in PHIV+ adolescents with similar findings to those PHIV+ adolescents enrolled in PHACS and other cohorts, especially regarding end-organ disease. Considering the relatively late start of ART compared to those in resource -rich countries, it is interesting that the PHIV+ adolescents in the CTAAC cohort do not have a more markedly increased burden of morbidity than their US counterparts. A possible explanation for this may be that the current generation of adolescents enrolled in CTAAC started on less toxic ART regimens than their US counterparts. In contrast with PHIV+ adolescents in resource rich settings, adolescents in the CTAAC cohort face a greater burden of infectious disease as evidenced by the high TB incidence. They also have a higher rate of hospitalization.

The implications of these findings are that further monitoring of this population is essential to see how this multi-morbidity will develop over their life-course. Screening and interventions to mitigate chronic morbidity need to be developed and incorporated into routine care for this population.

In addition, the high burden of TB and hospitalization needs to be addressed with existing preventive strategies and the development of new innovative strategies. As incident TB was associated with high HIV viral load, strategies focusing on adherence, optimal ART regimens and keeping adolescents in clinical care are of utmost importance.

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Annexes

Annex 1: Chronic comorbidities in children and adolescents with perinatally acquired HIV infection in sub-Saharan Africa in the era of antiretroviral therapy.
Annex 2: Ethics approval for PhD and Cape Town Adolescent Antiretroviral Cohort
Annex 3: Consent and Assent forms
Annex 4: Cape Town Adolescent Antiretroviral Cohort Case Report Forms

Chronic comorbidities in children and adolescents with perinatally acquired HIV infection in sub-Saharan Africa in the era of antiretroviral therapy

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Globally, 1·7 million children are living with HIV, of which 90% are in sub-Saharan Africa. The remarkable scale-up of combination antiretroviral therapy has resulted in increasing numbers of children with HIV surviving to adolescence. Unfortunately, in sub-Saharan Africa, HIV diagnosis is often delayed with children starting antiretroviral therapy late in childhood. There have been increasing reports from low-income settings of children with HIV who have multisystem chronic comorbidities despite antiretroviral therapy. Many of these chronic conditions show clinical phenotypes distinct from those in adults with HIV, and result in disability and reduced quality of life. In this Review, we discuss the spectrum and pathogenesis of comorbidities in children with HIV in sub-Saharan Africa. Prompt diagnosis and treatment of perinatally acquired HIV infection is a priority. Additionally, there is a need for increased awareness of the burden of chronic comorbidities. Diagnostic and therapeutic strategies need to be collectively developed if children with HIV are to achieve their full potential.

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Introduction

The HIV pandemic has been established for 40 years and, in 2018, 1·7 million children (aged <15 years) worldwide were estimated to be living with HIV, of which 90% were in sub-Saharan Africa. Most children with HIV have been infected by mother-to-child transmission.[1] Because of the remarkable scale-up of effective interventions that prevent mother-to-child transmission, the number of perinatally acquired HIV infections decreased from 280 000 in 2010 to 160 000 in 2018.[1] Additionally, access to combination antiretroviral therapy (ART; a combination of three drugs leading to durable viral suppression) has expanded globally over the past decade, resulting in a substantial decline in mortality and an increased life expectancy in children with HIV. Thus, escalating numbers of children, who would previously have died in infancy and early childhood from untreated HIV infection, are now surviving to adolescence.[2] HIV has therefore changed from a life-threatening illness to a chronic, treatable, albeit incurable, condition.

In high-income countries, adults on ART with well controlled infection have shown a range of comorbidities, including cardiovascular, renal, neurocognitive, and lung disease, which have been described and termed non-AIDS-defining illnesses. These comorbidities contrast with the opportunistic infections and malignancies that occur at advanced stages of HIV disease due to HIV-mediated immunosuppression. Likewise, there is now increasing recognition that children with HIV, including those taking ART, are at risk of developing chronic multisystem comorbidities and concomitant disability.[3,4] Some reports have suggested a trend from infectious events to non-infectious morbidities associated with inflammation, immunodeficiency, and drug toxicity as these children age.[5]

Many of these comorbidities will be driven by underlying inflammation associated with HIV infection. The spectrum of comorbidities in children might differ from that in adults, most likely related to the timing of HIV infection or ART initiation, or both, and the absence of traditional adult risk factors such as ageing, smoking, and drinking alcohol. Furthermore, there could be differences in the epidemiology of comorbidities in children with HIV in different settings. In high-income settings, combination ART became available in 1996, whereas in much of sub-Saharan Africa, paediatric ART was introduced after 2004. Additionally, children with HIV in sub-Saharan Africa start ART much later than

Key messages

- Despite antiretroviral therapy, long-standing HIV infection in children is associated with multisystem chronic comorbidities, particularly in sub-Saharan Africa where HIV treatment initiation is often delayed and occurs much later in childhood than in high-income settings
- Chronic morbidities in children with HIV are driven by underlying dysregulated immune activation associated with HIV infection, or are a sequela of infections or HIV treatment, or both
- HIV programmes have predominantly focused on delivery of antiretroviral therapy and much less attention has been paid to diagnosis and management of chronic comorbidities
- Validated tools for screening and diagnosis and evidence-based interventions for prevention and treatment of comorbidities need to be developed
- As well as earlier initiation of antiretroviral therapy, HIV care programmes need to identify and address the additional health needs of children with chronic complications, including educational support, rehabilitation, nutrition, psychosocial, and mental health support, if children with HIV are to have optimal health outcomes

Figure 1: **High-resolution CT scan of the lungs in a child with HIV**
Bilateral black and white lung pattern characteristic of mosaic attenuation, with decrease in the number and calibre of vessels (arrow). There is also atelectasis on the right and bronchiectasis.

those in high-income settings. In a global meta-analysis of children who entered HIV care before 10 years of age, the age at ART initiation in Africa was 7·8 years compared with 0·9 years in the USA.[6]

In this Review, we discuss the spectrum of chronic morbidities in children growing up with HIV, focusing on studies in sub-Saharan Africa, the likely pathogenesis underlying the development of comorbidities, the implications for HIV care and management, and implications for HIV programmes that have largely focused on ART delivery until now.

Chronic comorbidities related to HIV

Chronic lung disease

Although the incidence of acute pulmonary infections in children with HIV has declined in the past decade because of co-trimoxazole prophylaxis and ART, several studies have reported a substantial burden of chronic respiratory symptoms and signs in children growing up with HIV who are taking ART. Symptoms and signs include cough, breathlessness, reduced exercise tolerance, hypoxia,[7–9] and reduced lung function (predominantly airflow obstruction with little reversibility with bronchodilators).[10,11] These findings have been reported in the context of delayed HIV diagnosis and initiation of ART in late childhood.

High-resolution CT studies in children with HIV show decreased attenuation in a mosaic pattern as the predominant radiological finding with or without bronchiectasis (figure 1).[12,13] Notably, radiological findings consistent with lymphoid interstitial pneumonitis, the most common cause of chronic lung disease in children with HIV in the pre-ART era, have become rare in the ART era (from around 2004, when ART began to become available in Africa).[12,14] Decreased attenuation correlates strongly with reduced forced expiratory volume in 1 s (FEV_1) and, together with hypoxia and irreversible airflow obstruction, these findings are consistent with constrictive obliterative bronchiolitis.[12] Obliterative bronchiolitis is characterised by inflammation of the bronchiolar epithelium, leading to dense fibrous scarring with small airway obstruction, complicated by recurrent infections and bronchiectasis. This condition has been described following severe lower respiratory tract infections in children, often with adenovirus and more commonly in the southern hemisphere than in the northern hemisphere.[15] A South African study showed that radiological features of obliterative bronchiolitis were associated with a history of tuberculosis or severe pneumonia.[13] Plain chest radiography is insensitive for small airways disease: a definitive diagnosis usually requires high-resolution CT,[12] which is rarely available in low-income settings. Bronchiectasis is another irreversible cause of chronic lung disease in children with HIV and can occur as a sequela of lymphoid interstitial pneumonitis, recurrent pulmonary infections including tuberculosis, and possibly HIV infection itself.[16]

Reports of paediatric chronic lung disease associated with HIV mainly come from low-income settings. This difference in reporting might be due to a high prevalence of risk factors in these settings, including recurrent pulmonary infections in early life, delayed ART initiation, household air pollution, malnutrition, and stunting.[17] Malnutrition during the first year of life might be associated with decreased lung function at 1 year of age, and stunting is a marker of delayed somatic growth, therefore, it is possible that stunted children could have smaller lungs and reduced lung volume.[18]

Importantly, lung impairments and decreased lung function in childhood track through adult life and therefore pulmonary injuries in childhood not only prevent an individual from reaching full lung potential but also increase the risk of chronic lung disease in adult life (figure 2A).[19,21] A South African study showed that lung function tracked over 2 years in adolescents with HIV (aged 9–16 years) who were well established on ART.[10] In this study, early life pulmonary tuberculosis or previous admission to hospital for lower respiratory tract infections were associated with reduced lung function.

Availability of diagnostic modalities for chronic lung disease, such as spirometry and high-resolution CT, is scarce in low-income settings. Therefore, chronic respiratory symptoms are often empirically treated with repeated antibiotics and antituberculosis therapy in settings where there is a high prevalence of HIV and tuberculosis is common. The pathogenesis of chronic lung disease associated with HIV is poorly understood and the disease is without specific management guidelines. However, prevention of pulmonary infections can mitigate the burden of chronic lung disease in children with HIV and optimise lung health. Pulmonary infections can be prevented by ensuring routine vaccinations (including pneumococcal conjugate vaccine and annual influenza vaccine),[22] early ART initiation, continued co-trimoxazole prophylaxis and use of isoniazid prophylactic therapy, avoidance of exposure to tobacco smoke and indoor air pollution, and optimisation of nutrition.

Cardiovascular disease

Studies from low-income countries have reported a high burden of cardiac abnormalities associated with HIV in children with HIV taking ART. Prevalence estimates from these studies are wide, ranging between 14% and 89%, most likely reflecting differences in measurements and in the selection of participants.[23–25] The spectrum of abnormalities includes left ventricular systolic and diastolic dysfunction, left ventricular hypertrophy, left atrial dilatation, isolated right ventricular dilatation, conduction abnormalities, and in some cases, pericardial thickening or effusion (figure 3).[24–26] In a South African cohort of adolescents with HIV (aged 9–14 years), right ventricular dysfunction was the most common form of cardiopulmonary dysfunction; cardiopulmonary dysfunction was associated with lower body-mass index, height, and previous history of pulmonary tuberculosis compared with adolescents who did not have HIV.[27] Notably, in most studies, children were paucisymptomatic. A prospective study in Zimbabwean children with HIV on ART reported the incidence of left and right echocardiographic abnormalities as 3·52 and 5·64 per 100 person-years, respectively.[28] This study also reported that most abnormalities persisted at 18 month follow-up but children were either asymptomatic or their symptoms had not worsened.[28]

Much less attention has been given to assessment of vascular disease in children with HIV than the attention given to cardiac disease in these children, although there is evidence from high-income countries that HIV and ART, particularly regimens that are protease inhibitor based, are associated with subclinical atherosclerosis even in young individuals.[29] A South African study reported an increased risk of endothelial dysfunction in adolescents with perinatally acquired HIV compared with their age-matched peers who were HIV-negative.[30] Traditional risk factors for cardiovascular disease, including age, hypertension, smoking, and lipid abnormalities, do not play a substantial role in this age group, and HIV or ART, or both, might therefore play a larger role in the pathogenesis of cardiovascular disease.

The natural history and clinical significance of cardiovascular abnormalities are not clear. In contrast to findings from sub-Saharan Africa, a study from the USA reported a decline in rates of cardiomyopathy in the era of ART.[31] The underlying mechanistic pathways of cardiovascular abnormalities are not understood and the abnormalities reported could reflect impairment acquired before ART initiation. Surveillance and studies to investigate pathogenesis and progression are needed to understand whether these abnormalities are likely to result in an increased risk of premature cardiovascular disease as adolescents with HIV enter adulthood.

Renal and metabolic disease

Microalbuminuria is an early marker of glomerular injury and predicts further proteinuria development.

