Optimization of Pediatric Antiretroviral Therapy in sub-Saharan Africa: Timing of Initiation in HIV/TB Co-infected Children and Using Gains in Weight, Height, or CD4 Count to Monitor the Response

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology in the School of Public Health.

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ABSTRACT

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Optimization of pediatric antiretroviral therapy in sub-Saharan Africa: Timing of initiation in HIV/TB co-infected children and using weight, height, and CD4 gain to monitor the response

(Under the direction of Frieda Behets, PhD, MPH)

Backgroung: Antiretroviral therapy (ART) has revolutionized the treatment of HIV, but substantial drug interactions between anti-TB and ART and the lack of health care infrastructures complicate the management of HIV/TB co-infected children in resource poor countries. More than 50% of TB infected children in some high burden countries in sub-Saharan Africa are also infected with HIV, but the optimal timing of ART in those children is unknown. In addition, though the drug-interactions and the high pill burden to treat HIV and TB require strict monitoring, regular measurements of viral load that is routine for ART monitoring in developed countries is not always possible in sub-Saharan Africa. This study had two aims. 1) Construct reference charts for gains in weight, height, absolute CD4 count, and CD4% in the first 6 months of ART, and to test the value of the 3rd, 10th, 25th, 33rd, and 50th percentiles as predictors of subsequent death, virological suppression, or treatment failure. 2) Determine the effect of delaying ART for at least 15, 30, or 60 days in HIV/TB co-infected children on virological suppression and survival.

Methods: We used information from an observational clinical cohort of HIV-infected children who sought care at an outpatient clinic at Chris Hani Baragwanath Hospital in Soweto, South Africa. To construct the reference charts, we assumed a Box Cox

power exponential distribution for the 6 month gains in weight, height, CD4 count and CD4% and used the generalized additive model for location, scale, and shape to estimate the parameters of each of the four distributions. Hazard ratios for the association of the selected centiles with the three outcomes were estimated using Cox proportional hazard model. For aim 2, though per guidelines all HIV-infected children with TB were eligible for ART, the decision whether to initiate or delay ART for a given child was made at each visit and sicker children were initiated earlier. Moreover, some children for whom ART was delayed could die before it was possible to classify them for exposure. To control for the time-dependent confounding by indication and the lead time on survival, mortality hazard ratios were estimated using the inverse-probability-of-treatment-weighting of marginal structural modelling. Adjusted hazard ratios for virological suppression were estimated using multivariate Cox proportional modelling.

Results: Overall, information from 1394 and from 573 children was used for aim 1 and aim 2 respectively.

Children whose weight, absolute CD4, or CD4% gain were below the 33rd percentile for age or gender had poorer ART outcomes with a three to four-fold higher hazard of death, about 0.75 -fold lower hazard of virological suppression and about two-fold higher hazard of treatment failure.

Delaying ART tended to be associated with increased mortality: adjusted hazard ratios (aHR) for 15, 30, and 60 days delay were: 0.90 (95%CI: 0.30, 2.75), 1.05 (95%CI: 0.29, 3.75), 2.18 (95%CI: 0.64, 7.48) respectively. Delaying ART appear to be potentially detrimental for the hazard of viral suppression: aHR: 0.98 (95%CI: 0.76, 1.26), 0.95,

(95%CI: 0.73, 1.23), 0.84 (95%CI: 0.61, 1.15) for 15, 30, and 60 days delay respectively.

Conclusion: Six-month weight gain and CD4 cell gain (count and CD4%) below the 33rd percentile were equally strong predictors of poor ART outcomes suggesting that, pending construction of more generalizable charts, weight gain can be used in children on ART to discriminate those who are failing the treatment from those who are responding. Since delaying ART beyond 30 days appears to negatively affect both survival and viral response, the recommendation should be reevaluated and necessary delays should not exceed 30 days.

DEDICATION

To the memory of my parents Georgette Kapche and Engelbert Lawa

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For this dissertation, I used information from children receiving care at the Harriet Shezi Children's Clinic in Soweto, South Africa. In addition to the South African government, the clinic has received funding from the United States President's Emergency Plan for AIDS Relief (PEPFAR), the United Nations Children's Fund (UNICEF), the Elizabeth Glazer Pediatric AIDS Foundation (EGPAF), Rockefeller Brother's Fund (RBF), the South African business sector, and a host of individuals, schools and churches. The clinic is run by some of the most dedicated people I have ever worked with. I am extremely grateful to Dr Tammy Meyers for giving me the opportunity to meet and collaborate with her dedicated team, to Mpumi Majozi and her team of data capturers and their many Saturdays

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LIST OF ABBREVIATIONS AND SYMBOLS

HIV	Human Immuno-deficiency virus
AIDS	Acquired immune deficiency syndrome
TB	Tuberculosis
ART	Antiretroviral Therapy
HAART	Highly active antiretroviral therapy
LPV/r	Lopinavir boosted ritonavir
SA	South Africa
WHO	World Health Organization
PI	Protease Inhibitor
NRTI	Nuclease reverse transcriptase inhibitor
NNRTI	Non-nuclease reverse transcriptase inhibitor
GAMLSS	Generalized additive model for location, scale and shape
BCPE	Box-Cox power exponential
WAZ	Weight-for-age Z score
HAZ	Height-for-age Z score
IPTW	Inverse-probability-of-treatment weighting

I. INTRODUCTION

Worldwide, it is estimated that about **1500 new pediatric infections with the Human Immunodeficiency Virus (HIV) occur daily and more than 90% of those new infections are happening in sub-Saharan Africa** [1, 2]. Children acquire HIV mainly through mother to child transmission [3]. In the absence of any intervention, between 15% and 45% of infants born to mothers living with HIV will become infected (5–10% during pregnancy, 10–20% during labor and delivery and 5–20% through breastfeeding) [3]. Children who are infected earlier in life progress more rapidly to acquired immuno-deficiency syndrome (AIDS) and death. In the absence of antiretroviral therapy (ART), more than a third of HIV-infected infants will not see their first birthday, and less than half will survive the first 2 years of life [4]. In high burden countries of Southern Africa, HIV is today the leading cause of death among children. In South Africa for example, more than 5% of children 5 years or younger are HIV infected [5] and HIV accounts for more than half of all deaths among children under the age of 5 years [6].

The spread of HIV worldwide has coincided with the re-emergence of *Mycobacterium tuberculosis* (TB) infections[7]. Despite the fact that TB is an important cause of childhood morbidity and mortality worldwide, TB in children has been less of a public health priority and data on trends in childhood TB are scarce in the published literature and marred with methodological limitations. In sub-Saharan Africa, more than anywhere else, HIV/TB co-infection has devastating consequences on chill

health. **In some cross sectional studies of TB infected children in the region, HIV prevalence above 50% has been reported**, compared to less than 5% in industrializedsettings [8-10]. The pathogeneses of both HIV and TB explain why HIV infection is such a fertile ground for TB.

II. REVIEW OF THE LITERATURE

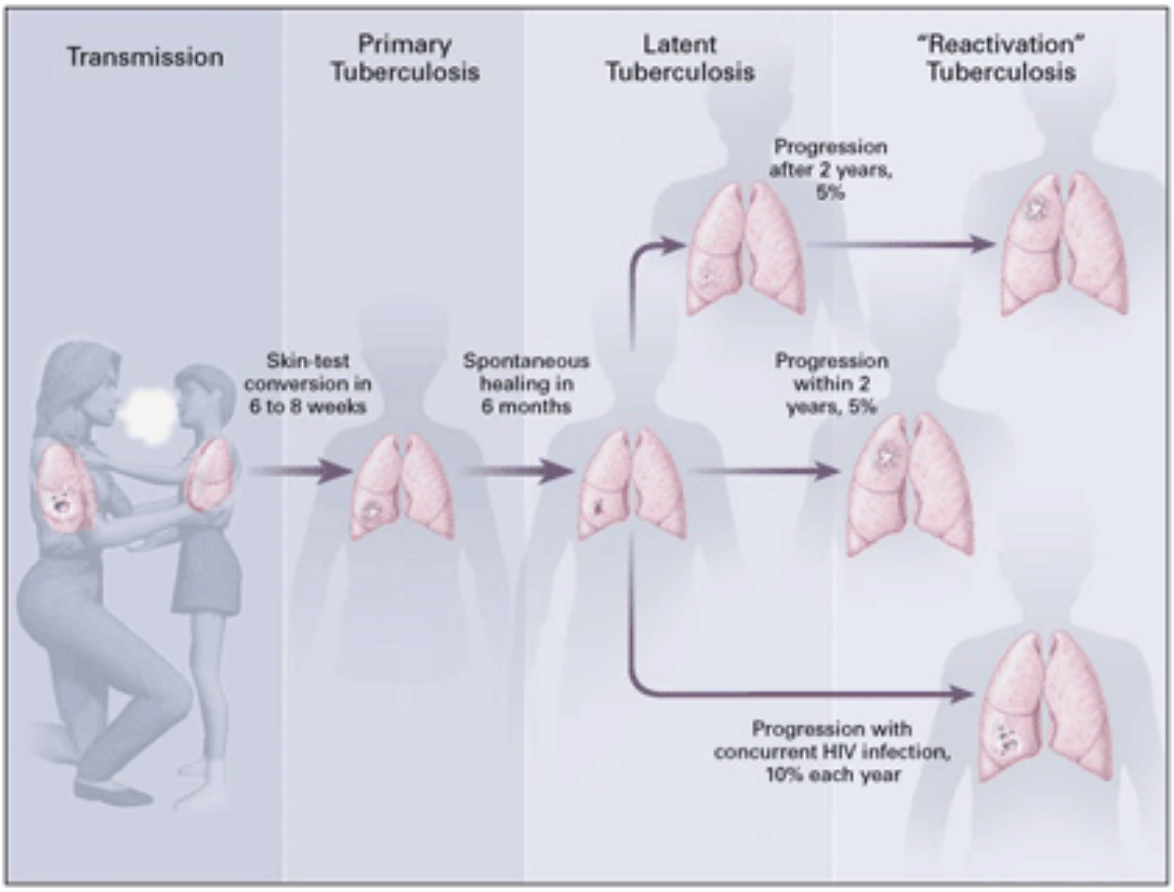
A. Pathogenesis of HIV and TB co-infection

1. Pathogenesis of tuberculosis

Tuberculosis is the world's second commonest cause of death from infectious disease, after HIV/AIDS. There were an estimated 8–9 million new cases of tuberculosis in 2000 [7]. TB is an airborne disease which is spread by droplet nuclei: particles of 1–5 µm in diameter that contain *Mycobacterium tuberculosis*. After expectoration by people with pulmonary or laryngeal tuberculosis during coughing, sneezing, singing, or talking, the particles can remain airborne for minutes to hours [11]. Once the infectious droplet nuclei are inhaled, *M tuberculosis* is then taken up by alveolar macrophages, initiating a cascade of events that result in either a successful containment of the infection or progression to active disease (primary progressive tuberculosis) (figure I.1). The T lymphocytes (particularly the CD4 positive T cells) are at the centre of this cascade. In fact, after being ingested by alveolar macrophages, *M tuberculosis* replicates slowly but continuously and spreads via the lymphatic system to the hilar lymph nodes. The infected macrophage releases interleukins 12 and 18, which stimulate T lymphocytes (predominantly CD4 positive T lymphocytes) to release interferon γ. In turn, interferon γ stimulates the phagocytosis of *M tuberculosis* in the macrophage. Interferon γ also stimulates the macrophage to release tumor necrosis factor α, an important factor in the granuloma formation. Activated T lymphocytes and macrophages form granulomas that

limit further replication and spread of the organism [11]. In most people (90%), unless there is a subsequent defect in cell-mediated immunity, the infection generally remains contained and active disease may never occur (fugure 1) [12]. However, when the host immune response cannot contain the replication of *M tuberculosis* associated with initial infection, either because of its immaturity as in young children or because of acquired immune deficiency as with HIV infection active disease occurs.

Figure II-1. Transmission of tuberculosis and progression from latent infection to reactivated disease



Source: **[12]**

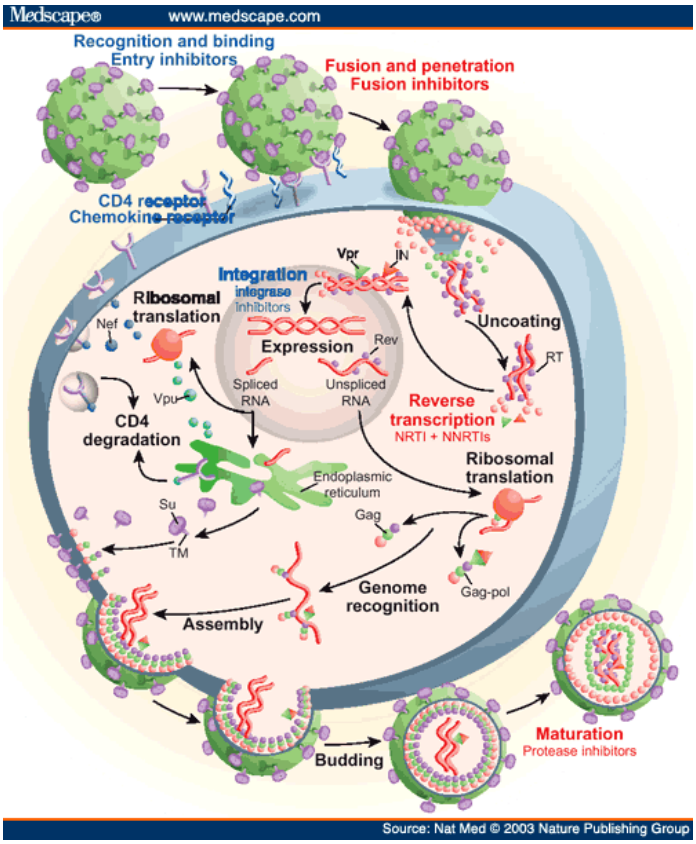
2. Pathogenesis of HIV

In children as in adults, **the critical basis for the immuno-pathogenesis of HIV infection is the depletion of the helper/inducer subset of T lymphocytes which express the CD4 phenotypic marker (the CD4 cell)**. The CD4 lymphocyte is the focal and critical cell involved directly or indirectly in the induction of most immunologic functions [13]. HIV has a selective tropism for the CD4 cell. A convincing body of evidence has suggested that the CD4 molecule is, in fact, the high-affinity receptor for the virus [14]. After HIV binds to the CD4 molecule, the virus is internalized and uncoated. Once internalized, the genomic RNA is transcribed to DNA by the enzyme reverse transcriptase. The proviral DNA, which can exist in a linear or circularized form, is integrated into the host chromosomal DNA. After integration of provirus, the infection may assume a latent phase with restriction of the cycle until the infected cell is activated. Once cell activation occurs, the proviral DNA transcribes viral genomic RNA and messenger RNA. Protein synthesis, processing, and virus assembly occur with budding of the mature virion from the cell surface (figure I-2). When active replication of virus occurs, the host cell is usually killed. This results in progressive depletion of CD4 cells with an expanded CD8 cell population, resulting in an inverted CD4/CD8 ratio. **Removal of CD4 cells from the immune system renders it progressively weaker and unable to contain secondary infections and results in profound immune suppression** [13, 14].

HIV infection in both children and adults causes a broad spectrum of diseases. In a meta-analysis of 17 studies in Europe and the United States of America, the most common AIDS events diagnosed were opportunistic infections (38%), followed by

serious recurrent bacterial infections (29%) and HIV encephalopathy (20%). The relative frequencies of these conditions were strongly age-related; *Pneumocystis jiroveci* and encephalopathy became proportionately less common, and serious recurrent bacterial infections proportionately more common, with increasing age. *Pneumocystis jiroveci* pneumonia (PCP) is a very common serious opportunistic infection in children with HIV-1 infection and is associated with a high mortality [15]. The pneumonia most often manifests between 3 to 6 months of age in infants with vertically-acquired infection [16]. Primary and reactivated tuberculosis is a major cause of morbidity and mortality among HIV-1-infected children residing in communities where infection with the pathogen is endemic [15].

Figure II-2. **The life cycle of HIV**



Link: http://www.medscape.com/content/2003/00/45/86/458640/art-nm458640.fig1.gif

B. HIV-TB co-infection in children

In general because of their immature immune system, children are highly susceptible to TB infection [17]. Following TB infection, children have a higher risk of progression to disease (extra-pulmonary and pulmonary) and death. Young infants have a particularly high morbidity and mortality from tuberculosis [17]. The effect of the HIV epidemic on the burden of tuberculosis in children has not been studied as well as in adults [7]. However, HIV is a major risk factor for childhood TB [18]. **Children may contract both HIV and TB from a dually infected mother**. Reported prevalence of HIV/TB co-infection in children range from less than 5% in industrialized settings to over 50% in some high-burden countries of southern Africa [8-10]. In HIV/TB co-infected children, the poor cell-mediated immunity increases the risk for disseminated tuberculosis disease, especially in advanced stages of HIV infection, resulting in higher mortality compared to HIV-negative children infected with tuberculosis [19]. **The risk of active tuberculosis in HIV co-infected children is related to CD4 count and indirectly to viral load [20] and restoration of cellular immunity with antiretroviral therapy reduces the susceptibility to tuberculosis** [21].

C. Antiretroviral Therapy in children

1. Treatment objectives

HIV causes a chronic infection which requires treatment for life once a child starts therapy. Current antiretroviral therapies do not eradicate HIV infection due to the long half-life of latently infected CD4 cells [22]. **Antiretroviral therapy for HIV-infected children aims at reducing HIV-related mortality and morbidity, restoring and**

preserving the immune function, maximally and durably suppressing viral replication, minimizing drug-related toxicity, maintaining normal physical growth and neurocognitive development, and improving the quality of life [22]. Today, six classes of antiretroviral drugs are available for HIV-1 therapy [23]. Two classes target the reverse transcriptase: the nucleoside reverse transcriptase inhibitors (NRTIs) and the non-NRTIs (NNRTIs). A third class – the protease inhibitors (PIs) target the viral protease. The newer classes: the fusion inhibitors, the entry inhibitors, and the HIV integrase strand transfer inhibitors are yet to be fully investigated in children [24]. Early results show enfuvirtide (fusion inhibitor) to be safe for long term use in children [25-29]. Currently, **the treatment of choice for HIV-infected children is a combination of at least 3 drugs**, which include at least 2 different classes of antiretroviral drugs [22, 24].

2. ART treatment in Children co-infected with TB

Because of the risk of rapid progression and dissemination of TB, it is recommended that in children co-infected with HIV and TB, TB treatment be initiated as soon as the diagnosis of TB is suspected [24]. The duration of TB treatment may vary from 6 to 12 months. Whatever the length of the treatment, rifampicin is recommended to be given for the entire duration [24]. **Rifampicin reduces the serum concentrations of most PIs by 80% or more, and NNRTIs by between 20% and 60%** [24]. Furthermore, the adverse events of anti-tuberculosis drugs and antiretroviral drugs are similar. For those reasons, it is recommended that initiation of ART in HIV-infected children with tuberculosis be delayed for at least 2-8 weeks after TB treatment initiation [24, 30]. The reason for the 2-8 weeks delay is to improve adherence and better differentiate potential side effects [30]. But the optimal timing of ART initiation in children co-infected with

HIV/TB is not known. The elevated incidence of TB and other secondary infections in HIV-infected children in sub-Saharan Africa coupled with the high pill burden (and the inherent challenge for adherence) and drug interactions underscore the need for strong monitoring of ART.

3. Monitoring of ART treatment

The prime target of antiretroviral drugs is the blocking of viral replication at multiple points in the viral life cycle (figure II-2). A quantitative and direct measure of the effectiveness of ART is the amount of viral particles that can be found in the various compartments of the body. The most commonly used of such measures is the plasma viral load. In clinical trials as well as in clinical practice, following initiation of ART, treatment effectiveness is measured as a substantial and sustained decrease in plasma HIV RNA concentration [21, 31-35]. Effectively blocking viral replication stops the depletion of CD4 cells and results in progressive increase in the absolute count of those cells as well as the re-equilibration of the CD4/CD8 ratio [31-39]. **The monitoring of pediatric ART in developed nations typically consists of the evaluation of CD4 count and viral load every 3-4 months** [22]. However, the measurement of viral load and/or CD4 cells count requires expensive and sophisticated technologies that cannot always be easily transferred to or sustained in all resource-limited settings.

HIV-infection has a severe negative effect on both height and weight gain in children. In the postnatal period, growth of HIV-infected children rapidly lags behind that of HIV-uninfected children. Decrements in both linear and ponderal growth are detectable by the age of 3 or 4 months [40]. This slow growth is sustained throughout childhood. In a prospective study of 282 term infants born to HIV-infected women in

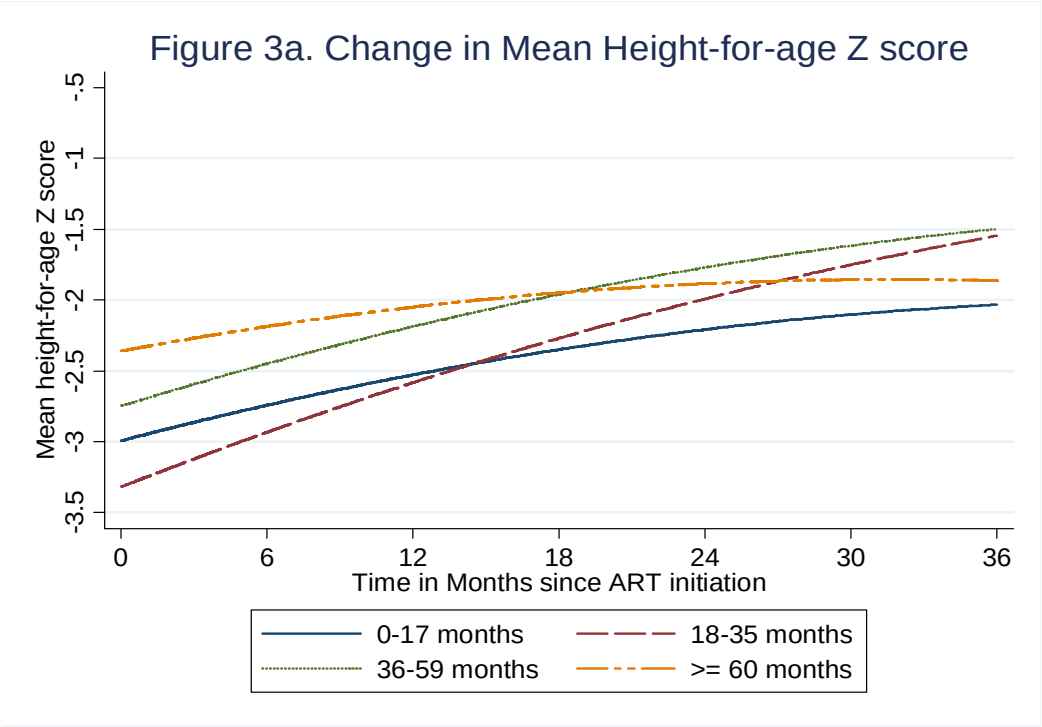
USA, Moye et al. found that HIV-infected children were 0.7 kg lighter and 2.2 cm shorter than HIV-exposed but uninfected children at 18 months of age [41]. Similarly in a retrospective study, Arpadi et al. found that 42% (14 of 33) of perinatally HIV-infected children >5 years old had a growth velocity of less than the 5th percentile (mean growth rate, 3 cm/year), an indication of severe growth failure [42]. Newell et al. also reported that by the age of 10 years, uninfected children in Europe were on average an estimated 7 kg heavier and 7.5 cm taller than infected children [43]. The mechanism through which HIV infection results in growth failure in children is complex and not well understood. However, **initiation of effective ART results in substantial but heterogeneous improvement in somatic growth.** In a previous analysis of data from 2102 children from our cohort in Soweto, South Africa, we found that initiation of ART was followed by a catch-up period of rapid growth of about a year (figures II-3) after which the average weight and height Z scores stabilized close to but below the expected mean of zero [37]. Younger children gained more substantial weights and for a longer period than older children. Similarly in a prospective cohort of 192 USA infant 24 months or younger, Nachman et al. found that younger children gained height more rapidly and children with greater baseline viral loads gained weight more rapidly [44]. Futhermore, in a retrospective analysis of a cohort of 1212 European children, Guillen et al. reported that children who successfully suppress viral replication have significantly higher z-score for weight and height [45]. In a retrospective analysis of cohort of 749 HIV-infected children receiving care in Uganda with average baseline weight-for-age z-score (WAZ) and height-for-age z-score (HAZ) of -3.2 and -2.7 respectively, the authors reported that children gain weight more rapidly than height

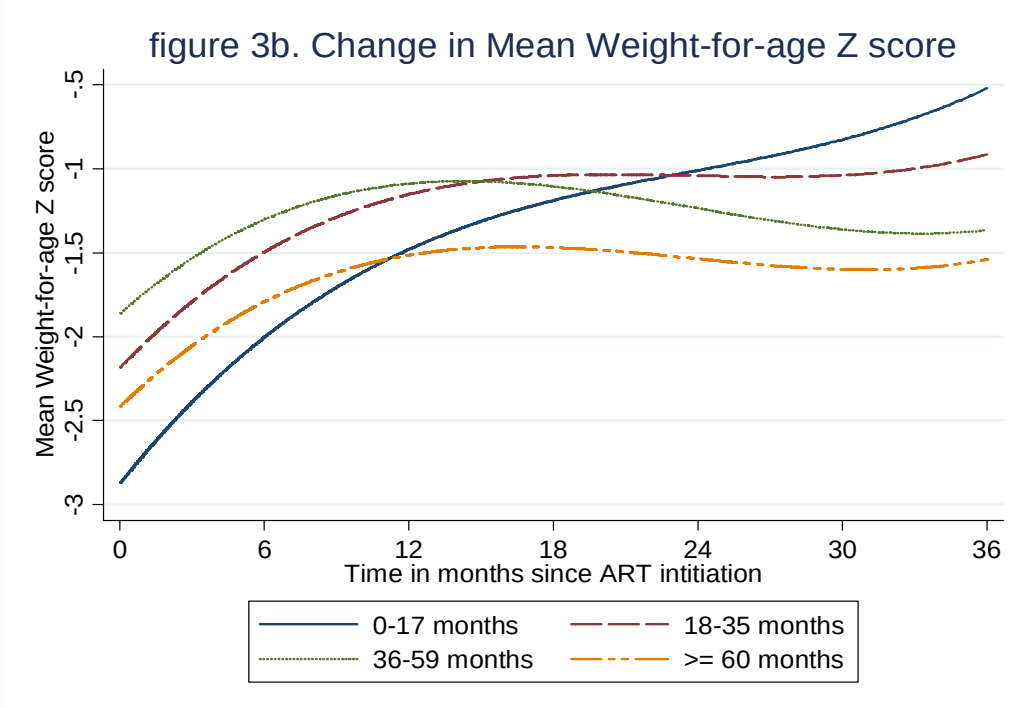
[46]. The ART regimen itself affects growth. In a prospective study comparing protease inhibitor (PI) and non PI containing regimens in 906 HIV-infected children in the United States with average baseline WAZ = -0.42 and HAZ = -0.90, the authors reported that the use of PIs was associated with per-year gains of 0.13 z scores in height and 0.05 z scores in weight relative to the expected growth with non–PI containing regimens [47]. The strong somatic growth observed after ART initiation and the fact that weight and height are relatively easy to measure make them a good alternative to viral load or CD4 count for the monitoring of ART.