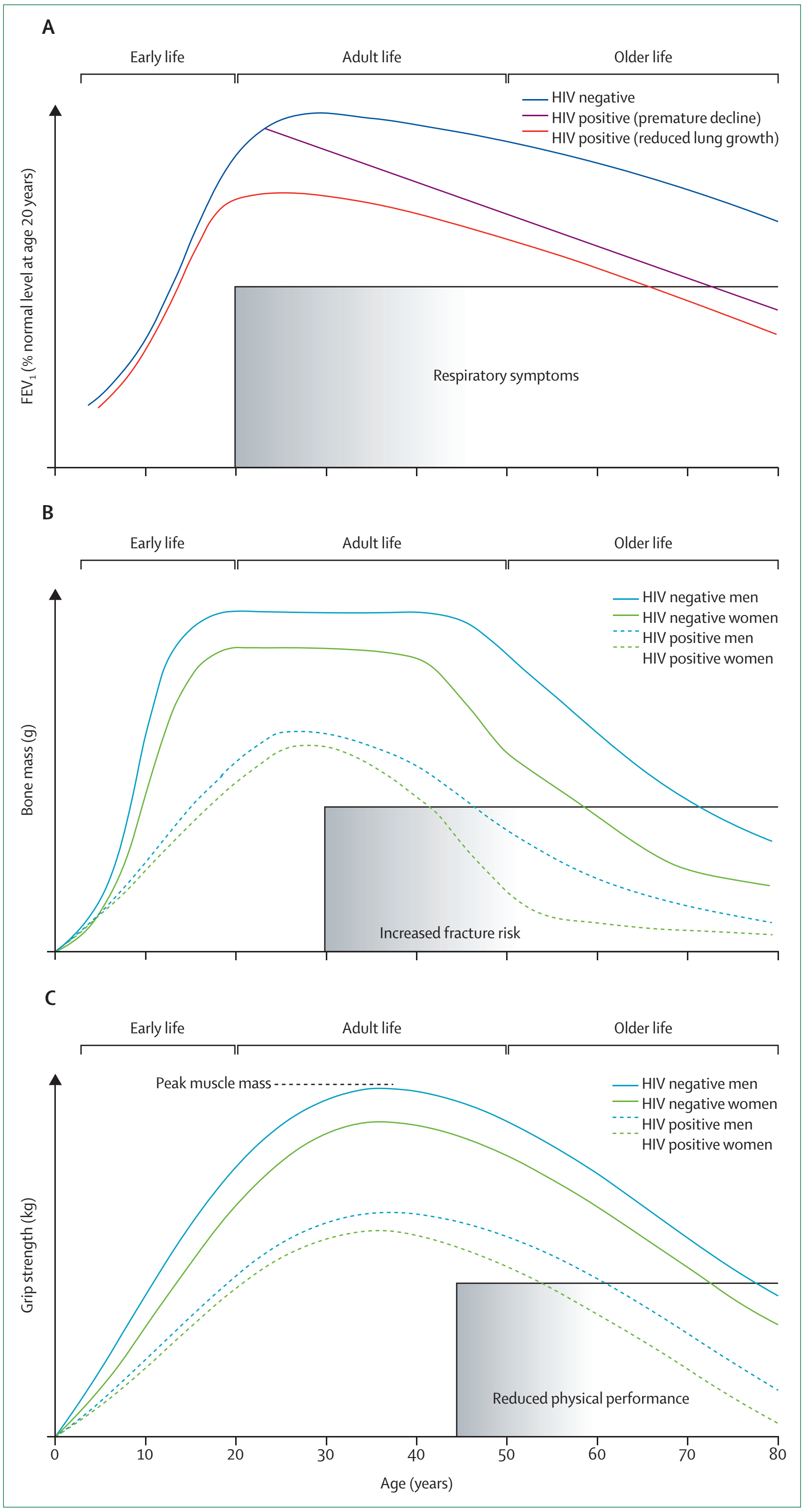

Figure 2: **Hypothesised effect of HIV infection across the life-course**
(A) Respiratory function (adapted from Weiss).[19] (B) Bone mass (adapted from Arpadi et al).[20] (C) Muscle strength.

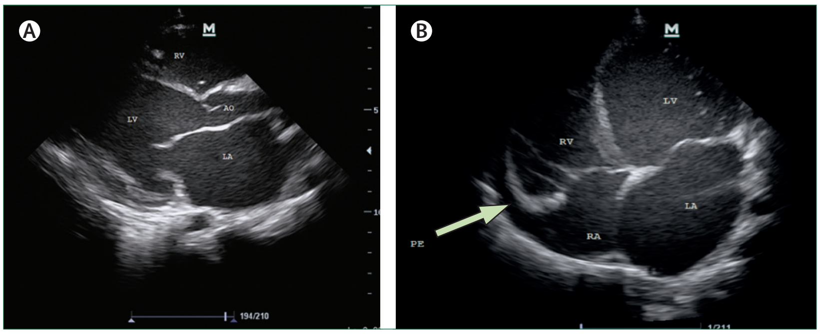

Figure 3: **Echocardiogram of a girl aged 12 years with HIV**
(A) Parasternal long axis view. (B) Apical four-chamber view showing pericardial effusion. AO=aorta. LA=left atrium. LV=left ventricle. RA=right atrium. RV=right ventricle. PE=pericardial effusion.

Two studies from Tanzania and South Africa reported a variable prevalence of 20% (49 of 240) and 8% (43 of 511), respectively, for microalbuminuria in children with HIV.[32,33] The children in the Tanzanian study were younger and more immunosuppressed than the children in the South African study, and microalbuminuria was strongly associated with immunosuppression and haematuria.[32] Notably, African people often carry *APOL1* variants G1 and G2, which are associated with increased odds of early renal disease, with HIV infection substantially augmenting the risk.[34]

Tenofovir disoproxil fumarate is associated with an adverse effect on renal function and wasting of low molecular weight proteins, phosphate, and glucose. Although slowly progressive, chronic kidney disease is uncommon.[35] Hyperphosphaturia secondary to tubular dysfunction can disturb renal-bone metabolic regulation, leading to progressive bone loss and hypophosphataemic osteomalacia, as observed in Fanconi syndrome.[35]

Older ART drugs, such as stavudine, didanosine, and early generation protease inhibitors, were associated with abnormal fat distribution (lipodystrophy or lipo-atrophy). Although the newer ART regimens are less toxic, children with HIV with established lipid abnormalities or abnormalities in fat distribution show little improvement from switching to newer, less toxic ART.[36] ART is also associated with dyslipidaemia and insulin resistance; however, data are sparse for children with HIV in sub-Saharan Africa. A South African study of children who attended HIV clinics on either a lopinavir and ritonavir or efavirenz based ART regimen (with 90 [90%] of 100 having taken stavudine previously) reported a 10% (10 of 96) prevalence of insulin resistance using the homoeostatic model assessment of insulin resistance (HOMA-IR) index, and similar prevalence of dyslipidaemias.[37] Overall, 40% (38 of 96) had either insulin resistance or at least one lipid abnormality. The adjusted mean LDL cholesterol increased by 0·24 mmol/L for each year of cumulative lopinavir and ritonavir exposure. Notably, the median body-mass index of participants was only 15·1 kg/m² (IQR 14·4–16·0) in children on lopinavir and ritonavir and 15·5 (IQR 14·6–17·0) in children on efavirenz where the healthy range is 18·0–24·9 kg/m². In a trial comparing three different regimens based on nucleoside reverse transcriptase inhibitors in children starting ART in Uganda, HOMA-IR score increased significantly in all three groups 48 weeks after ART initiation, and this increase correlated with monocyte activation.[38] Similarly, in another study, abacavir was associated with increased HOMA-IR score in adolescents.[39] Although the long-term effects of the renal and metabolic abnormalities reported in cross-sectional studies are not known, insulin resistance, dyslipidaemias, and abnormal fat distribution are recognised risk factors for cardiovascular disease, hence monitoring is required. Switching to newer protease inhibitors, such as atazanavir or darunavir, in children taking lopinavir might improve lipid profiles.[40]

Musculoskeletal disease

HIV infection is associated with impaired growth, manifested as stunting (impaired linear growth) and as delay of pubertal onset by up to a year.[41,42] Stunting is more profound in children with HIV in low-income settings than in those in high-income settings, most likely reflecting high background rates of undernutrition and intercurrent infections in children with HIV from low-income settings.[43]

Impaired growth could have a deleterious effect on musculoskeletal development and health across the life course. Puberty is a crucial period for musculoskeletal development and bone mass accrual. After cessation of linear growth and skeletal maturation, bone mass reaches a peak,[44] after which bone mass declines at varying rates throughout adulthood (figure 2B). Peak bone mass accounts for 60% of lifetime osteoporosis risk,[45] with a 10% decrease in peak bone mass doubling the risk of adult fracture.[46] Pubertal delay predicts lower adult bone mass and increases future osteoporotic fracture risk.[47] A systematic review of 32 studies reported an increased prevalence of low bone density in children with HIV, and that HIV appeared to be associated with decreased bone accrual throughout childhood and adolescence.[20] However, most studies were in high-income settings and varied with respect to comparison groups, methods of measurement, and adjustment for body size or growth retardation. A Zimbabwean study showed reduced size-adjusted (Z score ≤–2·0) lumbar spine bone density in 14% (14 of 97) and reduced total body less head bone density in 12% (12 of 97) of children with HIV aged 8–16 years taking ART.[48] Notably, this study used gold standard size-adjustment methods to analyse dual x-ray absorptiometry scans, which if ignored, underestimate bone density in stunted children.[49]

Certain ART drugs such as tenofovir disoproxil fumarate can cause accelerated bone loss most likely aggravated by low body mass and vitamin D deficiency.[35] Although studies have reported an association between tenofovir disoproxil fumarate and lower bone density in children with HIV, this association might not be sustained in the long term and longitudinal studies from sub-Saharan Africa are needed to determine the effect of tenofovir disoproxil fumarate on bone health in children.[35,48,50] Additional factors that are prevalent in children with HIV can further compromise bone health, including low muscle mass, poor nutrition, inadequate dietary calcium, vitamin D deficiency, and the pro-inflammatory milieu associated with HIV.[51–53] Muscle strength and bone strength are closely related; muscles exert forces on bone resulting in bone adaptation in size and strength.[54] HIV infection and consequent ill health could reduce physical activity levels, impairing muscle strength (figure 2C) and skeletal impact loading, and thus bone development.[55]

Although growth resumes after ART initiation, children who have more profound stunting and begin ART in late childhood have a delayed growth spurt and are typically unable to reach their height potential.[43,56,57] Age at ART initiation is an important predictor of bone density. In the Zimbabwean study, children with HIV starting ART after the age of 8 years had, on average, at least 1 standard deviation lower size-adjusted lumbar spine bone density.[48] This level of bone density reduction doubles fracture risk in adults.[58] Given the late average age at ART initiation of CWHIV in sub-Saharan Africa, these findings are concerning.[6]

Neurodevelopmental delay, neurocognitive disease, and mental health

In the pre-ART era, severe neurodevelopmental delay and HIV encephalopathy were common in children with HIV; the prevalence of neurocognitive impairment associated with HIV has declined in the ART era. Early ART initiation and viral suppression in infancy improves neurocognitive outcomes.[59] However, children with HIV who start ART outside infancy can have subtle to severe neurocognitive deficits. A prospective study of children aged 5–11 years from four countries in sub-Saharan Africa compared neuropsychological outcomes in children with HIV, children who had been exposed to HIV but were not infected, and children who had not been exposed to HIV. This study reported that children with HIV did worse in all cognitive domains than did the other two groups. More than 95% (239 of 246) of children with HIV had a suppressed HIV viral load and good immunological status (CD4 percentage greater than or equal to 25%), but only 1% (3 of 246) started ART in the first 6 months of life.[60]

An MRI study found that white matter structural abnormalities occur early after birth, and ART initiation by 8 weeks of age might be too late to prevent white matter abnormalities associated with HIV in the CNS.[61] Second-line ART, a high HIV viral load, low CD4 cell count, and poor cognitive function were associated with poor white matter integrity, measured by diffuse tensor imaging in children with HIV in a South African study.[62] A meta-analysis showed an association between HIV infection in children and adolescents and neurocognitive impairment, mainly in the domains of working memory, executive function, and processing.[63] This study also showed evidence of deficits in visual memory and visual-spatial ability. Geographical bias was notable, with only a third of studies coming from sub-Saharan Africa. The causes of neurocognitive impairment despite effective ART are likely to be multifactorial, including ongoing viral replication in the CNS and resulting neuro-inflammation, irreversible CNS injury before ART, and neurotoxic effects of ART; and could be compounded by socioeconomic and psychosocial factors.[64] Children with neurocognitive impairment can appear asymptomatic with deficits missed by routine testing. Screening tools and standardised definitions that are context-specific and have been culturally validated are scarce. However, a study in South Africa in 2019 has validated a youth international HIV dementia screen.[65]

Several studies report a high prevalence of mental health disorders in children and adolescents with HIV. A large, Ugandan study that recruited more than 1300 children and adolescents with HIV reported a 17·4% (233 of 1339) prevalence of any psychiatric disorder and a 9·6% (128 of 1339) prevalence of a behavioural disorder, most commonly attention deficit hyperactivity disorder. These disorders were more common in adolescents than in children and commonly occurred concurrently with each other.[66] Similarly, a South African study reported that adolescents with HIV had poorer functional competence, self-concept, and motivation; and higher levels of depression, disruptive behaviour, attention-deficit hyperactivity disorder symptoms, and clinically significant anger, compared with their peers who were HIV-negative.[67] Children with HIV face recurrent and cumulative psychosocial stressors that differ from other chronic childhood illnesses, such as stigma and discrimination, responsibility for welfare of siblings or other family members who are ill, illness and the death of their parents, and unstable guardianship. These stressors can hamper development of protective mechanisms and leave children psychologically vulnerable and ill-equipped for coping with challenges, most likely increasing the risk of mental health disorders.[67–69] It is possible that the neuropathological effects of HIV infection could augment risk.[70] Mental health disorders affect an individual's adherence to ART and are associated with an impaired quality of life, yet they typically receive little attention in the face of physical health concerns.