In resource poor settings where viral load or CD4 cells count are not available, **the WHO recommends that primarily clinical parameters be used for monitoring ART**, **particularly the improvement in somatic growth in children** who have been failing to grow [24].

However, **the problem with using somatic growth and to some extent CD4** cell count and CD4% as monitoring tools for ART in children **is that the** velocity at which children gain weight, height, and CD4 cells strongly depends on age or gender. Therefore, **reference value ranges change with age and/or gender** making the development of simplified protocols very difficult. A major limitation with standardized growth is that parameters from a reference population are needed for its calculation. In addition that measurements from patients have to be converted to standard values before they can be used, currently the available standards are cross-sectional and do not measure the velocity of somatic growth in HIV-infected children on ART [48, 49].

Figure II-3. Change in the average height- and weight-for-age z score in a cohort of 2102 children who received ART at the Harriet Shezi Children's clinic between April 2004 and March 2008





Source: [37]

So far, to our knowledge, all studies [41, 50-54] but one [55] that have assessed the usefulness of somatic growth as prognostic tools for HIV/AIDS disease progression or as a monitoring tool for ART response employed standardized growth.

In the study by Carey et al. using observations from 1338 HIV-infected children aged 3 months to 15 years who participated in one of four US clinical trials of pediatric anti-HIV therapies, the authors used statistical smoothing (locally weighted regression) of growth histories to derive velocity estimates, and used quantile regression to determine the distributions of physical growth velocities. Using the obtained distributions, the authors show that they could be used to predict HIV progression. But, children in the study were North American who were either on mono or dual therapy or not on ART at all [55].

D. Summary

In summary, of the 2 million children living with HIV worldwide, more than 90% are in sub-Saharan Africa where the epidemic of HIV is compounded by that of TB. In some high-burden countries of Southern Africa, more than 50% of TB-infected children are also HIV-infected. Despite the increasing availability of ART on the continent, the high pill burden and its potential negative effect on adherence, coupled with substantial drug interactions between anti-tuberculosis and antiretroviral drugs complicate the management of HIV/TB co-infection and call for closer monitoring of ART in those settings. But, frequent viral load and CD4 measurements that are standard for monitoring ART in developed countries are not always available or sustainable in some of the poorest regions. Alternative means for monitoring the response to ART that are easy to use and do not rely on sophisticated technologies are needed. In addition because of the drug interactions between anti-TB and antiretrovirals, it is currently

recommended to delay ART for at least 2 to 8 weeks after TB treatment initiation in HIV/TB co-infected children. But the effect of this delay on survival and response to ART as well as the optimal timing of ART initiation among those children are yet to be determined.

III. STATEMENT OF SPECIFIC AIMS

A. Study questions/specific aims

Research question 1: How much weight, height, or CD4 cell count (both absolute and percentage) gain in the first 6 months of ART is an indication of good ART response in HIV-infected children?

Specific aim 1: To construct reference distribution for the first 6-month weight, height, absolute CD4 count, and CD4% gains and assess the value of the 3rd, 10th, 25th, 33rd, and 50th percentile of each distribution as predictors of death, virological suppression, or treatment failure after 6 months of ART.

Research question 2: What is the optimal timing for ART initiation in HIV/TB co-infected children?

Specific aim 2: To determine the effect of delaying ART initiation after TB treatment initiation in HIV/TB co-infected children on virological suppression and survival respectively.

B. Hypotheses

Hypothesis for Aim 1

Children falling below the 3rd, 10th, 25th, 33rd, or 50th percentile of weight, height, CD4 cell gains at 6 months post-ART are at higher hazard of death, of not achieving viral suppression, or of failing antiretroviral treatment subsequently.

Hypothesis for Aim 2

In HIV/TB co-infected children, initiation of ART immediately after TB treatment will improve survival but decrease subsequent virological response to ART.

C. Rationale

Rationale for aim 1

The high pill burden and drug interactions between ART and other anti-infectious agents demand that children initiated on ART in the region, be closely monitored. But, frequent viral load measurements that are the standard for monitoring ART in developed countries are not always available in some of the poorest settings. Alternative means for monitoring ART that do not rely on sophisticated technologies are needed. Somatic growth and to some extent CD4 cell count have been recommended for use in those settings. But because, the absolute gain in weight, height, and CD4 count following ART initiation are strongly dependent on age or gender, reference distributions have to be constructed and cut-off points for decision making defined. The 6-month interval was chosen to match to the current recommended interval for repeating viral load and CD4 count and in accordance with the published literature covering growth velocity and HIV prognostic [55-57].

Rationale for aim 2

In sub-Saharan Africa where more than 90% of HIV-infected children worldwide live, prevalence of HIV among TB-infected children top 50% in some high burden countries. Despite increasing available of ART, this high prevalence of HIV/TB co-infection and substantial drug interactions that exist between anti-TB and ART have complicated the management of pediatric HIV in the region. Currently, though the recommendation is to delay ART initiation for at least 2 to 8 weeks after TB treatment initiation, the optimal timing of ART in those children is not known. The justification for the 2-8 weeks delay is to at least be able to distinguish side effects due to anti-TB from that due to ART drugs and therefore to improve adherence to ART and the virological response. Knowing the effect of currently recommended delay on survival and virologic suppression can help to improve the management of HIV/TB co-infected children.

IV. METHODS

A. Overview of methods

For aim 1, the Generalized Additive Model for Location, Scale and Shape (GAMLSS) and the Box Cox Power Exponential (BCPE) distribution were used to construct the distribution of 6 months post-ART initiation weight, height, CD4 cell count, and CD4% gains and the Cox proportional model was used to assess the association of the selected centiles of each distribution with the 3 outcomes considered.

For aim 2, the inverse-probability-of-treatment weighting method of marginal structural model was used to adjust for time-varying confounding by indication and potential lead time bias for the effect of ART delay on survival. Multivariate Cox model was used to assess the effect of ART delay on viral suppression.

B. Design

1. Data source

    a. Study site

The data used in these analyses are from an observational clinical cohort of HIV-infected children who sought and received care at Harriet Shezi Children's Clinic (henceforth the clinic) from April 1st, 2004 to March 31st, 2008. The clinic is a pediatric outpatient HIV clinic at Chris Hani Baragwanath Hospital (the hospital) in Soweto, South Africa. It was started in 1997 by Dr Tammy Meyers and later became known as the

Harriet Shezi Children's Clinic in 2000. The name Harriet Shezi was chosen in recognition of the first black matron of the hospital appointed in 1958, a symbol of triumph in the face of adversity and prejudice. While it is a government service, funding and support from international agencies including the United States President's Emergency Plan for AIDS Relief *(*PEPFAR*)*, the United Nations Children's Fund (UNICEF), Elizabeth Glazer Pediatric AIDS Foundation, Rockefeller Brother's Fund, the South African business sector and a host of individuals, schools and churches, have allowed the clinic to expand in response to the community's need for quality comprehensive pediatric HIV care services. This support enabled the antiretroviral treatment of children with AIDS even before free ART became available in the public sector. On April 1st, 2004 the South African government launched a massive program to roll out ART throughout the country with the ambitious goal of providing ART to all its HIV-infected citizens in need. The clinic was among the firsts to start HIV-infected children on ART [58]. As of March 2008, more than 3553 HIV-infected children have received care in the clinic among whom about 2216 had been initiated on ART in the government program, making it the largest pediatric HIV clinic in South Africa and one of the largest in the world. Harriet Shezi Children's Clinic has, since the year 2000, been an international research site for the International Maternal Pediatric and Adolescent AIDS Clinical Trials Network (IMPAACT), formerly known as the Pediatric AIDS Clinical Trial Group (PACTG).

b. Study population

Children in the clinic are referred primarily from the pediatric ward of the hospital, though referrals from other sites and services do occur. To be enrolled in the clinic,

documented HIV-infection is required. Because children who received HIV care before ART became free in the country might be different from those who accessed HIV care after April 2004, all analyses were restricted to children whose initial visit in the clinic occurred between April 1st, 2004 and March 31st, 2008. For aim 1, children had to have at least 6 months of follow-up on ART, a pre-ART measurement of either weight, height, CD4 count, and CD4% and another measurement between 5 and 7 months (needed to calculate the 6 months gain) (figure IV-1). For aim 2, they needed to have been initiated on TB treatment in the clinic before ART (figure IV-2). Children older than 15 years were excluded from all analyses.

Figure IV-1. Description of the cohort of children who received ART at Harriet Shezi Children's Clinic between April 2004 and March 2008 and had both baseline and 6 months post-ART initiation measurements for either weight, height, and CD4 cell count

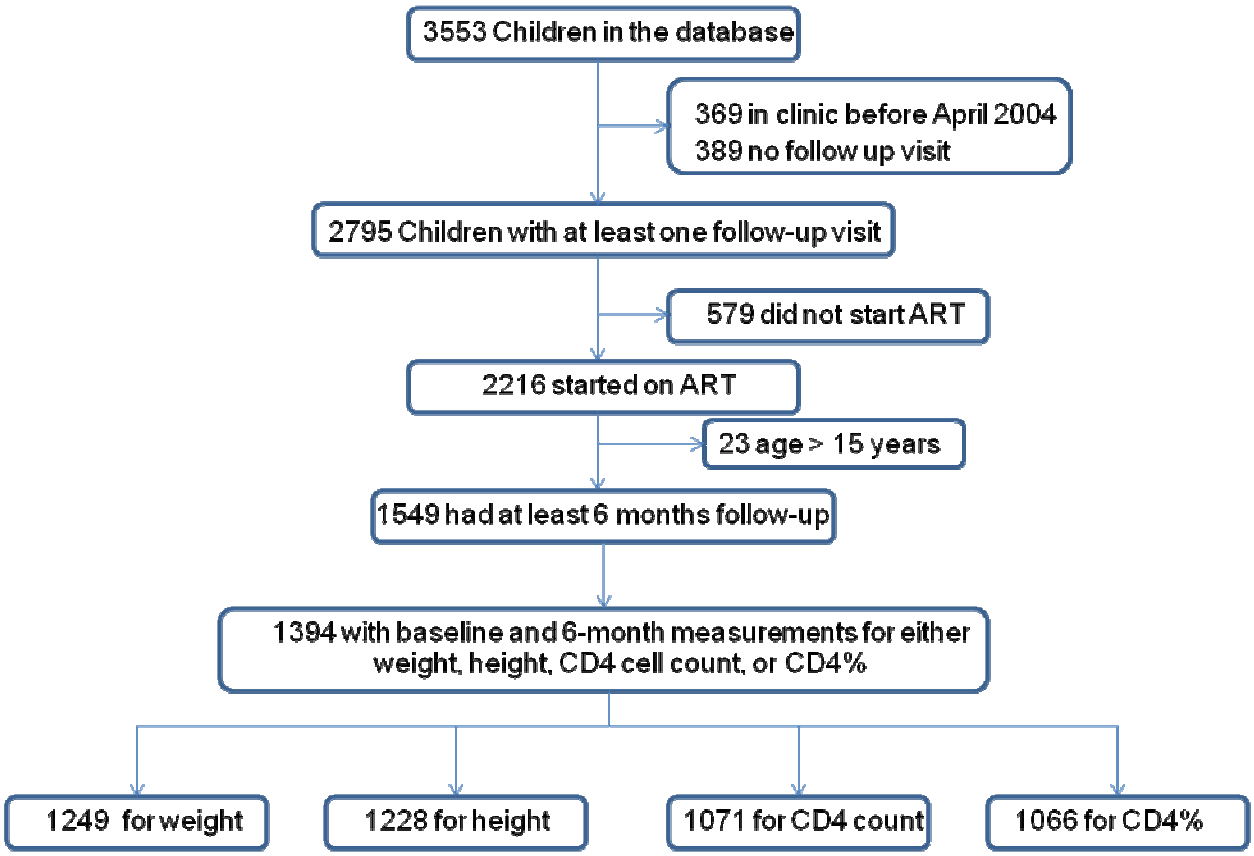
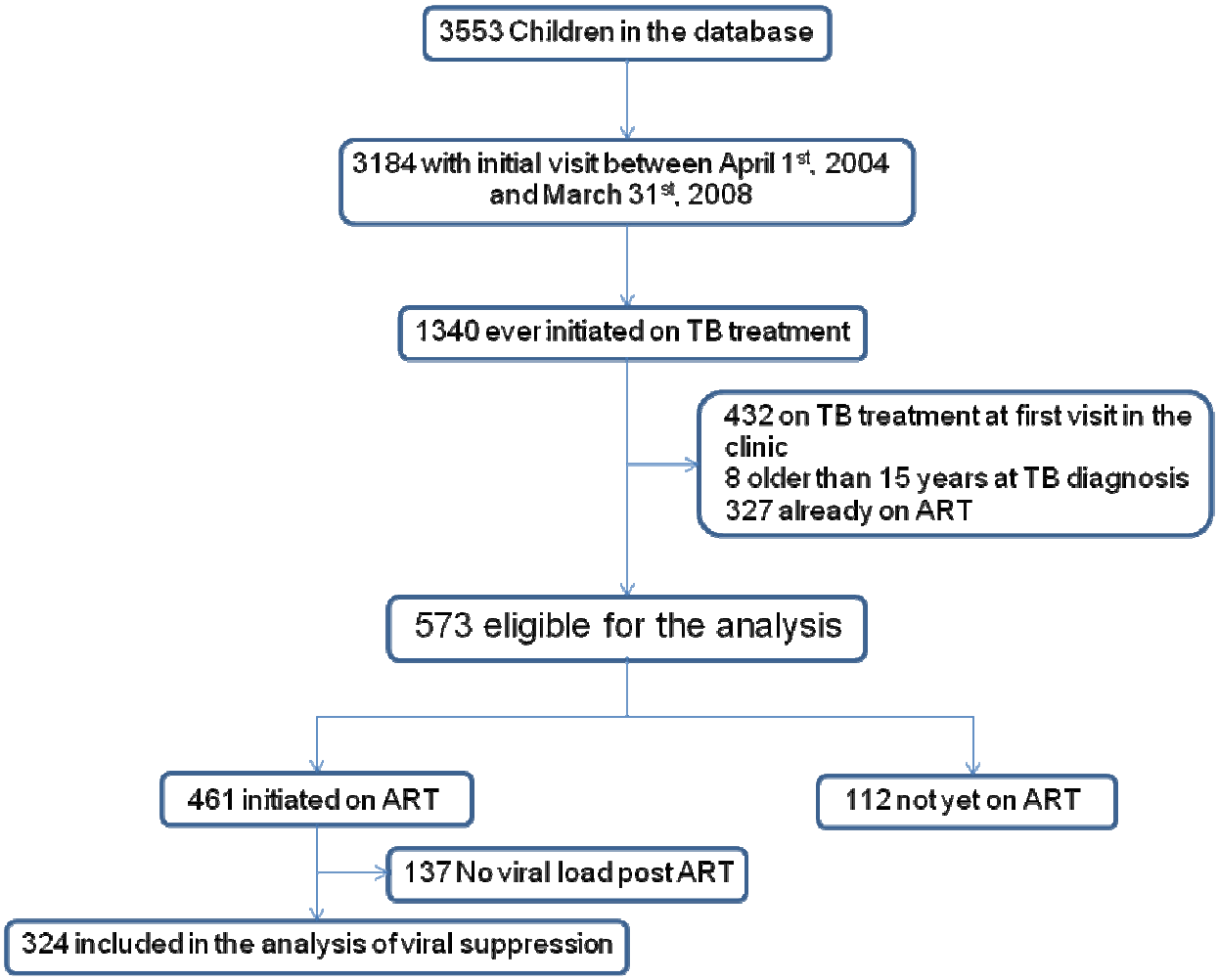


Figure IV-2. Description of the cohort of ART naïve children who were initiated on TB treatment at Harriet Shezi Children's clinic between April 2004 and March 2008



c. Treatment protocols

At the initial visit to the clinic, HIV-infected children were assessed for their clinical history and physical examination and their WHO clinical stage was determined. Children were initiated on ART and follow-up according to the national guidelines: an adaptation from the WHO guidelines for ART in children [57]. The first line regimen included stavudine, lamivudine, and ritonavir boosted lopinavir (LPV/r or Kaletra®) for children younger than 3 years of age or efavirenz for those over 3 years and over 10kg of

weight. Second line regimen included zidovudine, didanosine, and nevirapine for children 3 years or younger or Kaletra®/efavirenz for children older than 3 years. Children who were started on ART were clinically reevaluated at 1 month, at 3 months, and every 3 months thereafter or at any other time if clinically needed. However, the pharmacy dispensed a maximum of 2 month's supply of medication.

Children attending the clinic were routinely screened by clinicians for TB and other opportunistic infections prior to initiating antiretroviral therapy. In accordance with national pediatric guidelines [58], all children should have had a chest X-Ray, induced sputum or gastric washings for TB microscopy, culture and sensitivity and a tuberculin skin test (TST). In the clinic, TB was commonly diagnosed on clinical grounds including severe failure to thrive, prolonged (more than two weeks history of) cough, suspicious chest radiograph with or without a positive contact history or bacteriological diagnosis. Children with these features were often commenced on TB treatment prior to ART initiation even in the absence of a definitive TB diagnosis. Children with TB were treated according to national guidelines with a combination of rifampicin, isoniazid, and pyrazinamide for the initial 2 months followed by rifampicin + isoniazid for the remaining 4 months [59]. In children with TB diagnosed before ART, it is recommended ART be delayed for 4 to 8 weeks. The guidelines allowed ART to be started earlier in severe cases.

d. Data collection, Laboratory procedures, database, and defaulter tracing

Clinical information was collected at every scheduled visit and laboratory investigations (hematology, chemistry, viral load, and CD4 cell count) were done at baseline and at the 6-monthly visit unless otherwise indicated. Laboratory tests were conducted at the

central laboratory of the Chris Hani Baragwanath Hospital which is part of the National Health Laboratory Services. HIV was diagnosed using two ELISA tests for children 18 months or older; a polymerase chain reaction (PCR) was used to detect HIV-1 proviral DNA for younger children. Plasma viral loads were initially measured using The AMPLICOR HIV-1 MONITOR Test, v1.5 (Roche) with lower limits of detection at 400 RNA copies/ml and the upper bound at 750,000 RNA copies/ml and subsequently a NASBA based viral load assay (Nuclisens EasyQ, Biomerieux) with lower and upper limits of detection at 25 and 3 million RNA copies/ml. CD4 counts were measured using a flow cytometer (Beckman Coulter).

To track program performance indicators and appointment attendance, a locally developed electronic medical record was used to record patients' data. At the end of each clinic day, an on-site team of data capturers entered designated elements from clinical forms into the database. In addition, a list of patients who missed clinic appointments was generated and given to a full time on-site defaulter tracer who contacted them by phone (about 75% of kids/family members had a phone). In case a patient could not be contacted by phone, a home visit was scheduled. The outcome of the tracing and reasons for missed visits were recorded in the electronic record.

e. Data cleaning and Quality control

Between June and August 2007, the electronic database was reviewed for it completeness. Particular entries such as weight, height, CD4 count, viral load were compared to medical records and any mismatches between the information in the electronic database and the medical records were investigated. Data capturers and data managers were closely associated in this process and have continued this process on a

regular basis to ensure consistency of values in the electronic database and the medical records. From March 31st 2008, when the dataset was closed for this analysis and June 2008, the process was repeated again and any unreasonable value was resolved.

2. Method for the proposed Aims

a. Classification of exposure

1. Exposures for aim 1

Four exposures were considered in this analysis: the 5th, 10th, 33rd, and 50th percentile of the distribution of weight, height, absolute CD4 cell count, and CD4% gain in the first 6 months post-ART initiation. We used a 6-month interval to match the recommended interval for viral load or CD4 count measurements for monitoring purposes in the clinic and in accordance with the published literature covering growth velocity and HIV prognostic [55-57].

2. Exposure for aim 2

The main exposure considered for aim 2 was time from TB initiation to ART initiation categorized in 15, 30, and 60 days or less and greater (delayed ART). The 15, 30, and 60 days cut-offs were chosen to approximate the minimum 2-8 or 4-8 weeks delay between TB treatment initiation and ART recommended in the WHO or the South African guidelines [24, 57].

b. Classification of outcomes

Three main outcomes were considered in these analyses: time to death (survival), time to first virological suppression and time to treatment failure. For children whose exact

date of death was missing, the date of last visit in the clinic was used as date of death. Time to first virological suppression was defined as the date of the first viral load measurement below 400 HIV RNA copies/ml (virological suppression). Treatment failure was defined as either failure to achieve virological suppression after at least 1 year of HAART, failure to achieve virological suppression prior to switch to second line regimen, or 2 viral load measurements above 1000 RNA copies/ml after initial viral suppression. The third outcome was only considered for aim 1.

3. Data analysis

a. Descriptive statistics

The distributions of baseline (ART initiation for aim 1 or TB treatment initiation for aim 2) characteristics were compared between the outcomes or exposures categories using Pearson chi square for categorical variables. For continuous variables, an unpaired, 2-tailed t-test was used if the normal assumption held or Wilcoxon rank sum (Mann-Whitney) test otherwise. The normality assumption was empirically tested using the Kolmogorov-Smirnov test [60].

Kaplan-Meier curves were fitted to examine survival or failure functions of the 3 outcomes stratified by the exposures and baseline characteristics: sex, CD4 cell count (categorized according to WHO immune suppression classification [24]) in mild or not significant, advanced, and severe), WHO clinical stage (Stage I&II vs. III&IV), Weight-for-age z-score (categorized as <-3SD, -3 to -2SD, and >=-2SD), Viral load categorized at about its median, TB treatment, and age at initiation of ART categorized < 18 months and 18 - 35 months, 36 – 59 months, and 60 months and above at baseline. The cut

offs for age were chosen not only to match the treatment indication cut off and the published conventions, [61] but also to capture the variation in the mortality rates by age. Gender specific weight-for-age and height-for-age z score (WAZ and HAZ) were plotted against WHO growth charts for children younger than 5 years and against US Centers for Disease Control and Prevention (CDC) charts for children 5 years or older, because WHO charts were not available for older children [49, 62]. Log-rank test was used to assess if the time-to-event differs statistically among the stratification groups.[63]

b. Statistical modeling

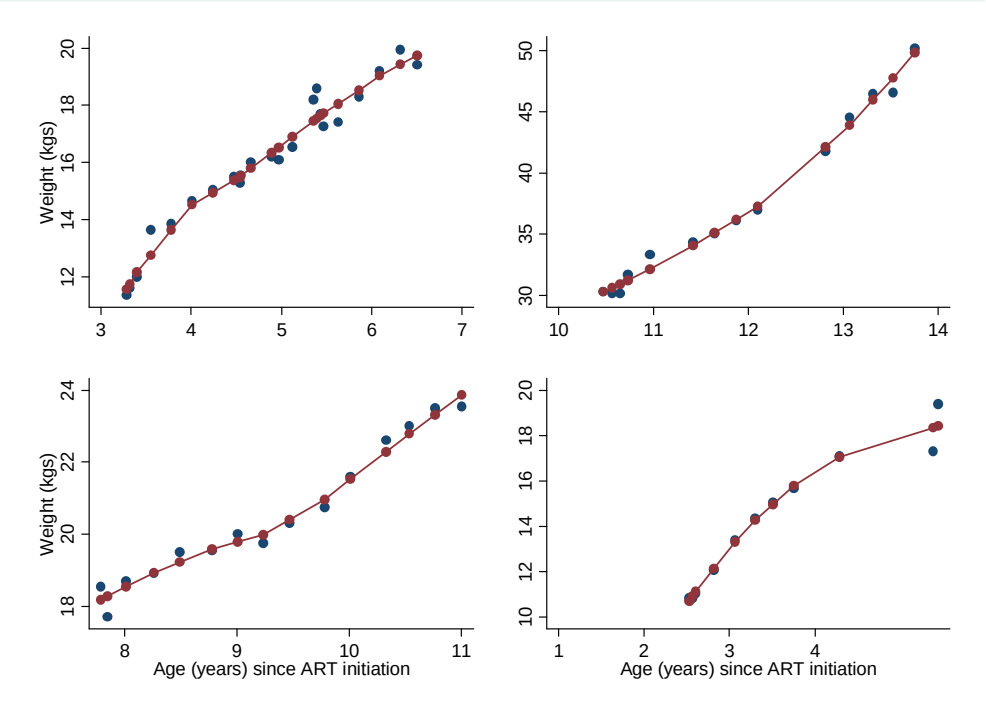
1. Modeling for aim 1

Estimation of the amount of weight, height, CD4 cell count, and CD4% gained in the first 6 months of ART

Biological and measurement variations between 2 consecutive measurements of weight and height, CD4 and CD4% can be important (figure IV-2, IV-3, IV-4). To minimize those variations, locally weighted quadratic regression was used to smooth the pattern of change in height, weight, and CD4 measurements over time after ART initiation for each individual. Locally weighted scatterplot smoothing (LOWESS) regression is an extension of the locally estimated scatterplot smoothing (LOESS) model [64] that performs linear regression on points in a data set weighted by a kernel centered at each given point X = x (in our case at each age point in the data). The estimated response curves: $H_i(t)$, $W_i(t)$, and $C_i(t)$ for weight, height, and CD4 (both absolute and percentage), where t represents any chronological age (in years) within the limits of observed follow-up time on the i^{th} participant were obtained for each individual child

(figure IV-2, IV-3, IV-4). The 6-month gain estimates were simply calculated as the differences $H_i(t_{i0} + 6) - H_i(t_{i0})$, $W_i(t_{i0} + 6) - W_i(t_{i0})$, and $C_i(t_{i0} + 6) - C_i(t_{i0})$, for weight, height, and CD4 respectively.