Malignancy

As children with HIV reach adolescence and become sexually active, they are at risk of acquiring human papillomavirus (HPV) infection, with certain subtypes (for example, HPV 16 and HPV 18) known to cause cervical cancer. The risk might be higher in those with HIV; in an Asian study in Thailand and Vietnam, perinatally infected adolescent girls had a higher prevalence of high-risk HPV and abnormal cervical cytology than adolescents who were not infected, after adjusting for age, sexual history, and pregnancy.[71] In a Kenyan study, the quadrivalent HPV vaccine was safe and highly immunogenic in boys and girls with HIV.[72] WHO recommends a three-dose series (at months 0, 1–2, and 6) for girls with HIV rather than the standard two dose series (given to immunocompetent girls younger than 15 years old), following studies that showed lower antibody titres after HPV vaccination in women with HIV compared with women who were not infected.[72,73] However, HIV testing before vaccination is not recommended, which could mean that adolescents with HIV miss the third dose if vaccination programmes are implemented in schools.

Children with HIV with advanced immunosuppression before ART initiation, or who started ART at an older age, have an increased risk of cancer compared with those with modest immunosuppression or who began ART in infancy.[74] Reliable incidence estimates of cancer in children with HIV are difficult to generate as many cancer registries do not report HIV status, and cancer incidence varies according to regions and study periods. Linked data from five paediatric ART programmes and four paediatric oncology units in South Africa showed an overall incidence of cancer of 82 per 100 000 person-years. The most common cancers were Kaposi's sarcoma with an incidence of 34 per 100 000 person-years and non-Hodgkin lymphoma with an incidence of 31 per 100 000 person-years.[75] The risk of developing cancer was reduced by 70% for children on ART, however, risk increased with age at start of treatment and immunodeficiency at enrolment. The risk of Kaposi's sarcoma is limited to children with HIV in, or from, sub-Saharan Africa. A study reported Kaposi's sarcoma incidence per 100 000 person-years of 81 in children from sub-Saharan Africa living in Europe, 86 in those in Eastern Africa, and 11 in those in southern Africa. There were no cases of Kaposi's sarcoma in Europe and Asia in children who were not from sub-Saharan Africa.[76] Data from Malawi suggest that incidence of Kaposi's sarcoma is increasing. The average annual number of new Kaposi's sarcoma diagnoses was 18 cases per year from 2006 to 2010, increasing to 25 cases per year from 2011 to 2015, despite improved access to ART.[77] Although this rise could be explained by increased awareness and detection, it is also possible that the cumulative risk of malignancy increases with age even in the era of ART. An older study from the USA, which followed up children for 10 years, showed that although the incidence of Kaposi's sarcoma and non-Hodgkin lymphoma decreased in the ART era, the risk of developing non-AIDS-defining cancers did not.[78] This increased risk of non-AIDS-defining cancers highlights the need for continued monitoring of children growing up with HIV. Access to comprehensive cancer services is scarce in most low-income settings and mortality is high.

Other comorbidities

Additionally, there are other comorbidities that are common in children with HIV, such as visual and hearing impairments and dental disease even in the ART era.[79–82] Skin disease (eg, seborrhoeic dermatitis, eosinophilic folliculitis, planar warts, and molluscum contagiosum) is severe and atypical in children with HIV, with children responding less well to treatment and relapsing more frequently than children who are not infected with HIV. Although incidence has declined in the ART era, skin conditions related to HIV are one of the most common management problems faced by health-care workers caring for children with HIV. ART is associated with risk of drug reactions and immune reconstitution inflammatory syndrome skin disease (unmasking of a new skin disease or paradoxical worsening of existing dermatological conditions).[83] These conditions receive little attention as they are not life-threatening. However, they can lead to complications and disability, reduced quality of life, and frequent clinic attendances, which place an additional burden on health services.

Pathogenesis of chronic comorbidities

The mechanisms underlying comorbidities associated with perinatally acquired HIV infection are largely unknown. Some of the underlying mechanisms might be shared with those reported in adult HIV infection, whereas others could be unique to the paediatric age group, in keeping with the distinct clinical features observed in this age group. Pathogenic mechanisms might also differ between populations in high-income and low-income settings.

In adults, the development of comorbidities associated with HIV is thought to be related to levels of persistent immune activation, predominantly reflecting activation of monocytes and macrophages, rather than activation of T cells.[84] Various biomarkers that are associated with adult comorbidities have been described, largely in cross-sectional studies.[85] These include inflammatory markers, such as IL-6, soluble tumour necrosis factor receptors 1 and 2 (soluble TNFR-1 and soluble TNFR-2), interferon-inducible protein 10 (IP10), and high sensitivity C-reactive protein; markers of monocyte activation, such as soluble CD14 and CD163; markers of indoleamine 2,3-dioxygenase-1 activity (IDO-1); markers of coagulation risk, particularly D-dimer levels; and markers of gut barrier dysfunction, such as zonulin and intestinal fatty acid binding protein. Tracking rising levels of these biomarkers in longitudinal studies better predicted the development of non-AIDS-defining illnesses than taking a single value at recruitment.[86] These findings suggest that impaired function of the gut barrier leads to translocation of microbial products from the gut into the circulation, where they activate monocytes and macrophages, initiating a cycle of chronic inflammation (figure 4).[85] This cycle appears to be related to the activation of a specific subset of inflammatory monocytes that express tissue factor: these monocytes can secrete high levels of pro-inflammatory cytokines and trigger the coagulation cascade, so are particularly implicated in coagulopathy associated with HIV.[87]

Additional factors that contribute to inflammation include vitamin D deficiency and cytomegalovirus co-infection.[88] Vitamin D deficiency is associated with immune activation levels in individuals with HIV, potentially mediated through its immunomodulatory effects on populations of monocytes and macrophages, dendritic cells, and B and T lymphocytes.[89] A trial of vitamin D supplementation in children with HIV who were deficient of vitamin D and well controlled on ART, led to significant reductions in both T-cell and monocyte activation.[90]

The relationship between comorbidities and cytomegalovirus infection is difficult to disentangle, as almost all individuals with HIV have cytomegalovirus co-infection. However, in the few studies that include people with HIV who were negative for cytomegalovirus, people with HIV and cytomegalovirus co-infection had higher plasma levels of IP-10, TNFR-2, and D-dimer,[91] and an increased risk of non-AIDS-defining illnesses, particularly cardiovascular and cerebrovascular disease.[92] Cohort studies have shown associations between inflammatory markers (which in turn are linked with the subsequent development of comorbidities) and the scale of immune response specific to cytomegalovirus (IgG concentration[93,94] and magnitude of T-cell response[95]). This suggests that subclinical cytomegalovirus reactivation or replication, or both, is reflected in elevated immune responses: however, it is important to control for ageing in these studies.[96] For children with HIV in low-income settings, especially when HIV diagnosis and ART initiation is delayed, primary cytomegalovirus infection most likely occurs in infancy at a time of uncontrolled HIV replication. African children acquire cytomegalovirus infection early in life, primarily through breastmilk exposure: studies in The Gambia showed 85% (239 of 281) of infants had acquired cytomegalovirus by 12 months of age, reaching 100% by 18 months.[97] Thus, their situation is distinct from that of adults who mainly acquire HIV after cytomegalovirus infection and would already have generated immune responses leading to viral control in the long term. Therefore, cytomegalovirus reactivation or reinfection might occur more frequently in children with perinatally acquired HIV who were diagnosed late, and could make a greater contribution to the pathogenesis of comorbidities. A study in Zimbabwe reported unexpectedly high levels of cytomegalovirus viraemia in older children with HIV.[98] The detection of cytomegalovirus DNA in plasma was associated with two of the major comorbidities described in older children (aged 6–16 years) with perinatally acquired HIV: chronic lung disease and stunting. Further studies are needed to determine whether similar findings are noted in other settings and if presence of cytomegalovirus DNA in the blood represents reactivation of latent infection or reinfection with new viral strains.

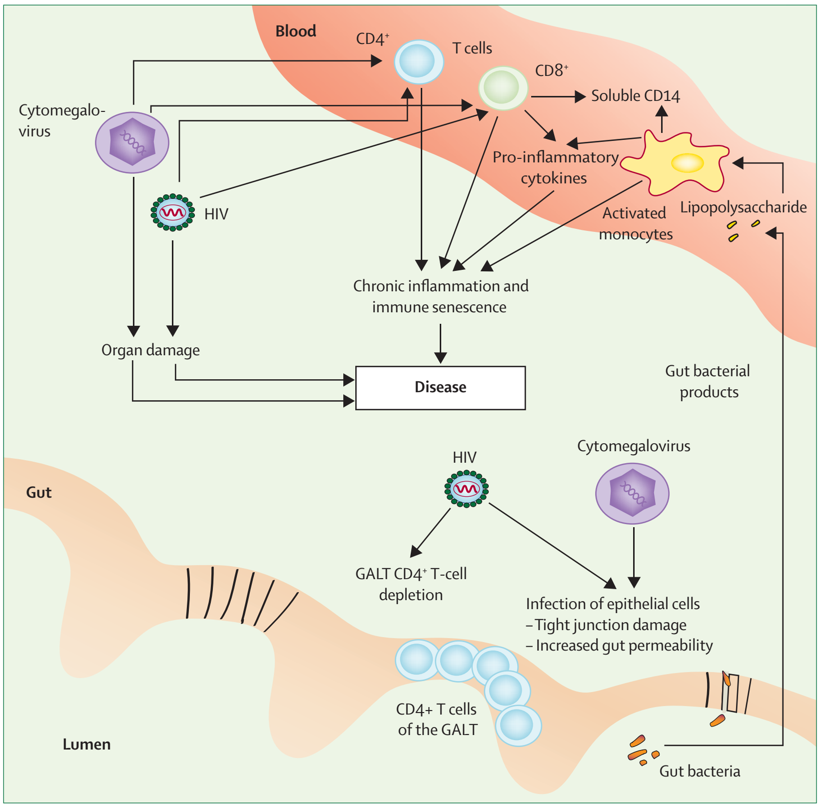

Figure 4: **Pathogenesis of comorbidities associated with HIV**
GALT=gut-associated lymphoid tissue.

The other key difference between children and adults is the greater potential for lymphopoiesis with enhanced thymic and bone marrow activity: paediatric slow progressors are characterised by what was described as supranormal thymic activity.[99] However, thymic infection leading to impaired function can occur in a proportion of children with HIV, which is associated with significantly more rapid disease progression.[100] Several studies suggest that children with perinatally acquired HIV have markers of premature ageing,[101] including shorter telomere length,[102] distinct epigenetic features of ageing,[103] and accumulation of senescent (CD28 negative and CD57 positive) and exhausted (PD-1 positive) T cells.[102] In children with HIV with these markers, there are also features of thymic dysfunction,[102] which could potentially indicate HIV infection of the thymus, leading to an inadequate response to turnover of T cells driven by immune activation.

In summary, comorbidities in children with HIV are likely to reflect persistent immune activation and premature ageing of the immune system, potentially driven by infection in early life with cytomegalovirus and

Panel: Addressing comorbidities associated with HIV in children and adolescents

Research priorities

- Epidemiology and clinical spectrum
- Pathogenesis
- Diagnosis and screening
  - Standard definitions of comorbidities based on population-specific normative ranges
  - When to start screening and frequency of screening
  - Age appropriate and culturally appropriate screening tools for mental health and neurocognitive disease
- Interventions for prevention and treatment
  - Preventive and therapeutic drugs—eg, antibiotics, antivirals, anti-inflammatory drugs, and vitamin D
  - Feasible and effective educational and mental health interventions
  - Interactions of drugs used for prevention or treatment, or both, with antiretroviral therapy
- Service delivery
  - How to integrate diagnosis and management of comorbidities associated with HIV within HIV services or maternal, newborn, and child health platforms, or both

Components of comprehensive HIV care

- Earlier initiation of antiretroviral treatment to prevent complications
- Monitoring growth, musculoskeletal, and neurocognitive development
- Screening for cardiac, lung, and renal disease
- Assessment of psychosocial status (schooling, guardianship) and mental health
- Management of common mental health disorders and psychosocial support
- Isoniazid and cotrimoxazole prophylaxis
- Optimal nutrition
- Catch up or revaccination according to WHO guidelines—eg, pneumococcal and influenza vaccination
- Human papillomavirus vaccination for adolescents
- Cervical cancer screening after sexual debut
- Referral to clinical specialties for management
- Liaison with disability and rehabilitation services
- School-based programmes to provide educational support
- Leverage existing early child development platforms for supporting children with HIV
- Linkage to community-based psychosocial support services

exacerbated by vitamin D deficiency in low-income settings (figure 4). Interventions aimed at reducing inflammation, cytomegalovirus suppression, or vitamin D supplementation, or a combination, could have potential for control or even reversal of comorbidities and merit further study.

Recommendations for policy and research

Although access to paediatric ART has increased substantially in the past decade, coverage in children lags behind that in adults with about 54% of children with HIV accessing treatment in 2018 compared with about 62% of adults globally.[104] Timely diagnosis and treatment of HIV infection in children remains an essential priority.