Figure IV-3. Weight response curves over chronologic age following ART initiation for 4 HIV-infected children estimated using locally weighted regression.



Points right the line (in red) are estimated the others (in blue) are the actual observed values

Figure IV-4 Height response curves over chronologic age following ART initiation for 4 HIV-infected children estimated using locally weighted regression

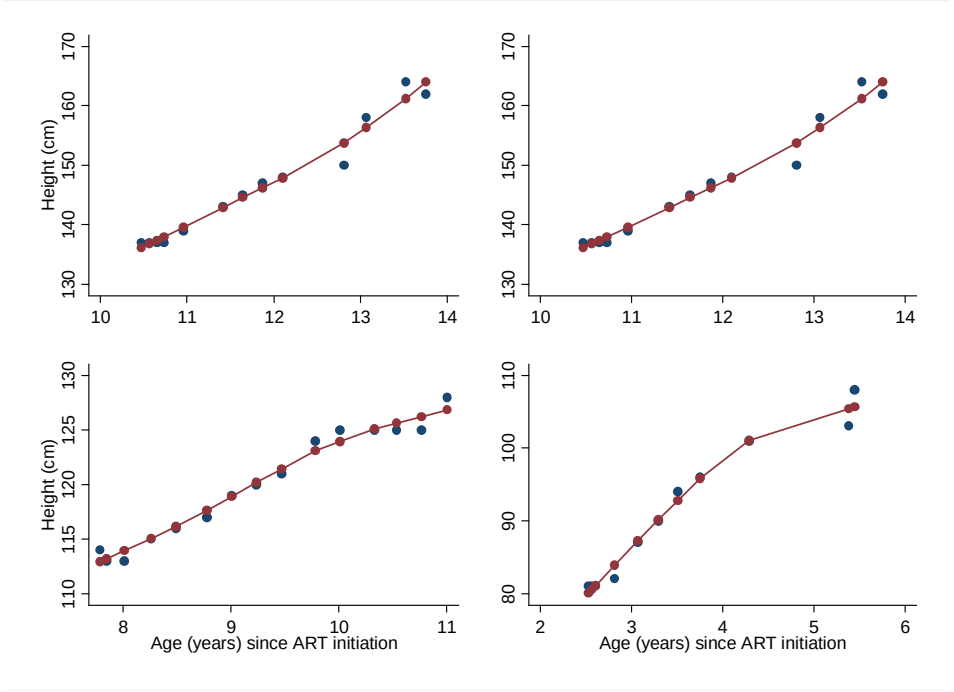
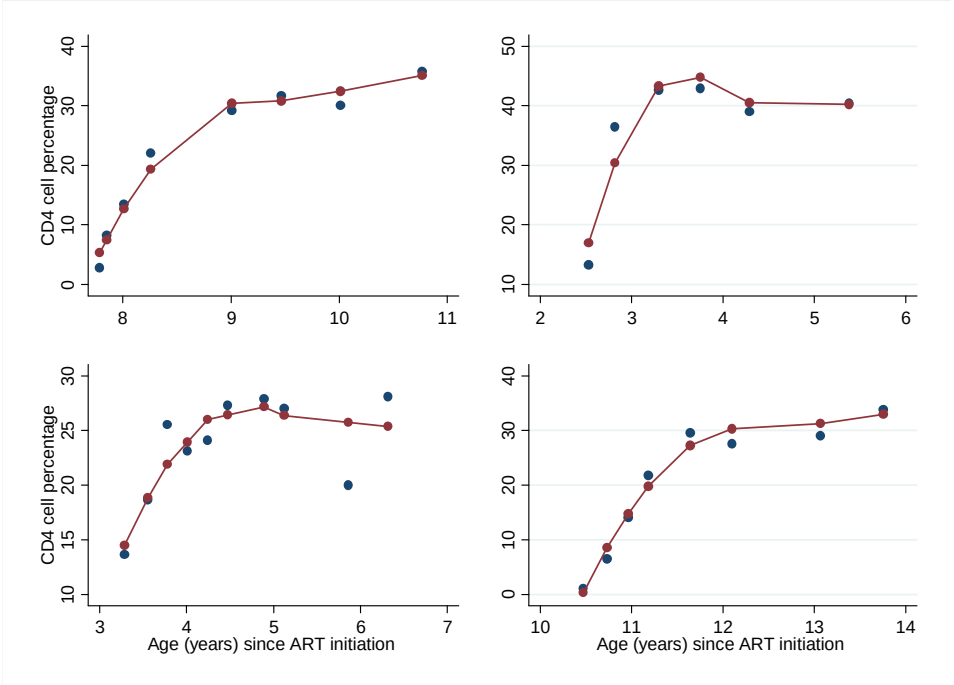
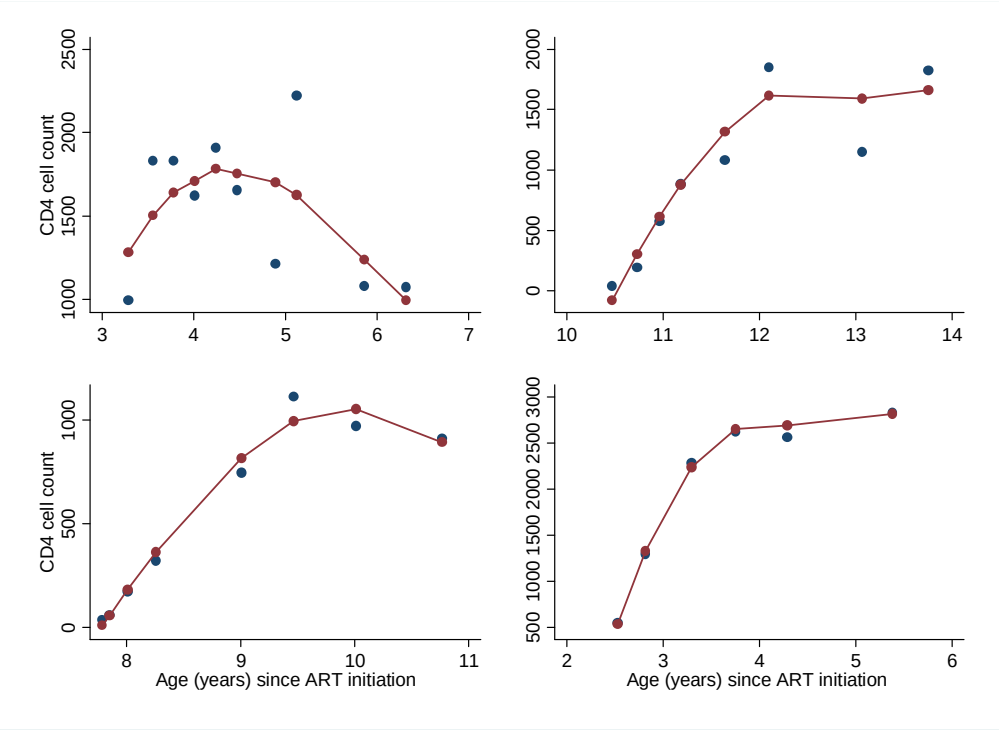


Figure IV-5. CD4% response curves over chronologic age following ART initiation for 4 HIV-infected children estimated using locally weighted regression.



Points right the line (in red) are estimated the others (in blue) are the actual observed values

Figure IV-6. CD4 cell count response curves over chronologic age following ART initiation for 4 HIV-infected children estimated using locally weighted regression.



Points right the line (in red) are estimated the others (in blue) are the actual observed values

Construction of age-specific reference distributions for the estimated 6 month gain in weight, height, CD4, and CD4 percentage

Growth curves have typically been constructed based on the assumption that heights, weights, and other similar measurements, are normally distributed. Age-specific mean and standard deviation curves, μ(t) and σ(t), are estimated and any chosen quantile curve for τ in the interval [0, 1] can then be constructed as

$$\hat{Q}(\tau \mid t) = \hat{\mu}(t) + \hat{\sigma}(t)\Phi^{-1}(t)$$

Where $\Phi^{-1}(t)$ denotes the inverse of the standard normal distribution function. Provided that observation are normally distributed at each age (t), these curves split the population into two parts with the proportion τ lying below the curve, and the proportion 1 - τ above the curve [65].

In children, the distribution of weight, height, or CD4 cell gain can be quite non-normal. To overcome this challenge, Cole and Green in 1992 proposed the Lambda, mu, and sigma (LMS) method [66]. For this method, the distribution at each covariate value is summarized by three parameters, the Box-Cox [67] power λ, the mean (median) μ, and the coefficient of variation σ**,** and the initials of the parameters give the name to the LMS method. To avoid grouping the data according to the value of covariate (in our case age), the method of maximum penalized likelihood is used to provide smooth estimates of the L, M and *S* curves directly with the three parameter constrained to change smoothly as the covariate changes.

However, this method assumes an underlying skewed normal distribution of the measurements so that a suitable power transformation [67] would render the distribution normal. The main problem with skewed normal distribution is the presence of kurtosis as the power transformation does not adjust for it. Although kurtosis is less important than skweness as contributor to non-normality, its presence can bias the estimated distribution. In the recent years, the LMS method has been expended to control for kurtosis with the introduction of Box-Cox power exponential (BCPE) distribution [68]. The BCPE distribution denoted BCPE (μ, σ, υ, τ), is a model for dependent variables Y exhibiting both skewness and kurtosis. The distribution is defined by a power transformation of Y (Y^{υ}) having a shifted or scaled standard power exponential distribution with parameter τ. So in addition to the 3 parameters of the skewed normal distribution, a fourth parameter is added for the BCPE distribution [68]. The BCPE distribution is also referred to as LMSP and has been adopted by the WHO as reference method for constructing the international growth curves [69].

LMS and LMSP are semi-parametric methods in the sense that to fit the models, a parametric distribution of Y has to be specified. Alternative methods to construct reference distributions that do not require any assumption on the parametric distribution of Y to be specified include non-parametric quantile regressions. In cases where transformation in semi-parametric methods can normalize the distribution of Y, results of the LMS or LMSP methods and non-parametric quantile regressions are comparable [65]. However, contrary to the semi-parametric methods, spacing between curves from non-parametric quantile regressions are not constrained to be related to each other and adjacent curves may cross, particularly at the ends of x axis. For this reason and because LMSP is the method of reference use by WHO, we chose the semi-parametric quantile regression method with BCPE as the parametric distribution for the construction of the distribution of 6-month gain in weight, height, CD4 count and CD4% and estimation of centile curves.

The generalized additive model for location, scale and shape (GAMLSS) was used to fit the chosen BCPE (μ, σ, υ, τ) distribution and estimate its different parameters with age at 6 month post-ART as explanatory variable. A GAMLSS assumes independent observations y_i for i = 1; 2; …; n with probability (density) function $f(y_i \mid \theta^i)$ conditional on $\theta^i = (\theta_{1i}; \theta_{2i}; \theta_{3i}; \theta_{4i}) = (\mu_i; \sigma_i; \nu_i; \tau_i)$ a vector of four distribution parameters, each of which can be a function to the explanatory variables. The first two parameters $(\mu_i; \sigma_i)$ are location and scale parameters, and the two remaining if any, are the shape parameters (skewness and kurtosis). The form of the distribution assumed for the outcome Y, $f(y_i \mid \mu_i, \sigma_i, \nu_i, \tau_i)$ is very general and the **gamlss package** in R can fit a

number of distributional families (including the BCPE) for both discrete or continuous variables [70, 71]. GAMLSS allows all the parameters of the distribution of the response variable Y to be modeled as linear or non-linear (smooth) functions of the explanatory variables. In this analysis all parameters were modeled as cubic splines function of age. To specify the GAMLSS model, in addition for choosing the family distribution for the response variable, the user has to choose the smoothing constant or degrees of freedom for each of the parameter in the model that is specified as a function of explanatory variables. The process for choosing those constants can be subjective and cumbersome. Rigby & Stasinopoulos propose a 3 steps systematic approach [68]. In the first step, the appropriate power for transforming the explanatory variable to improve the general fit of the model is considered. In this study, to simplify the interpretability of the constructed curves, we did not transform age, thought its transformation in some cases improved the fit substantially. In step two, taking one parameter at a time, the number of degrees of freedom for the smoothing constant for the parameter that minimize the generalized Akaike Information Criterion (GAIC) (a measure of global fit of the model) is selected. The last step is used to fine tune those numbers of degrees of freedom.

The best set of the combination of effective degrees of freedom obtained was used to fit the final model and the Q-statistic [72] and worm plots [73] were used for the diagnostic of potential region-wise and overall misfit (Appendice B).

The main assumption of the BCPE regression model is that after the appropriate transformations, the distribution of outcome is normally distributed independently of age. Therefore, residuals are expected to have a normal distribution that can be evaluated

using the usual residuals plots. However, the residual plots are not powered to detect age related departure from the model (i.e. a poor fit of the model at a given age point). The Q-statistic [72] and worm plots [73] have been proposed to this end. For Q-test, age is categorized in groups of approximate equal size and the Q-test statistics which are sensitive to age dependency are computed for the first four moments, z1, z2, z3, z4: values larger than 2 indicate a misfit of respectively the mean, variance, skewness, and kurtosis. The worm plot is a collection of detrended Q-Q plots, each of which applies to one of successive age groups. The data plot in each plot form a worm-like string. The shape of the worm indicate how the data differ from the assumed underlying distribution [73]. A flat worm indicates a good fit (Appendice A). Correction of those point-wise misfits were attempted by fine tuning the number of degree of freedom for the corresponding parameter [68].

Assessing the association between the weight gain and subsequent response to ART.

Cox proportional hazard models were used to estimate the effect of each of the selected centiles, adjusted for baseline characteristics. The main assumption of the proportional hazard model is that the hazard in the index group is proportional to the hazard in the referent group [74]. This proportional hazard assumption was formally evaluated for all covariates using the Kolmogorov-type supremum test [75]. All available baseline characteristics were entered in the full model for each of the chosen centile and using a backward selection procedure all variables that did not contribute significantly (Wald test p-value >0.05) to the fit of the model were dropped from the final model.

2. Modeling for aim 2

In aim 2, we wanted to estimate the effect of initiating ART within the first 15, 30, or 60 days of TB treatment initiation on mortality and virological response. The problem with this was that, though per guidelines, all children with TB are eligible to ART, the decision whether to initiate or delay ART for a given child was made at each visit based on clinical and immunological characteristics of the child at the visit. Hence children in advanced clinical stage who were more likely to die were initiated on ART earlier while initiation was delayed for those in better clinical status (figure IV-6). This introduces a time-dependent confounding by indication in the analysis. In addition, some children in who ART initiation was delayed could have died before they could be properly classified for exposure (i.e before they had at least 15, 30, or 60 days of follow-up after TB treatment). To adjust for this confounding and the potential lead time bias, we used inverse-probability-of-treatment weighting (IPTW) of marginal structural model [76-78]. The IPTW is an extension of the Horvitz-Thompson estimator. Stated in non mathematical terms, the Horvitz & Thompson estimator stipulates that for each probability sample (sample with known non zero probability of selection for each element), an unbiased estimate of the population mean or total can be obtained by weighting each observation in the sample by the inverse of its probability of selection [79]. The Horvitz-Thompson was subsequently expanded to any population parameter including regression coefficients [80]. Applications of the Horvitz-Thompson estimator are common in the causal inference literature [78, 81, 82]. However, contrary to surveys where the probabilities of selection are fixed and assumed to be known, in observational studies, these probabilities have to be estimated from the sample at hand using

measured covariates and mathematical modeling. This means that the resulted weights can be highly instable and the validity of the estimator depends on non verifiable assumptions such as no misspecification of the models for weights and no unmeasured confounding [78]. To limit the variability of the estimated probabilities or weights, Robins proposed using “stabilized weights” instead in the IPTW estimator [78].

To estimate the stabilized weights [83], time from TB treatment initiation to ART was treated as failure time and was categorized as less than 0.5 month, 0.5-1, 1-2, 2-3, 3-4, 4-5, 5-6, and 6 months of greater. The cut-off was chosen to match that of exposure and to ensure that there were enough observations in each interval to fit the models. For the numerator of stabilized weights, a pooled logistic model for the probability of initiating ART in each time interval was specified with severity of immunosuppression, log viral load, WAZ, and age at TB treatment initiation as fixed covariates. For the denominator, the same pooled logistic model as above was specified with severity of immunosuppression, log viral load, and WAZ as time-varying covariates. Apart from the initial visit, if a patient had more than 1 measurement for a time-varying covariate in a time interval, only the last measurement was considered. A necessary condition for correct model specification is that the stabilized weights have a mean of one [83].

Estimating the effect of ART timing on ART outcomes

Using the estimated stabilized weights, weighted Cox proportional models were fitted to obtain the crude and adjusted effect of the timing of ART initiation on survival or on virological suppression. All covariables included in the models were formerly assessed for the proportional hazard assumption using the Kolmogorov-type supremum test.[75]

All analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC) and all tests were conducted at 0.05 significance level.

Figure IV-7. Directed acyclic graph for the effect of ART delay on survival among HIV/TB co-infected children

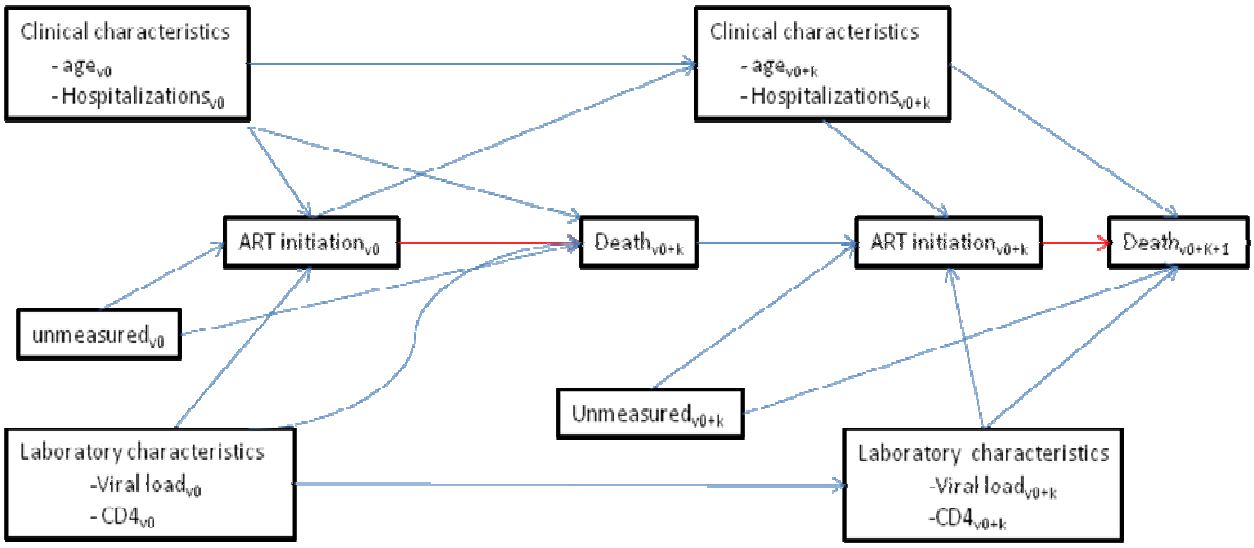
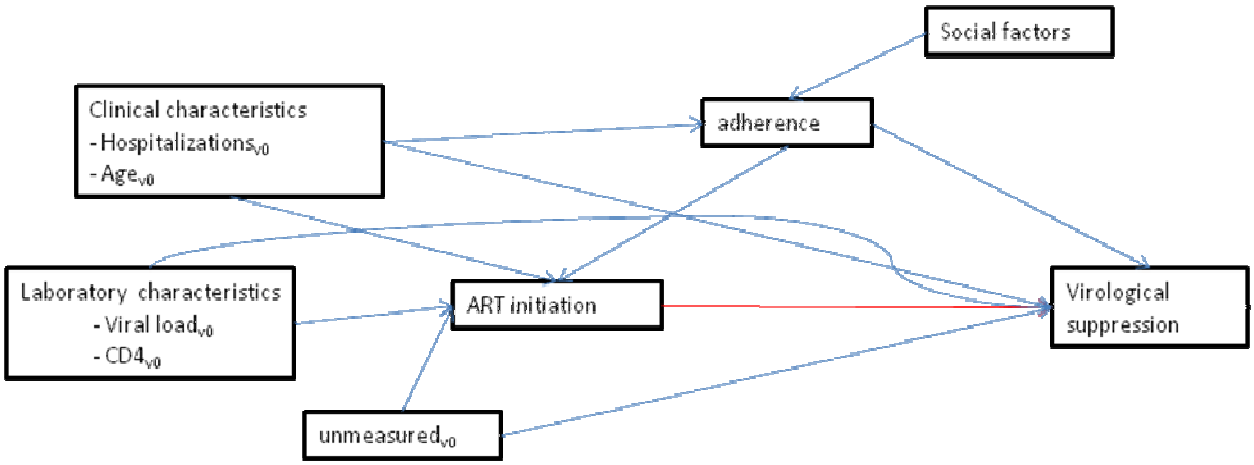


Figure IV-8. Directed acyclic graph for the effect of ART delay on virological suppression among HIV/TB co-infected children



V_k = visit k, where k = 0,1,2, … n from TB treatment initiation, tk = time from TB treatment initiation to visit k.

V. SIX-MONTH POST-HAART INITIATION GAINS IN WEIGHT, HEIGHT, AND CD4 COUNT AND SUBSEQUENT MORTALITY AND VIROLOGIC RESPONSE IN HIV-INFECTED SOUTH AFRICAN CHILDREN

A. Introduction

More than 2 million children live with HIV worldwide and more than 90% of them live in sub-Saharan Africa.[1] In the absence of antiretroviral therapy (ART), a third of children infected perinatally will not survive to their first birthday, and more than half will not survive their second birthday.[4] Despite recent international efforts to increase access to ART in low and middle income countries, the scaling up of ART in sub-Saharan Africa faces at least two major challenges.

First, in developed nations, the monitoring of ART consists of evaluating CD4 count and viral load every 3-4 months.[22] However, the measurement of viral load and CD4 cells count requires expensive and sophisticated technologies that cannot always be easily transferred or sustained in some of the poorest settings. In settings where viral load or CD4 cells count are not available, the World Health Organization (WHO) recommends that clinical parameters be used for monitoring ART, particularly the gains in weight or height in children.[24]

Second, in addition to the lack of the infrastructures, sub-Saharan Africa also faces an enormous shortage of trained health care personnel.[84] In the context of efforts to rapidly increase access to HIV services, the WHO recommends that specific tasks be

moved, where appropriate, from highly qualified health workers to health workers with shorter training and fewer qualifications.[85] For this task shifting to be efficient, simplified protocols with clear decisional algorithms must be available. Contrary to viral load, which has a clear and simple target cut-point (below detection limit), cut-points for weight, height, and CD4 cell gain that correlate with subsequent treatment outcomes have not been clearly established.

In this study, we aimed to construct percentile curves for gains in weight, height, absolute CD4, and CD4% in the first 6 months of ART, and to test the value of the lower centile curves (3^{rd}, 10^{th}, 25^{th}, 33^{rd}, and 50^{th}) as predictors of subsequent death, virological suppression, or treatment failure.

B. Methods

During the first 4 years (April, 2004 to March, 2008) of government sponsored HIV treatment and care program for HIV-infected children at the Harriet Shezi Children's Clinic, a pediatric outpatient clinic at Chris Hani Baragwanath Hospital, Soweto, Johannesburg, 2193 children 15 years or younger were initiated on ART. Of these, 1549 (70.6%) had at least 6 months of follow-up on ART and 1394 (90.0%) with baseline and 6 months follow up weight, height, absolute CD4, and CD4% data were included in this analysis. ART eligibility was in accordance with national guidelines [57]. The first line regimen included stavudine, lamivudine, and ritonavir-boosted lopinavir (LPV/r) for children 3 years or younger; or stavudine, lamivudine and efavirenz for those over 3 years and over 10kg of weight. Second line regimen included zidovudine, didanosine, and nevirapine for children 3 years or younger; zidovudine, didanosine, and

LPV/r or efavirenz for children older than 3 years. Children who were started on ART were clinically reevaluated at 1 month, at 3 months, and every 3 months thereafter or at any other time if clinically needed.

At each scheduled visit, children were assessed clinically and weight and height (children 2 years or older) or length (children < 2years) were recorded. Laboratory investigations (hematology, chemistry, viral load, and CD4 cell count) were done at baseline and every 6 months unless otherwise indicated.