In sub-Saharan Africa, there is a large cohort of children with HIV entering adolescence and adulthood that have had delayed ART initiation, and are at increased risk of multisystem impairments and earlier onset of comorbidities than are usually associated with ageing. To date, HIV care has mainly focused on delivery and sustainment of adherence to ART and there is an under-appreciation of the burden of multisystem comorbidities associated with HIV in children. Additionally, complex clinical issues place heavy demands on already over-stretched health-care systems, and optimum screening and management strategies are not well defined. In response to these issues, WHO convened two scoping meetings in 2014 and in 2019 to review available data and policies on management of major comorbidities associated with HIV, and evidence gaps in clinical management and programming.

Comprehensive HIV care should include diagnosis and management of comorbidities and consequent disability. The panel outlines suggestions for interventions and research priorities aimed at addressing comorbidities associated with HIV in children. In Africa, dedicated HIV services for children and adolescents are the exception rather than the rule. Provision of comprehensive HIV care will need to extend beyond centres of excellence to low-level health-care settings, integrate within existing HIV and maternal, newborn and child health platforms, and consider the physiological and psychosocial changes through childhood. Importantly, inclusion of guardians, teachers, and communities as equal partners will be crucial to optimally support children with HIV to achieve their full potential.

Search strategy and selection criteria

This is a descriptive review on comorbidities associated with HIV infection in children, informed by clinical experience and expert opinion. We searched PubMed for articles published from Jan 1, 2014, to July 31, 2019, with MeSH terms for HIV, Africa, and children and adolescents; and with MeSH and related terms for specific comorbidities (eg, cardiac disease and lung disease). We also looked for relevant publications among our personal files. We did not set any language limits. Articles resulting from these searches and relevant references cited in those articles were reviewed. The final reference list was generated on the basis of relevance to the broad scope of this review.

Contributors

RAF and LJF conceptualised and coordinated the Review. RAF wrote the chronic lung disease section with SR-J, and collated the manuscript. RAF and EDM provided clinical pictures. SR-J wrote the pathogenesis section. LJF wrote the malignancy section. CLG and RR wrote the

musculoskeletal section. EDM and HJZ wrote the cardiac disease section. JH wrote the neurocognitive and mental health section with input from LJF and RAF. JJ and CLG wrote the renal and metabolic disease section. WA and LM wrote the policy section with input from RAF and LJF. All authors, including All authors contributed to editing and approved the final version of the Review.

Declaration of interests

We declare no competing interests.

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Annex 2: Ethics approval for PhD and Cape Town Adolescent Antiretroviral Cohort

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

24 January 2018

HREC REF: 057/2018

Prof Heather Zar
Paediatrics Pulmonology
Red Cross Children's Hospital

Dear Prof Zar

PROJECT TITLE: SPECTRUM, PROGRESSION AND PREDICTORS OF MORBIDITY IN PERINATALLY-HIV-INFECTED ADOLESCENTS ON ANTIRETROVIRAL THERAPY (PhD-candidate-Dr L Frigati) sub-study linked to 051/2013

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

- We note that only secondary analyses of data will be used.

Approval is granted for one year until the 30 January 2019.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, Dr Lisa Frigati will also be involved in this study.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

057/2018

ANNUAL PROGRESS REPORT

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Additional number of participants still required	0

5. Refusals

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

6. Cumulative summary of participants

Total number of participants who provided consent	681
Number of participants determined to be ineligible (i.e. after screening)	63
Number of participants currently active on the study	490
Number of participants completed study (without events leading to withdrawal)	0
Number of participants withdrawn at participants' request (i.e. changed their mind)	27
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	0
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	135
This is unchanged since 2015. Please refer to main study HREC 651/2013	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	135

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:

Manuscripts on insulin resistance, renal abnormalities have been published
Since May 2019 – manuscript on multisystem disease has been published, manuscript on hospitalization submitted
Manuscript on TB incidence is being written

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

☑	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved

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

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Annex 3: Consent and Assent forms

Appendix 3a. Assent form

PATIENT INFORMATION AND ASSENT FORM FOR PARTICIPANTS FOR STUDY:

Cape Town Adolescent Antiretroviral Cohort (CTAAC)

You are invited to take part in a research study that is being done at Red Cross Children's Hospital. Please read this information which explains the project and ask the study staff or doctor any questions you have about the study.

You have been asked to take part in this study because you are HIV positive, between the ages of 9 and 14 years, on ARVs and you live in Cape Town. HIV is very common in South Africa and can affect different parts of the body in children of your age but very little about these effects in adolescence have been studied, this information will help us improve the treatment of other children your age with HIV in the future.

WHY IS THIS STUDY BEING DONE?

We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. The study will take place over 4 years during which we will follow you up and monitor your health. Approximately 600 adolescents will take part in this study. At any time during this study, if we find that you have a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHAT WILL HAPPEN TO YOU IF YOU JOIN THE STUDY?

If you take part in the study the following will happen:

1. You will have to attend study visits held at Red Cross War Memorial Children's Hospital every 6 months for the next 4 years.
2. The first visit at Red Cross Memorial Children's Hospital will last 4-5 hours. At the first visit and once a year you will be asked to complete a questionnaire. The questionnaire asks you questions about you, your family, where you live, your school, your health and your medication.
3. A study doctor or nurse will ask to examine you; this will take more or less 10 minutes, to look at your general health. This is similar to an examination whenever you go to the clinic.
4. We will ask to take about 3 teaspoons of blood once a year. We will do several tests on this blood and if you give your permission some of this blood will be kept in a fridge to be tested later on.
5. We will ask to take a special video of your heart. It is not painful, but you have to lie still on a bed for about 15 minutes while the picture of the heart is made. We will only do this test at the first and last study visit.
6. We will ask to do another test to look at your veins, this is painless and very similar to having your blood pressure measured, using a special machine.
7. We will ask you to have a chest X-ray. This is when we take a picture of your lungs, it is painless and just like the X-rays done in the hospital and clinics.

8. We will ask you to breathe into a special machine to see how well your lungs are working.
9. We will ask you to walk as fast as you can back and forth on a flat surface like a hallway for 6 minutes.
10. We will ask you to give us sputum (phlegm) so that we can test for TB and other infections. If you are unable to cough enough phlegm we will give you a mask to breathe in a water vapour to make you cough.
11. We will ask to do a nasal swab, this means we will take a small stick with cotton wool at the tip and insert it into the front part of your nose to test for bacteria and infection.
12. We will ask to do an ultrasound, this means we will put a little bit of gel on your stomach and chest and a probe that will take a video of your organs, this is a quick and painless procedure.
13. We will ask you to do another ultrasound of your joints with a bit of gel and a probe on your knuckles, wrist and the heel of your foot. It is quick and painless though the gel may be cold.
14. We will ask to do a hearing test to test for any hearing problems. This means you will put in earphones and listen to beeping sounds at different volumes and tell us when you hear the sounds. This is a quick and painless test.
15. We will ask you to give us some urine (wee) in a bottle to test how your kidneys are working and for commonly abused drugs (like cannabis/weed and 'tik') and other substances and if you give your permission we will store a sample for further testing in the future. If it is found that your urine has tested positive for any of these drugs, we will inform you and your parent/legal guardian/caregiver of these results and we will then refer you to the Cape Town Drug Counselling Centre for counselling and advice on how to stop using drugs.
16. We will ask you to give us some stool (kaka) that we will collect in a container. The stool (kaka) is being collected so that we can study it to see what it is made up of and to see if the medicines you take have any effects on the stool (kaka) . This can help us to understand how you are growing and also understand any illnesses that you may have. To collect the stool, we will put a container in the toilet. You will sit on the toilet as you usually do to provide the stool. You will leave it in the bathroom and the study nurse will collect it afterwards.
17. We will also ask you to participate in another study, this study will have a separate form similar to this form that will explain the study and ask your permission to include you in the study. You may choose to participate in only one or both of these studies. We will also ask your parent/legal guardian/caregiver for permission to take part in one or both of these studies.
18. At the other visits (every 6 months) you will again be asked a few questions about yourself, your family, your house and anything that has changed since your last visit. You will also be asked to be examined again like at your first visit. The 6 monthly visits will be much shorter than the first visit and will take about 1 to 2 hours.

WHAT ARE THE ADVANTAGES OF BEING IN THIS STUDY?

If we find that you have an untreated health problem during, you will be told the result and you will be sent to the appropriate clinic/hospital to give you the right treatment.
We are not taking over your treatment and you should still go to your ARV clinic for your regular clinic visits. We will inform your doctor of any abnormal results we find during the study.
With this new information we learn through this study it will help us to improve the treatment of HIV-infected children in the future.

WILL THIS STUDY HURT OR MAKE ME FEEL BAD?

You will have some discomfort from the needle when blood is taken. We will try to do the blood test at the same time as other blood tests and using a cream that dulls the pain from the needle. Only a small amount of blood will be taken. You may also have a bit of discomfort when you have to give us the sputum sample, as the mask with the water vapour can make you cough a lot but we will monitor you carefully and give you the necessary treatment if this happens. The nasal swab is painless but it may cause you to sneeze but this will stop by itself. The gel from the echo and ultrasound might be slightly cold but is otherwise painless. You may feel tired during the Walk test but if this happens you can tell the study nurse and you can then stop and rest.

Some of the questions we ask you during your visits might be of a personal nature and will include questions on drug abuse, alcohol and tobacco use and sexual behaviour however these questions will be asked in a private room by trained study staff and you may choose to answer these questions alone or with your parent/legal guardian/ caregiver present. You may also choose not to answer these questions. The information we get from these questions will only be revealed to your parent/legal guardian/caregiver if it affects your health in a negative way or endangers your life or the life of another person.

HOW LONG WILL YOU BE IN THE STUDY?

You will be seen at Red Cross Hospital every 6 months for 4 years. That means you will have to come to Red Cross 9 times over 4 years.

DO YOU HAVE TO BE IN THE STUDY?

You may choose to be in this study or not. If you choose not to be in the study then you will get regular treatment as usual at your ARV clinic.

WHAT DO I DO IF I HAVE QUESTIONS?

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

Professor Heather Zar at (021) 658 5111 or the study medical officer at (021) 658 5520

If you have any questions about your rights as a research participant, you may contact the following member of the ethics committee:

Professor Marc Blockman at (021) 406 6338

CONFIDENTIALITY

Your study records will be kept confidential. Your name will not appear in any publication of this study. As a participant in this study it is very important to be able to contact you and therefore we will need to collect detailed tracking information like your address and at least two phone numbers where we might get hold of you. Please take note that even when contacting friends or neighbours we will never give them the reason that we are looking for you.

STORAGE OF SAMPLES

If any of the blood, sputum, nasal swabs or urine samples I have provided for this research project are unused or leftover when the project is completed

☐ I give my permission for my samples to be stored indefinitely and used in future research of any type which has been properly approved including genetic testing and other research.

☐ I give permission for my samples to be stored indefinitely and used in future research but only for research on HIV.

☐ I give permission for my samples to be stored indefinitely and used in future research except for research about ______________________________________.

OR

☐ I wish for my samples to be destroyed immediately.

I have read and understood this form. My questions have been answered. I am willing to participate in this study.

I, ________________________ (please print name) agree to participate in this study.

Participant signature: ______________ Date and Time: DD/MMM/YYYY at - -H - -

Participant Enrolment number: __________

Study staff name (print): _________________ Study staff signature: ____________

Date and Time: DD/MMM/YYYY at - - H - -

Annex 3b. Consent form

Parent /Legal Guardian/Caregiver Information Sheet and Consent Form

Cape Town Adolescent Antiretroviral Cohort (CTAAC)

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

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

WHY IS THIS STUDY BEING DONE?

We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. The study will take place over 4 years during which we will follow-up your child and monitor his/her health. At any time during this study, if we find that your child has a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHO CAN TAKE PART IN THE STUDY?

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

WHAT DO I HAVE TO DO IF I TAKE PART?

Your child will need to attend study visits held at Red Cross War Memorial Children's Hospital every 6 months for the next 4 years.

Enrolment and annual (yearly) visits

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

1. You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, HIV treatment history, and other aspects of health, both in the past and at present.

2. Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting 10 minutes approximately, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

3. Your child will be asked to give blood. We will collect about 15 millilitres (3 teaspoons) of blood once a year. This blood will be used to test for HIV in the body (viral load), the body's response to HIV infection (CD4 cell count), the fats in the blood (lipogram), the body's degree of health or general inflammation (Full blood count, Liver and Kidney function, C-reactive protein, Calcium, Magnesium Phosphate, Albumin), TB infection and autoantibodies like ANA, anti-cardiolipin antibody and anti-dsDNA. Antibodies are made by the body to help fight infection. Sometimes rather than fighting infection these antibodies attack the body and are called autoantibodies. There are specific autoantibodies that cause specific diseases like Lupus. It is possible to have these autoantibodies even before one gets sick with a disease and these antibodies can sometimes be present without actually causing any disease. If permission is given some of this blood may be stored for future tests and studies.