Statistical Analysis

Construction of percentile curves of the 6-month gains in weight, height, CD4, and CD4%

For each individual child, with n sequence data of weight, height or length, CD4 count, and CD4% measurements, the response curves were obtained by smoothing the n measurements over the chronological age at the time of the measurement using locally weighted quadratic regression. The 6-month estimates of weight, height, CD4, and CD4% gain were obtained by subtracting the response curve estimates at 6 months from the estimates at ART initiation. [86]

To obtain the reference percentile curves, we used methods similar to those used by the WHO to construct recent international growth curves. [69] The 6-month estimates of weight, height, CD4 count, and CD4% gain were regressed on chronological age using the generalized additive model for location, scale and shape, a method that requires a parametric distribution assumption for the response variable while allowing the modeling of the distribution parameter as nonparmetric (smooth) functions of the explanatory variables [87]. For the response variable, we assumed a Box-Cox power exponential

distribution with four parameters relating to location (μ, median), scale (σ, coefficient of variation), skewness (υ, transformation for symmetry), and kurtosis (τ, power exponential parameter), respectively[68]. To specify the model, the user must choose the number of degrees of freedom (df) to be used for each parameter. Starting with the simplest model that includes age and the fitting of μ and σ curves while keeping the degree of freedom for υ and τ fixed at zero, we searched for df(μ) and then df(σ) that minimized the global deviance as indicated by the generalized Akaike Information Criterion (with penalty 3 for each degree of freedom used). In the next step, using the df(μ) and df(σ) selected in the previous, we sequentially searched for the df(υ) and df(τ) that minimized the global deviance. In the last step, Q statistic [72] and worm plots [73] were used to fine tune the selected df(μ), df(σ), df(υ), and df(τ) [68].

The selected optimum set of degrees of freedom was used to fit a final model and the age-specific mean and standard deviation curves, μ(t) and σ(t), were estimated. Centile curves for selected centile τ in the interval [0, 1] were then constructed as $\hat{Q}(\tau \mid t) = \hat{\mu}(t) + \hat{\sigma}(t)\Phi^{-1}(t)$ [65]. Where $\Phi^{-1}(t)$ denotes the inverse of the standard normal distribution function. These analyses were done using the gamlss package in R [70, 71]. Growth velocity is usually assessed in pediatric HIV using reference values from the Fels Institute cohort.[88-91] Estimates of the 3rd, 50th, and 90th percentile of the six-month post-ART gains in weight and height were compared to that of a cohort of 818 non-HIV infected and otherwise healthy white American children from the Fels Institute. [56, 92]

Association of lower percentiles with subsequent responses to ART

Three outcomes were considered: time to death (survival), time to first virological suppression and time to treatment failure. For two children whose exact date of death was missing, the last

visit in the clinic was used as date of death. Virological suppression was defined as the first viral load measurement below 400 HIV RNA copies/ml. Treatment failure was defined as failure to achieve virological suppression after at least 1 year of ART, failure to achieve virological suppression prior to switch to second line regimen, or 2 viral load measurements above 1000 RNA copies/ml after initial viral suppression.

Kaplan Meier survival curves stratifying by the selected lower centiles (3^{rd}, 10^{th}, 25^{th}, 33^{rd}, 50^{th}) of the 6 month weight, height, CD4, and CD4% gains and log-rank test were used to assess the association with each of the outcomes.[63] For each of the 3 outcomes, five Cox proportional hazard models were fitted with each of the 5 selected centiles as predictors. Baseline WHO clinical stage (stage I/ II and III/ IV); level of immunosuppression (mild or not significant, advanced, and severe according to age specific CD4% and/or CD4 count values);[24] viral load (≥ or <5 log copies); tuberculosis treatment at ART initiation, age at ART initiation (< 1.5 years, 1.5-3 years, 3-5 years, 5-8 years, and 8-15 years) and weight-for-age z score (WAZ) (≥ minus 2SD, -2 to – 3SD, and <-3SD) [48, 49] were included in the initial model. Using a stepwise backward selection procedure and Wald test, all covariates that did not contribute significantly to the fit of each model were dropped. The Hazard ratio and 95% confidence interval (CI) from each of the final models were reported. All variables included in the model met the proportional hazard assumption formally evaluated using the Kolmogorov-type supremum test [75].

Because attained weight- and height-for-age percentile curves are commonly available and familiar to health care workers (Road-to-Health), to evaluate whether attained growth can be used directly to monitor ART response, we also assessed the association between the lower percentile of the attained weight- and height-for-age at six months post-ART in our cohort and subsequent treatment outcomes. Analyses were done using SAS 9.1 (SAS Institute, Cary, NC). All tests were conducted using a 2-sided 0.05 significance level, without correction for multiple comparisons (or uncertainty due to model selection).

C. Results

Description of the cohort

As of March 31, 2008, 54 (3.7%) of the 1394 children included in the analysis had not been seen in the clinic for more than 6 months (Loss to follow-up), 18 (1.3%) were dead, and 66 (4.7%) have been transferred out. Of the 1394 children, 699 (50.2%) were male, 872 (67.4%) in WHO clinical stage III&IV, 1120 (81.9%) were severely immuno-suppressed, 772 (58.3%) had viral load > 5 log RNA copies/ml, 378 (27.9%) had WAZ <-3SD, 512 (38.1%) had HAZ <-3SD, 353 (25.3%) were on TB treatment, and 262 (18.8%) were 17 months or younger at the time of ART initiation (table 1).

Of the 1394 children, 1249 (89.6%) had sufficient baseline and follow up data on weight, 1228 (88.1%) on height, 1071 (76.8%) on CD4 count, and 1066 (76.5%) on CD4%. To avoid the influence of extreme values, 22 (1.8%) observations for weight, 17 (1.4%) for height, 16 (1.5%) for CD4 count, and 6 (0.6%) for CD4% were excluded. The distribution of baseline characteristics did not vary by availability of follow–up data.

Six-month weight and height gain and their distribution compared to that of normal white American children of the Fels Institute cohort

In the first 6 months post-ART, the median weight gain among boys was 2.73 kg among 1 year-olds, decreased to 1.41 kg among 4 year-olds, after which it increased to 3.05 kg among 14 year-olds (table 2, figure 1). Similarly, median weight gain in girls was 2.63 kg among 1 year-olds, decreased to 1.37 kg among 5 year-olds, and rose to 3.16 kg among 15 year-olds. The median height gain decreased from 8.54 cm among 1 year-old to 1.79 cm among 12 year-old males, and from 8.58 cm among 1 year-old to a 2.18 cm among 12 year-old females (table 2, figure 2).

Compared to the gain observed among healthy US children (Fels Institute Cohort), the six-month weight gain whether at the lower or upper tails of the distribution was consistently higher in our cohort of HIV infected South African children initiating ART, and the height gain particularly in the lower tails of the distribution was substantially and consistently lower, with differences amounting to up to 2 cm for the 3rd percentile (table 2).

Six-month CD4 count and CD4% gain

The CD4 count and CD4% improved substantially after ART initiation. While the gain in absolute CD4 count decreased with increasing age (from 672 cells among 1 year-olds to 310 cells among 6 year-olds and 154 cells among 15 year-olds), the gain in CD4% remained relatively stable across ages, ranging from 9.1% among 1 year-olds to 7.5% among 15 year-olds. (figure 3). Children below the 3rd percentile all had negative gains, and those below the 10th percentile barely maintained their baseline level of absolute CD4 or CD4% count (figure 3).

Association between the 3rd, 10th, 25th, 33rd, and 50th percentile of six-month post-ART weight, height, and CD4 gains and survival

Of the 1394 children included in the analysis, 18 (1.3%) deaths occurred over the 2792.2 years of follow-up, corresponding to a mortality rate of 6.4 (95%CI: 4.1, 10.2) deaths per 1000 child-years between 6 months and 47 months. Children in the lower percentile of weight, CD4 count, and CD4% gain at 6 months of ART had statistically higher crude hazard of death than those with greater gains (table 3). After adjustment for WHO clinical stage, WAZ, and TB treatment at ART initiation, the hazard ratios comparing children below the 33rd percentile of weight, CD4, and CD4% gains to those

above were 4.52 (95%CI: 1.47, 13.90) for weight, 3.03 (95%CI: 1.01, 9.11) for CD4 count, and 2.60 (95%CI: 0.87, 7.74) for CD4%. Hazard ratios increased with lower percentiles. There was no association between six-month height gain and survival.

Association between the 3rd, 10th, 25th, 33rd, and 50th percentile of six-month post-ART weight, height, and CD4 gain and subsequent viral suppression

Almost all children achieved virological suppression, with Kaplan Meier estimates of 84.4% by 12 months and 96.4% by 24 months. Children in the lower percentile of weight, CD4 count, and CD4% gain at 6 months of ART were at lower hazard of virological suppression than those at higher percentiles (table 3). After adjustment for age (weight), or age and baseline WHO clinical stage (CD4 count and CD4%), the hazard ratios for viral suppression comparing children below the 33rd percentile to those above for weight, CD4, and CD4% gain at 6 months of ART were 0.80 (95%CI: 0.70, 0.91), 0.79 (95%CI: 0.69, 0.92), and 0.73 (95%CI: 0.63, 0.84), respectively (table 3). The hazard ratios for viral suppression were smaller with lower percentiles. Height gain in the first 6 months of ART was not associated with virological suppression.

Association between the 3rd, 10th, 25th, 33rd, and 50th percentile of six-month post-ART weight, height, and CD4 gain and subsequent treatment failure

The Kaplan Meier estimates of the proportion of children who failed treatment rose from 0.1% by 12 months to 5.5% by 24 months and 20.1% at 36 months. Children in the lower percentiles of the distribution of weight, CD4 count, and CD4% gain at 6 months of ART were at increased hazard of treatment failure. After adjustment for WHO clinical stage and severity of immunosuppression at ART initiation, the hazard ratios for treatment failure comparing children with weight gain below the 33rd percentile to those

with gain above was 1.56 (95%CI: 1.07, 2.28) (table 3). For children falling below the 33rd percentile of CD4 and CD4% gain, the hazard ratios were 1.46 (95%CI: 1.06, 2.30) and 2.43 (95%CI: 1.66, 3.57), respectively, after adjustment for WHO clinical stage and WAZ (table 3). As for survival, the strength of the association increased with lower percentiles.

Association between the 3rd, 10th, 25th, 33rd, and 50th, percentile of attained weight and height for age (Road-to-Health chart) at six months post-ART and subsequent treatment outcomes

Six-month after ART, the median weight-for-age and height-for-age attained in our cohort was below the 8th (WAZ = -1.41) percentile for weight and below the 1st (HAZ = -2.37) percentile for height on the Road-to-Health chart. Children below the third percentile for weight tended to be at higher risk for death (aHR 4.63, 95% CI 0.78, 27.42), but there was no correlation between weight or height for age at 6 months of ART and virological suppression or treatment failure (table 4).

D. Discussion

It is well established that poor nutritional status and stunting at time of ART initiation are correlated with poor prognosis of HIV infection in children.[50, 55] Monitoring height and weight is routinely performed in the follow up of children, but there are no reference values for weight or height gain among children receiving ART.[24] We constructed reference curves for monitoring 6 month gains in weight, height, CD4 count, and CD4% in children initiating ART, and demonstrated that lower percentiles of CD4% gains, weight and CD4 count, but not height, were associated with treatment outcomes, with increasing strength of association with decreasing percentiles.

In our cohort of South African children, six-month weight gain was consistently and substantially higher than that observed in the Fels cohort of white American children enrolled prenatally and followed-up between 1929 to 1978, while six-month height gain was consistently lower across all ages and gender.[56, 92] Similar differences have been observed with South African children born between April and June, 1990, (when the HIV prevalence among pregnant women was 0.7%),[93] suggesting that the differences between our cohort of HIV infected children and healthy US children may only in part be due to the presence of HIV infection.

The Fels reference values have been used in several pediatric antiretroviral drug trials in the US. [88-91] Our results demonstrate that using the Fels cohort as a reference for HIV infected children in South Africa, may result in an important underestimation of the number of children at risk of poor ART outcomes. For example, a boy who initiates ART at age 18 months and gains 1.5 kg in the first 6 months has a weight gain of the 90th percentile (+1SD) on the Fels cohort growth velocity curves, but scores below the 10th percentile (-1SD) on our newly developed reference curves of weight gain for HIV infected children on ART. A close follow up of this child would be warranted as his weight gain predicts a 6.7-fold hazard of death, 1.8 fold hazard of virologic failure and reduced likelihood (adjusted HR 0.8) of virological suppression, compared to children with gender- and age-specific weight gain above the 10th percentile.

In contrast to six month weight gain plotted on our newly developed reference charts for HIV- infected children, weight at six months of ART plotted on standard weight-for-age charts, did not appear appropriate for the purpose of monitoring ART response in our cohort. In our cohort, though as expected children with weight-for-age or height-for-age

below the 3rd percentile were at increased hazard for subsequent mortality, there was no correlation with subsequent virological suppression or treatment failure.