4. Your child will be asked to undergo echocardiography. This is a video of the child's heart. This video is called an echocardiogram or "echo". For this, we will put some gel and a probe on your child's chest. The doctor will see your child's heart move on a screen. A short video of this movement will be recorded. We will also check your heart rate and rhythm (the way in which your heart is beating). We will also look at the arteries in your child's neck to see if they are harder or thicker than usual. This will take less than 5 minutes. We will only do this test at the first visit, at the two year visit and the last study visit.

5. Your child will be asked to undergo endothelial pulse amplitude tonometry (Endo-PAT). This is a painless procedure very similar to having blood pressure measured, using a special machine. This will measure the thickness of your child's veins and how easily the blood flows through the veins. This test will be done at the first visit and then yearly.

6. Your child will be asked to have a chest x-ray. This is a painless procedure to take a picture of your child's lungs. This helps to check if your child has a lung problem which needs treatment. An X-ray will be done at the first visit and yearly.

7. Your child will be asked to provide a urine sample to test for kidney function and to test for substances that may affect his/ her health and if permission is given a sample will be stored for future testing. Urine will be tested for commonly abused drugs and exposure to smoking. If any of these are detected in the urine we will inform you and your child of these results, you will both receive counselling and you will be referred to the Cape Town Drug Counselling Centre for treatment.

8. Your child will be asked to give us some stool (kaka) that we will collect in a container. The stool (kaka) is being collected so that we can study it to see what it is made up of and to see if the medicines your child has taken has any effects on the stool (kaka). This can help us to understand how your child is growing and also understand any illnesses that they may have. To collect the stool, we will put a container in the toilet. Your child will sit on the toilet as they usually do to provide the stool. They will leave it in the bathroom and the study nurse will collect it afterwards.

9. Your child will be asked to undergo lung function testing. This involves breathing into a special machine to see how the lungs are working. Your child will do 3 different blowing tests. One test measures inflammation in the lungs and takes only 1 or 2 minutes. A second test measures the size of the lungs and how well they are working and involves breathing oxygen for the middle part of the test. The third test measures the flow of air out of the lungs when your child blows as hard and fast as she/he can. If your child cannot blow air well out of their lungs we will give your child an asthma medicine (bronchodilator) to open their chest. After 5 minutes we will repeat the blowing test, to see if this treatment helps your child and what kind of lung problem your child might have.

10. Your child will be asked to do a 6 minute walk test to measure their ability to do exercise. This involves your child walking back and forth on a flat surface such as a hallway for 6 minutes. We will monitor their oxygen level and blood pressure before and after the test.

11. Your child may be asked to undergo sputum induction to test for TB and other infections. This involves having a nebuliser treatment to open your child's chest and make him / her cough. We will then ask your child to cough some phlegm up into a container or suction him/ her to get phlegm if he/ she cannot cough this up. The phlegm will be sent to the laboratory for tests for TB and other germs.

12. Your child will be asked to have a nasal swab done. This procedure involves putting a small stick with cotton wool at the tip into your child's nose to collect mucus. We will test this for bacteria and infection.

13. Your child will be asked to undergo a bedside ultrasound, it involves putting a little bit of gel on your child's stomach and chest and a probe that will take a video of your child's organs, this is similar to the ultrasound that is done when you are pregnant and is quick and painless.

14. Your child will be asked to undergo another ultrasound of the joints with a bit of gel and the probe on their knuckles, wrist and the heel of their foot. It is also quick and painless though the gel may be cold.

15. Your child will be asked to have a hearing test done. This involves your child putting in earphones and listening to beeping sounds at different volumes and telling us when he/she hears the sounds. This will help us to test whether or not your child has a hearing problem. This is a quick and painless test.

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

1. You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, HIV treatment history, and other aspects of health.

2. Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting about 10 minutes, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

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

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

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

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

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

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

1. Blood drawing: Your child will experience discomfort from the needle when blood is taken. Where possible this blood test will be done at the same time as other blood tests and using an anaesthetic cream to dull the pain from the needle. Only a small amount of blood will be taken.
2. Endo-PAT: This will be similar to having a blood pressure done- that is for 5 minutes, a tight bandage will be placed on the upper part of both arms while measurements are done on the fingers. This is slightly uncomfortable but not painful.
3. Echocardiography: It is not painful, but will need your child to lie still for about 15 minutes. Gel will be used to help make the pictures clearer and this will be slightly cold.
4. Lung function testing: Lung function testing is not painful. Your child may feel a little dizzy for a short time when they blow out hard and fast. All the testing will be done by a trained technologist who is experienced in guiding young people in these tests. Breathing oxygen feels similar to breathing room air and is safe.
5. 6 minute Walk Test: The object of this test is to walk as far as possible in 6minutes. This is a painless test but your child may feel short of breath. We will monitor their oxygen level and blood pressure before and after the test and if your child feels tired they can stop to rest.
6. Chest X-ray: This is painless but may cause your child some discomfort as it requires sitting still for less than 5 minutes. An X-ray will be done at the start and at the end of the study, unless your child has an illness for which an X-ray is needed.
7. Induced Sputum: Your child may experience minor side effects such as increased coughing, vomiting, wheezing or mild bleeding from the nose. A trained nurse will do this in a special room.
8. Nasal swab: This is a painless procedure but may cause some sneezing that will stop quickly.
9. Bedside ultrasound: The gel might be slightly cold but it won't hurt.

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

WILL I RECEIVE ANY PAYMENT?
You will receive R200 for the visits when you and your child visit the study to compensate you for transport costs and time associated with the study.

CAN I AND/OR MY CHILD REFUSE TO TAKE PART IN THE STUDY?
If you or your child do not wish to participate, you or your child can refuse now or at any time in the future. Your child may also refuse to participate despite you having given consent for him/her participation. Even if you and your child decide not to take part, your child will receive the same health care, including ART, through your local health facility.

CONFIDENTIALITY

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

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

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

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

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

Prof Marc Blockman at (021) 406 6338

STORAGE OF SAMPLES

If any of the blood, sputum, nasal swabs or urine samples my child has provided for this research project are unused or leftover when the project is completed

☐ I give my permission for my child's samples to be stored indefinitely and used in future research of any type which has been properly approved by the Human Research Ethics Committee including genetic testing and other research.

☐ I give permission for my child's samples to be stored indefinitely and used in future research but only for research on HIV.

☐ I give permission for my child's samples to be stored indefinitely and used in future research except for research about ____.

OR ☐ I wish for my child's samples to be destroyed immediately.

I have read this consent form (or have had it explained to me), all my questions have been answered, and I agree for my child to take part in this study.

______________________________	______________________________	DD/MMM/YYYY at - -H - -
Parent/Legal Guardian/Caregiver (Print)	Parent/Legal Guardian/Caregiver signature/Thumbprint)	Date and Time

____________________	________________________	DD/MMM/YYY at - -H- -
Study Staff (Print)	Study Staff's Signature	Date and Time

Please complete the Witness page if the participant's Parent/Legal Guardian/Caregiver is unable to read or write.

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

Participant Enrolment number: __________________

Please complete the following:

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

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

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

Participant Enrolment Number:					

<u>EXAMINATION CRF</u>

A. ANTHROPOMETRY

BASIC		
1.	Weight(Kg)	
2.	Height(cm)	

CIRCUMFERENCES		
3.	Head (cm)	
4.	Mid- upper arm (cm)	
5.	Hip (cm)	
6.	Waist (cm)	
7.	Mid- thigh (cm)	

SKINFOLD THICKNESS		Reading
8.	Bicep (mm)	
9.	Tricep (mm)	
10.	Sub-scapular (mm)	
11.	Supra-iliac (mm)	

Participant Enrolment Number:					

B. GENERAL EXAMINATION

Vital signs		
		Value
1	Temperature	°C
2	Heart Rate	bpm
3	Respiratory Rate	/min
4	Blood Pressure	/
5	Oxygen Saturation	% in RA
General appearance and examination		
6	Any dysmorphic features present?	☐ Yes ☐ No
	If Yes, please describe:	
7	Glasses?	☐ Yes ☐ No
8	Skin ☐Normal ☐Eczema ☐Impetigo ☐Molluscum ☐Ringworm ☐Scabies ☐Warts ☐Other	
	If Other, please describe:	
9	Skin turgor	☐Normal ☐Reduced
10	Capillary refill time	☐Normal (<2 s) ☐Slow (>2s)
11	Nails ☐Normal ☐ Beau's lines ☐Clubbing ☐Splinter haemorrhages ☐Spoon-shaped ☐ Fungal infection (onychomycosis) ☐ Nail bed infection (panonychia) ☐Other	
	If Other, please describe:	
12	Hair	☐Normal ☐Sparse ☐Hair loss(alopecia) ☐Tinea Capitus ☐Other
	If Other, please describe:	
13	Generalised lymphadenopathy	☐Yes ☐No
14	Jaundice	☐Yes ☐No
15	Pallor	☐Yes ☐No
16	Cyanosis	☐Yes ☐No

Participant Enrolment Number: | | | | | |

	If Yes, please indicate the following:	☐Peripheral	☐Central
17	Pedal Oedema	☐Yes	☐No
18	Any medical devices visible? (VP shunt, PEG, O2 etc.)	☐Yes	☐No
	If Yes, please describe:		
19	Please assess lipodystrophy at each site using the scale below. ☐Face ☐Trunk ☐Limbs ☐Buttocks		
	0 – No fat changes; 1 – Possible minor changes, noticeable only on close inspection; 2 - Moderate changes, readily noticeable to an experienced clinician or a close relative who knows the child well; 3 – Major changes, readily noticeable to a casual observer.		

C. EYES; EAR, NOSE & THROAT

Site		Findings	
1.	Ears	Hearing aid?	☐Yes ☐No
		Left external ear	Right external ear
		☐Normal ☐Otitis externa ☐ Other, specify:	☐Normal ☐ Otitis externa ☐ Other, specify:
		Left Tympanic Membrane	Right Tympanic Membrane
		☐Normal ☐ Dull ☐Bulging ☐Inflamed ☐Perforated ☐Acute Otitis media ☐ Chronic Otitis media ☐ Excessive wax - Not visualized ☐Other	☐Normal ☐ Dull ☐Bulging ☐Inflamed ☐Perforated ☐Acute Otitis media ☐ Chronic Otitis media ☐ Excessive wax- Not visualized ☐ Other
	If Other, please specify:		
2.	Nose ☐Normal ☐Enlarged turbinates ☐Rhinorhoea ☐Polyps ☐Other		
	If Other, please specify:		
3.	Throat ☐Normal ☐Pharyngitis ☐Tonsillitis ☐Enlarged tonsils ☐Other		
	If Other, please specify:		

Participant Enrolment Number:					

D. ORAL HEALTH

1.	Parotid enlargement	☐No	☐Yes, if yes, ☐Unilateral or ☐Bilateral	
2.	Oral candidiasis	☐No	☐Yes	
3.	Angular chelitis	☐No	☐Yes	
4.	Orofacial herpetic lesions	☐No	☐Yes, if yes,☐ herpes labial ☐herpetic stomatitis	
5.	Mucosal ulcerations	☐No	☐Yes	
6.	Missing teeth	☐No	☐Yes	
7.	Caries	☐No	☐Yes	
8.	Other	☐No	☐Yes, Please specify:	

E. JOINTS

1.	Swelling or pain in any joint? ☐No ☐Yes, if yes, please describe each site and pathology: ____________________ ____________________

Participant Enrolment Number:					

F. RESPIRATORY EXAMINTION

RESPIRATORY SIGNS		
1.	Was there a cough?	☐Yes ☐No (Go to Q3)
2.	If yes, please describe.	☐Dry ☐Wet ☐Barking
3.	Was stridor present?	☐Yes ☐No
4.	Was there an audible wheeze?	☐Yes ☐No
5.	Was nasal flaring present?	☐Yes ☐No
6.	Was there a tracheal tug?	☐Yes ☐No
7.	Were there subcostal recessions present?	☐Yes ☐No
8.	Were there intercostal recessions present?	☐Yes ☐No
9.	Was there a chest deformity present?	☐Yes ☐No (Go to Q11)
10.	If yes, please describe.	☐Pectus Excavatum ☐Pectus Carinatum ☐ Harrison Sulcus ☐Other, ________________
11.	Were there any palpable rattles?	☐Yes ☐No
12.	Was there hyperinflation present?	☐Yes ☐No
13.	Was chest auscultation abnormal?	☐Yes ☐No (Go to section G)

FINDINGS			
		Left Side	Right Side
15.	Wheeze	☐Yes ☐No	☐Yes ☐No
16.	Crackles/crepitations	☐Yes ☐No	☐Yes ☐No
17.	Decreased breath sounds	☐Yes ☐No	☐Yes ☐No
18.	Bronchial breath sounds	☐Yes ☐No	☐Yes ☐No

Participant Enrolment Number:					

G. CARDIOVASCULAR EXAMINATION

PULSES - Were these pulses present?		Left Side	Right Side
1.	Carotid palpable	☐Yes ☐No	☐Yes ☐No
2.	Brachial palpable	☐Yes ☐No	☐Yes ☐No
3.	Radial palpable	☐Yes ☐No	☐Yes ☐No
4.	Femoral palpable	☐Yes ☐No	☐Yes ☐No
5.	Popliteal palpable	☐Yes ☐No	☐Yes ☐No
6.	Dorsalis Pedis palpable	☐Yes ☐No	☐Yes ☐No

CARDIOVASCULAR EXAM		
7.	JVP	☐ Normal ☐ Elevated ☐ Not visualized ☐ Other
		If other, please describe:
8.	a) Apex	☐ Normal ☐ Displaced ☐ Not palpable ☐ Other
		If other, please describe:
	b) Right Ventricular Heave	☐ No ☐ Yes
	c) Epigastric pulsation	☐ No ☐ Yes
9.	Heart Sounds	☐ Normal ☐ Gallop ☐ Pericardial rub ☐ Thrill ☐ Other additional sounds (e.g. snaps, clicks etc.)
		If Other, please describe:
10.	Murmur	☐ No ☐ Yes
		If Yes, please describe type of murmur, position best heard and radiation: Type of murmur: ☐ Systolic ☐ Diastolic Position best heard: __________ Radiation: __________

Participant Enrolment Number:					

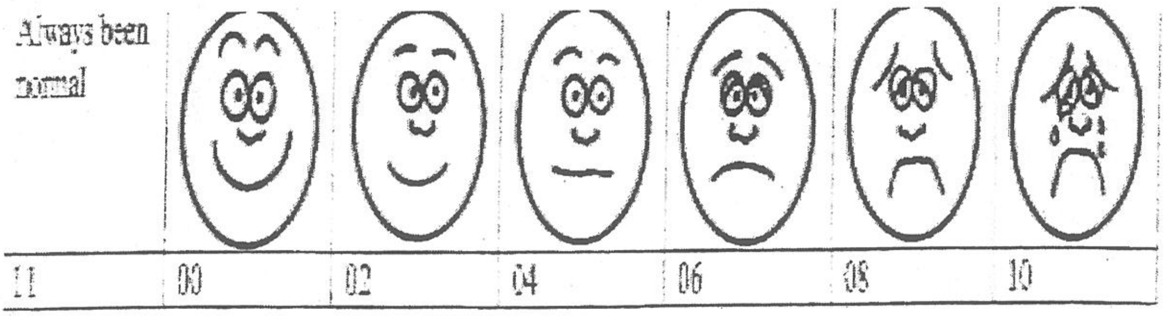
H. ABDOMINAL EXAMINATION

1.	Hepatomegaly	☐No ☐Yes, if yes indicate liver span __________cm
2.	Splenomegaly	☐No ☐Yes
3.	Any scars?	☐No ☐Yes, if yes, please describe: ______________________
4.	Any ascites present?	☐No ☐Yes, if yes, please indicate if this is: ☐Acute ☐Chronic
5.	Any other masses palpable?	☐No ☐Yes, if yes, please describe: ______________________

I. CENTRAL NERVOUS SYSTEM EXAMINATION

Peripheral neuropathy

1. Have you ever had pain, burning, pins and needles or numbness in your hands or feet? If yes, tell us how the pain is NOW, if the pain has gone away, score 00, if you've never had pain score 11.



Subjective severity scale:

☐R	☐L

2. Location of above symptoms

   Use score of:
   - 0= none
   - 1=feet or hands (only)
   - 2=extends to ankles (or wrists)
   - 3=extends above ankle (wrist) but not above knee (elbow)
   - 4=extends to knees (elbows)
   - 5=extends above knees (elbow)

☐R	☐L

3. Ankle reflexes

   Use score of:
   - 0= absent
   - 1= hypoactive
   - 2= normal deep tendon reflex
   - 3= hyperactive (significant spread)
   - 4= clonus
   - 8= unable/did not assess

☐ R	☐ L

Motor

4. Muscle bulk

   Use score of:
   - 0=normal muscle bulk
   - 1=diffuse atrophy
   - 2= symmetrical distal atrophy (just hands or feet)
   - 3= symmetrical proximal atrophy (thigh or arms)
   - 4= asymmetrical atrophy
   - 9= Unable to evaluate or did not assess

   ☐

5. Involuntary movement type (observe face, trunk limbs at rest)

   Use score of:
   - 0=normal
   - 1=myoclonus
   - 2= athetosis
   - 3= chorea
   - 4= hemiballismus
   - 5= complex

   ☐

6. Involuntary movement site

   Use score of:
   - 0=not present
   - 1=orofacial
   - 2=neck
   - 3=trunk
   - 4=upper limbs/hands
   - 5= lower limbs

   ☐

Participant Enrolment Number:					

7. Motor tone (resistance to motor movement while relaxed)

   Use score of:
   - 0=normal
   - 1=diffuse increase
   - 2=increase in legs only
   - 3=increased in one limb or one side
   - 4= decreased diffusely
   - 5= decreased in legs only
   - 6= decreased asymmetrical
   - 7= other
   - 9= unable to evaluate or did not assess

8. Tone quality

   Use score of:
   - 0=normal muscle
   - 1=clasp knife
   - 2= leadpipe
   - 3= cogwheel

9. Strength

   Use score of:
   - 0=normal
   - 1=weakness, able to offer resistance
   - 2=moderate weakness, maintains posture against gravity not resistance
   - 3=severe weakness, able to move with gravity eliminated
   - 4=muscle flicker only
   - 5= absences of movement
   - 9=unable to evaluate or did not assess

Gait

10. Gait coordination

    Use score of:
    - 0=normal gait
    - 1=mild impairment(evident only on rapid turns or tandem)
    - 2=moderate impairment(clear difficulty of unassisted gait)
    - 3=severe impairment(walking only with assistance)
    - 4= non-ambulatory
    - 5= weakness precludes assessment of gait coordination
    - 9= unable to evaluate or did not assess

11. Gait appearance

    Use score of:
    - 0=normal
    - 1=predominant abnormality wide- based or weakness of foot dorsiflexion
    - 2=predominant abnormality is bilateral spasticity
    - 3=predominant abnormality is hemiparetic gait
    - 4= unable to walk
    - 9= unable to determine or did not assess

Participant Enrolment Number: | | | | | |

Coordination

12. Limb coordination

TEST:
a) rapid opposition to first and second fingers
b) rapid wrist rotation
c) rapid foot tapping

Use score of:
- 0=normal
- 1=mild slowness or clumsiness(compared to examiner movement is slightly slower, fatigues or breaks down earlier)
- 2=moderate slowness or clumsiness
- 7= weakness precludes assessment
- 9= unable to evaluate or did not assess

Upper extremity ☐

Lower extremity ☐

Reflexes

13. Deep Tendon reflexes

Use score of:
- 0=normal
- 1=increased diffusely
- 2=increased in legs only
- 3=increased in one limb or on one side
- 4= decreased diffusely
- 5= decreased distally and symmetrically
- 6=decreased or absent ankle jerks only
- 7=decreased asymmetrically
- 9= unable to evaluate or did not assess

☐

14. Jaw Jerk

Use score of:
- 0=absent
- 1=present
- 2= unusually brisk(2-3+)
- 9= unable to evaluate or did not assess

☐

15. Grasp reflex

Use score of:
- 0=absent
- 1=unilateral
- 2=bilateral
- 9= unable to evaluate or did not assess

☐

Participant Enrolment Number: | | | | | |

16. Palmo-mental reflex

Use score of:
- 0=absent
- 1=present
- 2=prominent
- 9= unable to evaluate or did not assess

☐

17. Glabellar tap

Use score of:
- 0=absent
- 1=present
- 2=prominent
- 9= unable to evaluate or did not assess

☐

18. Plantar response (Babinski)

Use score of:
- 0=flexor(down going great toe)
- 1=extensor(up going great toe)
- 2=absent
- 9= unable to evaluate or did not assess

☐R ☐L

Other tests

19. Neck stiffness

Use score of:
- 0=normal
- 1=increased tone
- 9= unable to evaluate or did not assess

☐

20. Smooth pursuits [have the participant follow finger a minimum of 2 times horizontally from side to side and 2 times vertically from up to down]

Use score of:
- 0=normal
- 1=interrupted pursuits, frequent corrections
- 2= cannot perform pursuits or sustained nystagmus present
- 9=unable to evaluate or did not assess

☐

21. Facial Strength

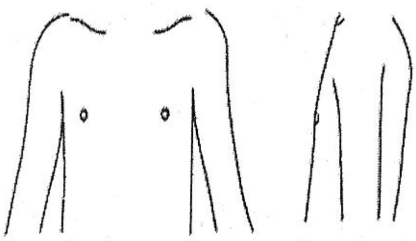
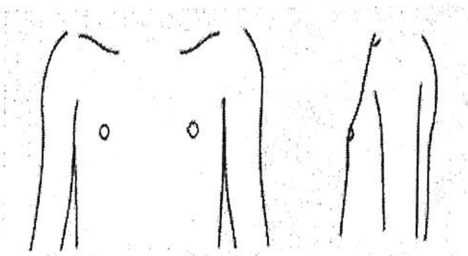
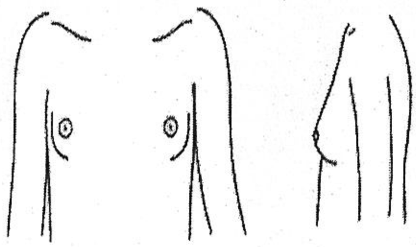
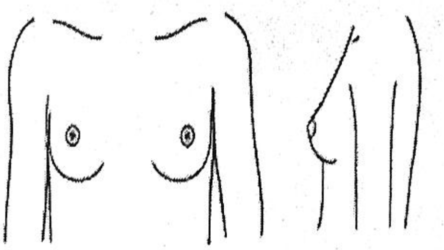
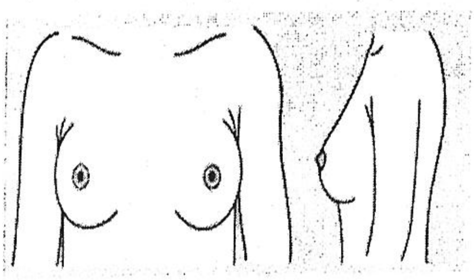
Use score of:
- 0=normal
- 1=mild asymmetry or equivocal weakness
- 2= definite paresis or palsy
- 9=unable to evaluate or did not assess

☐

Participant Enrolment Number: | | | | | |

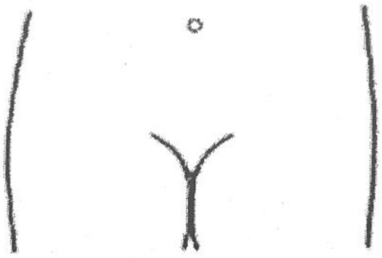
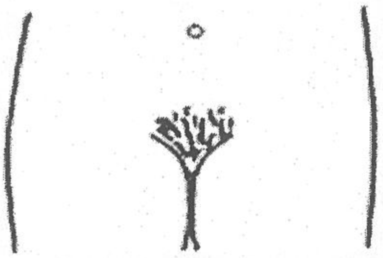
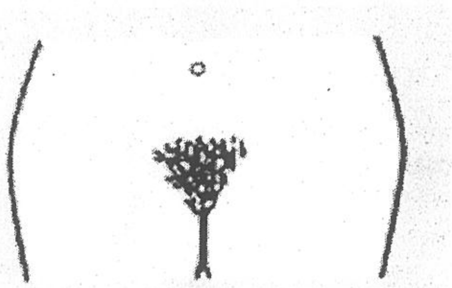
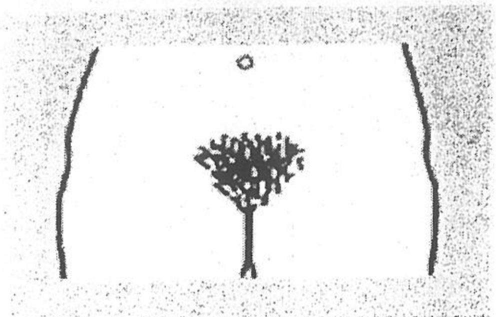
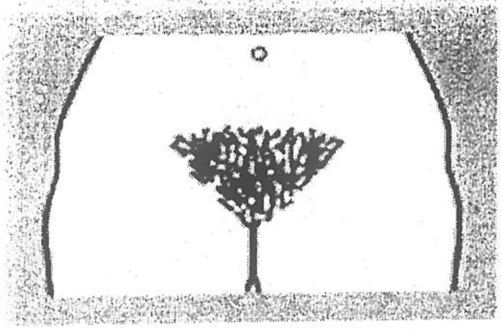
J. TANNER STAGES AND SEXUAL DEVELOPMENT

Please only complete this table if the participant is FEMALE.

BREAST DEVELOPMENT			
I		The nipple is raised a little. The rest of the breast is still flat	☐
II		This is the breast bud stage. In this stage, the nipple is raised more than in Stage 1. The breast is a small mound.	☐
III		The areola (the area around the nipple) is larger than in Stage 1. The breast and areola are both larger than in Stage 2. The areola does not stick out away from the breast	☐
IV		The areola and the nipple make up a mound that sticks up above the shape of the breast. NOTE: This stage may not happen at all for some girls. Some girls develop from Stage 3 to Stage 5, with no Stage 4.	☐
V		This is the mature adult stage. The breasts are fully developed. Only the nipple sticks out in this stage. The areola has moved back in the general shape of the breast.	☐

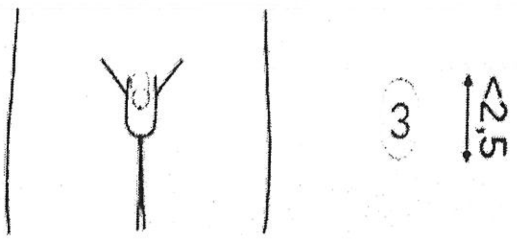
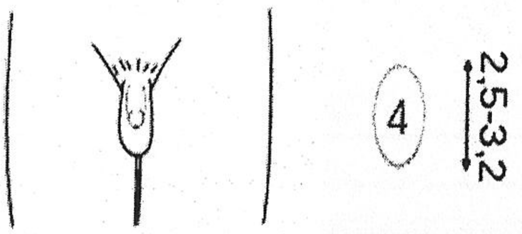
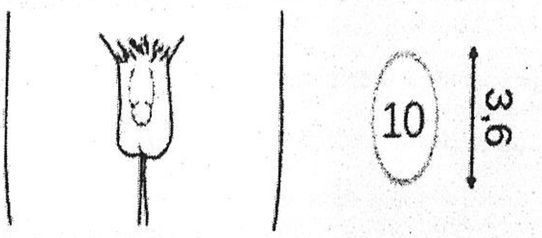
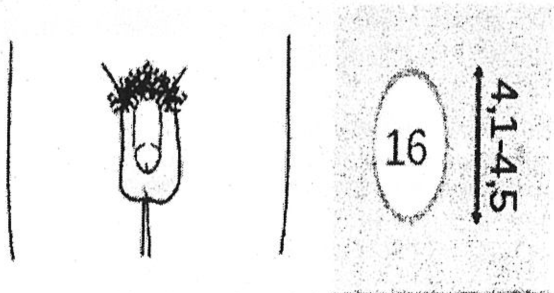
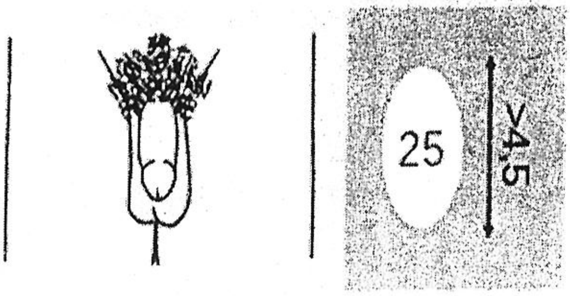
Participant Enrolment Number:

Please only complete this table if the participant is FEMALE.

PUBIC HAIR			
I		There is no pubic hair at all.	☐
II		There is a little soft, long, and lightly-coloured hair. This hair may be straight or a little curly.	☐
III		The hair is darker in this stage. It is coarse and more curled. It has spread out and thinly covers a bigger area.	☐
IV		The hair is now as dark, curly, and coarse, as that of an adult female. The area that the hair covers is not as big as that of an adult female. The hair has NOT spread out to the legs.	☐
V		The hair is now like that of an adult female.	☐

Participant Enrolment Number:					

Please only complete this table if the participant is MALE.

TESTICLE GROWTH			
I		This is the preadolescent stage. There is no pubic hair. The penis and testicles are about the same size as in childhood.	☐
II		The penis, the testicles and pubic hair start to grow. Sparse and fine pubic hair grows at the base of the penis. The colour of the skin around the testicles starts turning red.	☐
III		Pubic hair becomes darker, coarser and curlier. The penis continues to grow wider and longer. The testicles continue to grow larger.	☐
IV		The penis continues to grow getting wider and longer. Pubic hair becomes adult type, except there is less. The testicles continue to grow larger. The penis gland (head) is more developed.	☐
V		Penis is at its full adult size. Pubic hair is thick spreading to the thighs. The penis gland (head) is fully developed.	☐

Participant Enrolment Number:					

K. AUDIOMETRY

1. Was audiometry done?

☐ **Yes, please complete the table below.**	☐ **No**

2. If No, please give a reason: ____

	Frequency at 250 Hz	Frequency at 500 Hz	Frequency at 1000 Hz	Frequency at 2000Hz	Frequency at 4000Hz	Pass/ Fail
RIGHT EAR THRESHOLD	______ dB	______ dB	______ dB	______ dB	______ dB	Pass/ Fail
LEFT EAR THRESHOLD	______ dB	______ dB	______ dB	______ dB	______ dB	Pass/ Fail
Please note that if any participant has a threshold of more than 25 dB, the participant has failed the hearing screening and will need appropriate referral.						

Date CRF completed	DD / mm / YYYY
CRF completed with a translator	☐ Yes ☐ No
Name of translator	
Name of person completing CRF	
Signature	

Participant Enrolment Number:					

INTERNATIONAL HIV DEMENTIA SCALE (IHDS)

Memory-Registration:

Give four words to recall (dog/inja, hat/umnqwazi, bean/imbotyi, red/bomvu) – 1 second to say each. Then ask the patient all four words after you have said them. Repeat words if the patient does not recall them all immediately. Tell the patient you will ask for recall of the words again a bit later.

1. Motor Speed:

Have the patient tap the first two fingers of the non-dominant hand as widely and as quickly as possible.

Use score of:

- 4 = 15 in 5 seconds
- 3 = 11-14 in 5 seconds
- 2 = 7-10 in 5 seconds
- 1 = 3-6 in 5 seconds
- 0 = 0-2 in 5 seconds

Score: ☐

2. Psychomotor Speed:

Have the patient perform the following movements with the non-dominant hand as quickly as possible:
1) Clench hand in fist on flat surface.
2) Put hand flat on surface with palm down.
3) Put hand perpendicular to flat surface on the side of the 5th digit.
Demonstrate and have patient perform twice for practice.

Use score of:

- 4 = 4 sequences in 10 seconds
- 3 = 3 sequences in 10 seconds
- 2 = 2 sequences in 10 seconds
- 1 = 1 sequence in 10 seconds
- 0 = unable to perform

Score: ☐

Participant Enrolment Number:					

3. Memory-Recall:

Ask the patient to recall the four words. For words not recalled, prompt with a semantic clue as follows: animal (dog); piece of clothing (hat); vegetable (bean); colour (red).

Use score of:

- 1 point for each word spontaneously recalled
- 0.5 points for each correct answer after prompting
- Maximum: 4 points

Score: ☐

4.Self-report cognitive symptom questionnaire – ask the patient the questions below, including the examples. The patient should answer either "yes" or "no" to each question. Circle the participant's response.

Now I am going to ask you some questions. For each question, please answer "yes" or "no".

a)	Have you been more forgetful compared to other children in your class or your friends? For example: you can't remember things/stuff like your classmates can	Yes	No
b)	Are you more easily distracted compared to other children in your class or your friends? For example: you cannot pay attention for very long like your classmates can.	Yes	No
c)	Have you noticed that you think more slowly compared to other children in your class or your friends? For example: it takes you longer to find a solution to a problem than it normally would take your classmates.	Yes	No
d)	Have you noticed that your hands are clumsy, compared to your classmates/friends? For example: you drop things more.	Yes	No
e)	Do you move slower than your classmates/friends? For example: you can't get up fast, or it takes you longer to do thing/stuff.	Yes	No

Count the number of "yes" responses. ___ / 5

Use score of:

- y-IHDS-CSQ scoring: add items 1-3 then subtract the total for item 4. (e.g.: (1 + 2 + 3) – 4 = IHDS-CSQ total score) to give a total score out of a maximum of 12 points.
- Score of ⊴0 indicates possible dementia

TOTAL IHDS SCORE	/12	Pass/ Fail

Participant Enrolment Number:					

LAB RESULTS CRF

A. CD4 ABSOLUTE COUNTS AND PERCENTAGES

Visit number	Specimen number	Date done	CD4	Result
Visit 1 (Enrolment)			CD4 count	
			CD4 %	%
Visit 3 (12 months)			CD4 count	
			CD4 %	%
Visit 5 (24 months)			CD4 count	
			CD4 %	%
Visit 7 (36 months)			CD4 count	
			CD4 %	%

B. HIV VIRAL LOAD RESULTS

Visit number	Specimen number	Date done	Result
Visit 1 (Enrolment)			Copies/ml
Visit 3 (12 months)			Copies/ml
Visit 5 (24 months)			Copies/ml
Visit 7 (36 months)			Copies/ml

Participant Enrolment Number:					

C. FULL BLOOD COUNT RESULTS

FBC	Date	Result
WCC		X10 ^g/L
HB		g/dL
MCV		
MCH		
Platelet count		X10 ^g/L

D. LIVER FUNCTION TEST RESULTS

LFT	Date	Result
ALT	DD / MMM / YYYY	
ALP	DD / MMM / YYYY	
Total bilirubin	DD / MMM / YYYY	

Participant Enrolment Number:					

E. SPUTUM RESULTS

Sputum Lab number	Date	AFB smear	TB Culture requested	If yes, TB culture result.	Resistance pattern
		☐ Positive ☐ Negative	☐ Yes ☐ No	☐ Mycobacterium TB ☐ Non-Tuberculous Mycobacterium ☐ M Bovis ☐ Negative ☐ Other: ______	☐ Resistant to INH only ☐ Resistant to Rifampicin only ☐ Multi-drug resistance ☐ Extreme drug resistance ☐ Sensitive
		☐ Positive ☐ Negative	☐ Yes ☐ No	☐ Mycobacterium TB ☐ Non-Tuberculous Mycobacterium ☐ M Bovis ☐ Negative ☐ Other: ______	☐ Resistant to INH only ☐ Resistant to Rifampicin only ☐ Multi-drug resistance ☐ Extreme drug resistance ☐ Sensitive
		☐ Positive ☐ Negative	☐ Yes ☐ No	☐ Mycobacterium TB ☐ Non-Tuberculous Mycobacterium ☐ M Bovis ☐ Negative ☐ Other: ______	☐ Resistant to INH only ☐ Resistant to Rifampicin only ☐ Multi-drug resistance ☐ Extreme drug resistance ☐ Sensitive
		☐ Positive ☐ Negative	☐ Yes ☐ No	☐ Mycobacterium TB ☐ Non-Tuberculous Mycobacterium ☐ M Bovis ☐ Negative ☐ Other: ______	☐ Resistant to INH only ☐ Resistant to Rifampicin only ☐ Multi-drug resistance ☐ Extreme drug resistance ☐ Sensitive

Participant Enrolment Number:					

F. LIPOGRAM RESULTS

Visit number	Specimen number	Date done	Results	
Visit 1 (Enrolment)			☐ Random ☐ Fasting	Total cholesterol: ________ Triglycerides: ________ HDL: ________ LDL: ________
Visit 3 (12 months)			☐ Random ☐ Fasting	Total cholesterol: ________ Triglycerides: ________ HDL: ________ LDL: ________
Visit 5 (24 months)			☐ Random ☐ Fasting	Total cholesterol: ________ Triglycerides: ________ HDL: ________ LDL: ________
Visit 7 (36 months)			☐ Random ☐ Fasting	Total cholesterol: ________ Triglycerides: ________ HDL: ________ LDL: ________

G. hs-CRP RESULTS

Visit number	Specimen number	Date done	Results
Visit 1 (Enrolment)			
Visit 3 (12 months)			
Visit 5 (24 months)			
Visit 7 (36 months)			

Participant Enrolment Number:					

H. U&E and CMP

	Date	Result
Sodium		mmol/l
Potassium		mmol/l
Urea		mmol/l
Creatinine		µmol/l
Calcium		mmol/l
Albumin		g/l
Calcium (Corrected)		mmol/l
Magnesium		mmol/l
Inorganic Phosphate		mmol/l

Date CRF completed	
Name of person completing CRF	
Signature	

Participant Enrolment Number:	☐☐☐☐☐

OTHER RESULTS CRF

A. URINE DIPSTIX

Was a Urine Dipstix done?	☐ No, if no, go to Section B. ☐ Yes
Date Urine Dipstix done	DD / mmm / YYYY
Urobilinogen	☐ Normal (enter this for results reported as 1 mg/dL or less) ☐ 2 mg/dL ☐ 4 mg/dL ☐ 8 mg/dL ☐ 12 mg/dL
Glucose	☐ Normal (negative) ☐ + (250 mg/dL) ☐ ++ (500 mg/dL) ☐ ≥ 1000 mg/dL (select this for results reported as +++ or ++++) ☐ Trace (50 - 150 mg/dL)
Bilirubin	__.__ mg/dL (mg of bilirubin/decilitre) ☐ negative
Ketones	☐ Negative ☐ + (10 - 25 mg/dL) ☐ ++ (40 - 50 mg/dL) ☐ > 100 mg/dL (select this for results reported as +++) ☐ Trace (5 mg/dL)
Specific Gravity	_.___
Blood	___ ery/uL (erythrocytes/microliter) ☐ negative If blood result is non-negative: Heolysis? ☐ negative ☐ positive
pH	_._
Protein	☐ Negative ☐ + (30 mg/dL) ☐ ++ (100 mg/dL) ☐ ≥ 300 mg/dL (select this for results reported as +++ or ++++) ☐ Trace
Nitrites	☐ Negative ☐ Positive (select this for results reported as "trace" or "positive")
Leukocytes	☐ Negative ☐ + (25 WBC/ul) ☐ ++ (75 WBC/ul) ☐ +++ (500 WBC/ul) ☐ Trace

Participant Enrolment Number:					

B. TST RESULT

Was a TST done?	☐ No, if no, go to end of CRF. ☐ Yes
Date TST done	DD / MMM / Y Y Y Y
Date TST read	DD / MMM / Y Y Y Y
Clinic where TST was read	
Read by whom	
Diameter	mm
Result	☐ Positive ☐ Negative ☐ Not read ☐ Non-reactive

Date CRF completed	DD / MMM / Y Y Y Y
Name of person completing CRF	
Signature	

CTAAC Participant Enrolment Number:					

LUNG FUNCTION CRF

Study Details		
1	Time arrived *(24 hour clock)*	HH : MM
2	Time finished (*24 hour clock)*	HH : MM
3	Date of Birth	DD/MM/YYYY
4	Height	cm
5	Weight	kg
6	Room temperature	^{0}C
7	Pressure	hPa
8	Humidity	%
8	Position	☐ Sitting ☐ Standing
9	Additional information:	

Equipment Calibration – EasyOnePro			
Date	Time	Within 3 %	File Reference
DD/MM/YYYY	HH : MM	☐ yes ☐ no	See Cal Log

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

See attached results for values				
Quality				Comment
Effort	15	☐ Maximal ☐ Submaximal	16	
Acceptable* **meets SOP criteria: start and end of test, free from artefact*	17	☐ Yes ☐ No	18	
Repeatable *<150ml difference best two FVC and FEV1*	19	☐ Yes ☐ No	20	

CTAAC Participant Enrolment Number:					

Bronchodilator			
	Time given *(24 hour clock)*	HH:MM	
	Dose of bronchodilator	mcg	

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

See attached results for values

Quality				Comment
Effort		☐ Maximal ☐ Submaximal		
Acceptable* **meets SOP criteria: start and end of test, free from artefact*		☐ Yes ☐ No		
Repeatable *<150ml difference best two FVC and FEV1*		☐ Yes ☐ No		

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

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

CTAAC Participant Enrolment Number:					

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

Participant Enrolment Number:					

ECHOCARDIOGRAM CRF

BASIC INFORMATION		
1.	Date of Birth	DD / MMM / YYYY
2.	Weight(Kg)	
3.	Height(cm)	
4.	BSA (m²)	
5.	Blood Pressure (mmHg)	/

ECHOCARDIOGRAM

LV dimensions

IVSd			.			mm
LVIDd			.			mm
LVPWd			.			mm
IVSs			.			mm
LVIDs			.			mm
LVPWs			.			mm
AO			.			mm
LA			.			mm
LA:AO			.			ratio
LV mass						g
LV mass indexed						g/m^2
LVOT diameter			.			mm
LVOT VTI			.			cm

LV function

LVEF			.		%	2D assessment
LVEF			.		%	Simpsons
LVSF			.		%	M-Mode

RV function

TAPSE	.		cm	RVEF (tapse)			.		%
FAC			%	RVEF (volume)			.		%

Participant Enrolment Number: | | | | | |

Valves

	Yes	No	If yes, state severity and pathology
MR	☐	☐	
MS	☐	☐	
AS	☐	☐	
AR	☐	☐	
PS	☐	☐	
PR	☐	☐	
TR	☐	☐	
TS	☐	☐	

Pericardium

	Yes	No	If yes, state severity and pathology
Pericardial effusion	☐	☐	

RV

TR max PG:			.			mmHg	
IVC							
Est. JVP from IVC			.			mmHg	
Est. PAP:			.			mmHg	
RVOT VTI			.			cm	
PVR			.			ratio	
PVR			.			Wood units	
PV AT						ms	acceleration time
Tei Index			.				

Tissue Doppler and diastolic function LV :

Mitral valve inflow E/A ratio:			.			
E-wave peak velocity:			.			m/s
E-wave deceleration time:						ms
Medial E':			.			cm/s
Lateral E':			.			cm/s
E/E' medial:			.			
E/E' lateral:			.			
Medial MAPSE Trace:			.			cm
Lateral MAPSE Trace:			.			cm

Tissue Doppler and diastolic function RV:

Tricuspid valve inflow E/A ratio:			.			
E-wave peak velocity:			.			m/s

Participant Enrolment Number: | | | | | |

E-wave deceleration time:						ms
Medial E':			.			
Lateral E':			.			
E/E' medial:			.			
E/E' lateral:			.			
TAPSE trace:			.			cm

Right heart and atrial dimensions:

Parasternal long-axis:

RVIDd (mid)			.			mm
RVIDs (mid):			.			mm
RVOTd:			.			mm
RVOTs:			.			mm
RV wall thickness			.			mm

Apical 4-chamber:

RVIDd (base):			.			mm
RVIDs (base):			.			mm
TV annulus:			.			mm
RV length (diastole):			.			mm
RV length (systole):			.			mm
RV area (diastole):			.			cm^2
RV area (systole):			.			cm^2
RVEDV:			.			ml
RVESV			.			ml

RA dimensions

RA length			.			mm
RA end-systolic area			.			cm^2

LA dimensions

LA length			.			mm
MV annulus:			.			mm
LA end-systolic area			.			cm^2

Participant Enrolment Number:					

Carotid measurements

Left common carotid IMT	.			mm
Left carotid bulb IMT	.			mm
Left internal carotid IMT	.			mm

Right common carotid IMT	.			mm
Right carotid bulb IMT	.			mm
Right internal carotid IMT	.			mm

Formulas

BSA	((Ht (cm) x Wt (kg)) / 3600)$^{1}/_{2}$
RVEF (volume)	RVEDV – RVESV / RVEDV x 100
RVEDV RVESV	0.85 (A^2/L) 0.85 (A^2/L)
FAC	(RVAd – RVAs) / RVAd x 100
PVR ratio	TR/RVOT VTI
PVR (Woods units)	(TRVmax/RVOT TVI) 10 + 0.16.

ECHOCARDIOGRAM REPORT SUMMARY:

Normal Structure: ☐ Abnormal Structure: ☐

Normal function: ☐ Abnormal function: ☐

Referral required: ☐ Details: ______________________________

Date CRF completed	
Name of person completing CRF	
Signature	

Participant Enrolment Number:					

INTERCURRENT EVENTS CRF

A. CLINIC VISITS

1. Which clinic do you attend for routine ARV appointments?

Name of clinic: ____________________ Date of last visit: DD / MMM / YYYY

2. Do you usually attend the clinic alone?

☐ Yes ☐ No

3. Have you been to a clinic for reasons other than your routine ARV appointments since your last study visit?

☐ Yes ☐ No, if No go to section B.

4. What was the reason(s) for attending the clinic?

____

5. What clinic(s) did you attend?

Name of clinic:	Date of visit:
1.	
2.	
3.	

6. Did you receive treatment other than your usual medication?

☐ Yes ☐ No, if No go to section B.

7. What treatment did you receive (any available information e.g. type of medication, colour, formula etc.)?

1.	Name of treatment:	
	Date treatment started	DD / MMM / YYYY
	Date treatment completed	DD / MMM / YYYY or: ☐ ongoing
2.	Name of treatment:	
	Date treatment started	DD / MMM / YYYY
	Date treatment completed	DD / MMM / YYYY or: ☐ ongoing
3.	Name of treatment:	
	Date treatment started	DD / MMM / YYYY
	Date treatment completed	DD / MMM / YYYY or: ☐ ongoing

Participant Enrolment Number:					

B. CURRENT ARV's

1. Did you refill your ARVs at your last clinic visit? ☐ Yes ☐ No

2. Are you still taking the same ARV's that you were taking at the last study visit?

   ☐ Yes, Go to section C. ☐ No

3. What ARV's are you currently taking?

1.
2.
3.
☐ Not sure.

4. Why were your ARV's changed?

   ____

C. CURRENT MEDICATION

1. Are you currently taking any other treatment than your usual ARV's and treatment you've already told us about?

   ☐ Yes ☐ No, if No go to section D.

2. What treatment are you currently taking (any available information e.g. type of medication, colour, formula etc.)?

1.	Name of treatment:	
	Date treatment started	DD / MMM / YYYY
	Date treatment completed	DD / MMM / YYYY or: ☐ ongoing
2.	Name of treatment:	
	Date treatment started	DD / MMM / YYYY
	Date treatment completed	DD / MMM / YYYY or: ☐ ongoing
3.	Name of treatment:	
	Date treatment started	DD / MMM / YYYY
	Date treatment completed	DD / MMM / YYYY or: ☐ ongoing

Participant Enrolment Number:					

D. HOSPITAL ADMISSIONS

1. Have you been admitted to hospital since your last study visit?

☐ Yes	☐ No, if No go section E.

2.

1.	Name of hospital:	
	Date of admission:	DD / MMM / YYYY
	Duration of admission:	Days/weeks
	Where in the hospital did you stay while admitted?	☐ Ward ☐ ICU ☐ Both
	What type of unit were you admitted to?	☐Paediatric ☐Adolescent ☐ Adult
	What was your diagnosis (e.g. Pneumonia, URTI, UTI, AGE, Meningitis, TB etc.)?	
2.	Name of hospital:	
	Date of admission:	DD / MMM / YYYY
	Duration of admission:	Days/weeks
	Where in the hospital did you stay while admitted?	☐ Ward ☐ ICU ☐ Both
	What type of unit were you admitted to?	
	What was your diagnosis (e.g. Pneumonia, URTI, UTI, AGE, Meningitis, TB etc.)?	☐ Yes ☐ No
3.	Name of hospital:	
	Date of admission:	DD / MMM / YYYY
	Duration of admission:	Days/weeks
	Where in the hospital did you stay while admitted?	☐ Ward ☐ ICU ☐ Both
	What type of unit were you admitted to?	
	What was your diagnosis (e.g. Pneumonia, URTI, UTI, AGE, Meningitis, TB etc.)?	☐ Yes ☐ No

E. SURGERY

1. Have you had any operations since your last study visit?

☐ Yes	☐ No, if No go section F.

2. What type of operation did you have? ______________________
3. Name of hospital where operation was done: ___________________
4. **Date of Operation:** DD/MM/YYYY

F. TB CONTACTS AND TREATMENT

Current TB contacts

1. **Family members or household contact:** Since your last study visit, have any of your **family members** (parents, grandparents, or siblings) or household members been diagnosed with TB?

☐ Yes, if yes please complete table below.	☐ No

Specify name(s), relation to participant; date diagnosed and place where diagnosis was made:

Name	Relation to participant	Date diagnosed	Place where diagnosed
1.		DD/ MMM/YYYY	
2.		DD/ MMM/YYYY	
3.		DD/ MMM/YYYY	
4.		DD/ MMM/YYYY	

2. Participant TB prophylaxis and treatment

a) TB prophylaxis

Have you been on TB treatment for prevention since the last visit?	☐Yes ☐No, go to c).

b)

Date prophylaxis started	DD / MMM / YYYY
Date prophylaxis completed	DD / MMM / YYYY or: ☐ ongoing
Where was treatment initiated?	Name of clinic/hospital: _______________

c) TB treatment

Have you been on TB treatment since the last visit?	☐Yes ☐No, go to section G.

d)

Date treatment started	DD / MMM / YYYY
Date treatment completed	DD / MMM / YYYY or: ☐ ongoing
Where was treatment initiated?	Name of clinic/hospital: _______________

Participant Enrolment Number:					

G. PREGNANCY

1. Is the participant female?

☐ Yes	☐ No (Go to the end)

2. What was the approximate date of your last menstrual period?
   Date: MM/DD/YYYY

3. Since the last clinic visit 6 months ago have you been pregnant?

☐ Yes	☐ No (Go to the end)

4. If yes, what is the outcome of the pregnancy?
   ☐ Currently pregnant
   ☐ Live birth
   ☐ Elective Termination (go to the end CRF)
   ☐ Spontaneous miscarriage (go to the end CRF)

5. Actual/expected date of delivery? MM/DD/YYYY

6. Where did/will the birth will occur?

   ____

7. Were there (if no longer pregnant) or are there any pregnancy complications?
   ☐ Diabetes

   ☐ High blood pressure
   ☐ Anaemia
   ☐ Hyperemesis (excessive vomiting)
   ☐ Eclampsia
   ☐ Pre-Eclampsia
   ☐ Pelvic inflammatory disease
   ☐ Asthma
   ☐ HIV (testing positive for the first time)
   ☐ Other (specify): ______________________________________

Date CRF completed	DD / MMM / YYYY
CRF completed by	☐ Participant only ☐ Caregiver only ☐ Participant and Caregiver together
Name of study staff completing CRF	
Signature	

Participant Enrolment Number:					

LAB RESULTS-GLUCOSE AND INSULIN CRF

A. Date of Visit 1 DD / MMM / YYYY

B. Date of lab testing DD / MMM / YYYY

C. Glucose and Insulin

Visit number	Specimen number	Glucose and Insulin	Result
Visit 1 (Baseline visit)		Glucose	mmol/L
		Insulin	mIU/L

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