CD4% gained in the first 6 months of ART showed the strongest association with poor ART outcomes. Monitoring gains in CD4% are particularly attractive as the CD4% gain was relatively stable across ages. In our cohort, children whose CD4% gain was less than 6 % after 6 months of ART fell below the 33rd percentile of CD4% gain and had a 2.6-folds increased hazard of death, 2.4-fold greater hazard of treatment failure and were less likely (adjusted HR 0.7) to achieve viral suppression compared to children who gained 6% or more in CD4%. However, as with viral load, access to CD4% measurement is not widespread in resource poor settings, limiting its usefulness in monitoring ART response.

Our analysis has many strengths including a large sample size and a long follow-up (median follow-up time on ART 25 months), and the use of three different outcomes limiting the impact of potential errors that might result from measurement of each. Our analysis also has a number of limitations. First, we only focus on one interval: the first 6-month of ART, chosen to match the recommended time for viral load or CD4 measurement [57] and in accordance with previous studies [50, 55]. Second, most children were referred after hospitalization, with few children being referred from primary health care clinics, thus selecting for children that may have been sicker at ART initiation and have greater gains in weight, height, and CD4 cell count if they survive the early weeks of treatment.[37] Although children in our cohort do not appear to differ substantially at ART initiation from children in other cohorts in the region [38, 61], reference curves constructed from a more representative population are needed. Third,

despite ART treatment some children had negative weight, height, CD4, and CD4% gains at 6 months. Although negative gains in CD4 cell count and weight can occur, negative height or length gains are likely due to measurement error occurring under routine conditions in a busy clinic. It has been shown that because of measurement errors, a single height velocity measurement even at 12 months lacks the precision to provide a reliable index of current growth, particularly in short children.[94] Third, some important confounders like adherence were not measured and could not be adjusted for.

Conclusion: We demonstrated that though weight gain is an excellent tool for monitoring the early response to ART, growth references from non HIV-infected children do not properly discriminate HIV-infected children with inappropriate growth response to ART. Our results also suggest that even with the reference distributions we constructed, the usual -2SD (3rd percentile) cut-off for growth failure definition misses a lot of children that are at high risk of failing ART and that substantially higher cut-offs should be considered. Gain in CD4% in the first six month of ART was the best predictor of poor subsequent ART outcomes. But in areas with limited access to viral load or CD4 measurement, weight gain post-ART using our newly developed reference distributions for HIV-infected children on ART is a good alternative.

Table V-1. Characteristics at ART initiation of 1394 children included in the analysis of six-month weight, height, CD4 cell gain*

Characteristic at ART initiation	Frequency	Percentage
Sex		
Male	699	50.18
Female	694	49.82
WHO Clinical stage		
1 or 2	422	32.61
3 0r 4	872	67.39
Severity of immunosuppression (WHO)		
Mild or not significant	158	11.55
Advanced	90	6.58
Severe	1120	81.87
Viral load		
>=100 000 copies/ml	772	58.31
<100000 copies/ml	552	41.69
Weight-for-age z score†		
>=-2SD	668	49.34
-2 to -3SD	308	22.75
< -3SD	378	27.92
Height-for-age z score†		
>=-2SD	430	32.02
-2 to -3SD	401	29.86
< -3SD	512	38.12
TB treatment at ART initiation		
No	1041	74.68
Yes	353	25.32
Age at ART initiation		
< 1.5 years	262	18.79
1.5 – 2.9 years	239	17.14
3.0 – 4.9 years	254	18.22
5.0 – 7.9 years	349	25.04
8.0 – 15.0 years	290	20.8
Time on ART in months median (IQR)	25.2	(17.9 to 33.5)‡

*Children were included if they had at least 6 months of follow-up on ART and at least a pre-ART and a 6-month measurement needed to estimate the six-month gain. Those children were part of a cohort of HIV-infected children who were initiated on ART at HSCC between April, 2004 and March, 2008. IQR = interquartile range.
†Gender specific weight- and height- for-age z score were obtained by plotting the weight measurements at baseline against the Word health organization (WHO) weight- and height-for-age charts for children <= 5years and against the Center for Disease and Control prevention (CDC) for children older than 5 years. ‡ Minimum = 6 months, Maximum = 47 months

Table V-2. Six-month weight, height gains in a cohort of 1394 HIV-infected children who were initiated on ART at HSCC (Soweto) between April, 2004 and March, 2008 compared to six-month gains among white non HIV-infected North American children of the Fels Institute cohort[†]

	Age (in years)														
Percentile	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
6-month weight gain (Kg/6 months)															
Male															
3rd Fels	1.20	0.38	0.28	0.24	0.14	0.07	0.21	0.20	-0.46	0.01	0.15	-0.36	0.10	-0.61	-2.99
HSCC	1.16	0.62	0.26	0.08	0.08	0.12	0.09	0.09	0.14	0.20	0.27	0.37	0.55	0.77	-
50th Fels	**2.20**	**1.13**	**0.97**	**0.95**	**1.06**	**1.18**	**1.26**	**1.52**	**1.56**	**1.70**	**1.73**	**2.00**	**2.81**	**3.33**	**3.24**
HSCC	**2.83**	**2.08**	**1.59**	**1.38**	**1.45**	**1.58**	**1.61**	**1.68**	**1.83**	**2.02**	**2.23**	**2.51**	**2.94**	**3.47**	**-**
90th Fels	3.13	1.65	1.52	1.46	1.76	2.01	2.25	2.54	2.95	3.14	3.51	4.24	5.29	5.71	5.73
HSCC	4.04	3.16	2.59	2.38	2.53	2.77	2.88	3.06	3.38	3.75	4.18	4.74	5.54	6.59	-
Female															
3rd Fels	1.30	0.41	0.24	0.11	0.05	0.05	0.16	-0.08	0.14	-0.10	-0.50	-0.41	-1.53	-2.15	-3.61
HSCC	1.13	0.58	0.29	0.15	0.04	0.00	0.02	0.04	0.02	0.02	0.04	0.05	0.02	-0.09	-0.24
50th Fels	**2.19**	**1.21**	**1.02**	**0.98**	**1.03**	**1.12**	**1.18**	**1.36**	**1.47**	**1.57**	**2.01**	**2.74**	**2.15**	**1.83**	**1.00**
HSCC	**2.63**	**1.91**	**1.56**	**1.44**	**1.37**	**1.40**	**1.57**	**1.76**	**1.90**	**2.10**	**2.40**	**2.74**	**3.04**	**3.24**	**3.16**
90th Fels	3.02	1.80	1.59	1.54	1.73	1.97	2.27	2.73	2.97	3.48	3.94	5.11	4.15	4.13	3.45
HSCC	3.95	3.08	2.69	2.60	2.55	2.66	2.97	3.30	3.59	3.96	4.49	5.11	5.67	6.10	6.40
6-month height gain (cm/6 months)															
Male															
3rd Fels	5.89	3.26	2.33	2.14	2.08	1.99	1.99	1.69	1.77	1.80	1.49	1.49	1.61	1.53	0.65
HSCC	3.40	1.73	1.20	1.16	1.02	0.75	0.50	0.38	0.23	0.03	-0.12	-0.25	-0.17	-0.07	-
50th Fels	**8.51**	**5.40**	**4.05**	**3.59**	**3.53**	**3.35**	**3.19**	**3.02**	**2.84**	**2.70**	**2.52**	**2.73**	**3.29**	**4.01**	**3.19**
HSCC	**8.54**	**5.52**	**4.55**	**4.51**	**4.26**	**3.74**	**3.25**	**3.05**	**2.76**	**2.35**	**2.07**	**1.79**	**1.98**	**2.23**	**-**
90th Fels	10.14	6.85	5.38	4.41	4.41	4.10	3.93	3.75	3.52	3.44	3.70	4.07	5.22	5.21	4.66
HSCC	11.64	7.71	6.45	6.38	6.06	5.43	4.83	4.61	4.26	3.75	3.43	3.08	3.39	3.76	-
Female															
3rd Fels	5.94	3.72	2.54	2.50	2.20	2.07	1.96	1.62	1.81	1.63	1.67	1.67	0.36	-0.28	-0.62
HSCC	2.01	1.81	1.17	0.86	0.67	0.43	0.13	0.00	-0.08	-0.20	-0.44	-0.41	-0.35	-0.19	-
50th Fels	**8.49**	**5.59**	**4.13**	**3.65**	**3.52**	**3.30**	**3.08**	**3.01**	**2.84**	**2.91**	**3.06**	**3.32**	**2.76**	**1.33**	**0.68**
HSCC	**8.58**	**6.21**	**4.97**	**4.41**	**4.10**	**3.65**	**3.05**	**2.84**	**2.74**	**2.54**	**2.29**	**2.18**	**2.45**	**2.98**	**-**
90th Fels	10.34	7.09	5.25	4.49	4.36	4.10	3.81	3.85	3.53	3.83	4.33	4.52	4.09	3.11	1.75
HSCC	11.64	8.63	7.07	6.39	6.03	5.48	4.72	4.49	4.39	4.16	3.85	3.75	4.15	4.94	-

[†]Serial data from 818 normal white American children from the Fels Longitudinal Study [56]. HSCC: Harriet Shezi Children's Clinic

Table V-3. Association between lower percentile of weight, height, absolute CD4 count, and CD4% gain at 6 months of ART and time to death, virological suppression, and treatment failure in a cohort of 1394 HIV-infected children from Soweto, South Africa

	Time to Death after 6 months		Time to Viral suppression**		Time to Treatment Failure£	
	HR (95 Confidence Interval)		HR (95 Confidence Interval)		HR (95 Confidence Interval)	
	crude	Adjusted*	crude	adjusted†	crude	adjusted‡
Weight						
3rd	8.32 (2.32, 29.84)	6.90 (1.81, 26.20)	0.71 (0.52, 0.98)	0.69 (0.48, 0.98)	1.83 (0.75, 4.51)	2.56 (1.04, 6.32)
10th	6.04 (2.02, 18.04)	7.12 (2.29, 22.11)	0.83 (0.68, 1.02)	0.79 (0.63, 0.98)	1.57 (0.86, 2.85)	1.84 (1.00, 3.38)
25th	3.60 (1.26, 10.27)	5.29 (1.76, 15.88)	0.86 (0.75, 0.99)	0.82 (0.71, 0.96)	1.21 (0.80, 1.83)	1.31 (0.86, 2.01)
33rd	2.85 (0.99, 8.21)	4.52 (1.47, 13.90)	0.83 (0.73, 0.94)	0.79 (0.70, 0.91)	1.44 (1.00, 2.07)	1.56 (1.07, 2.28)
50th	2.38 (0.75, 7.59)	2.89 (0.87, 9.56)	0.87 (0.78, 0.98)	0.84 (0.74, 0.95)	1.30 (0.89, 1.84)	1.47 (1.01, 2.16)
Height						
3rd	1.62 (0.21, 12.38)	1.95 (0.25, 15.20)	1.00 (0.77, 1.30)	0.97 (0.74, 1.29)	0.15 (0.02, 1.06)	0.22 (0.03, 1.55)
10th	2.76 (0.87, 8.81)	2.26 (0.68, 7.45)	1.08 (0.91, 1.28)	1.02 (0.89, 1.17)	0.81 (0.49, 1.36)	0.84 (0.49, 1.44)
25th	1.36 (0.46, 4.07)	1.35 (0.44, 4.14)	1.08 (0.95, 1.23)	1.03 (0.91, 1.17)	0.74 (0.49, 1.11)	0.73 (0.48, 1.13)
33rd	1.25 (0.43, 3.61)	1.30 (0.44, 3.87)	1.08 (0.96, 1.22)	1.03 (0.91, 1.17)	0.77 (0.53, 1.12)	0.76 (0.51, 1.13)
50th	1.72 (0.58, 5.12)	2.15 (0.65, 7.08)	1.05 (0.93, 1.18)	0.99 (0.87, 1.12)	0.75 (0.52, 1.07)	0.75 (0.51, 1.10)
CD4 count						
3rd	2.70 (0.35, 2.69)	5.56 (0.69, 44.83)	0.84 (0.59, 1.20)	0.86 (0.58, 1.28)	0.48 (0.07, 3.44)	-
10th	3.74 (1.04, 13.42)	5.40 (1.46, 20.00)	0.57 (0.45, 0.72)	0.54 (0.42, 0.69)	3.37 (2.01, 5.64)	3.42 (1.96, 5.99)
33rd	2.95 (1.02, 8.49)	3.03 (1.01, 9.11)	0.80 (0.70, 0.91)	0.79 (0.69, 0.92)	1.43 (0.99, 2.05)	1.56 (1.06, 2.30)
50th	1.59 (0.53, 4.73)	1.51 (0.49, 4.66)	0.92 (0.81, 1.04)	0.90 (0.79, 1.03)	1.21 (0.84, 1.75)	1.23 (0.82, 1.83)
CD4 count Percentage						
3rd	11.01 (3.07, 39.50)	19.43 (4.88, 77.38)	0.52 (0.35, 0.77)	0.50 (0.32, 0.78)	7.00 (3.51, 13.96)	8.75 (3.93, 19.48)
10th	8.31 (2.88, 23.97)	9.11 (3.03, 27.34)	0.65 (0.52, 0.81)	0.59 (0.47, 0.76)	4.28 (2.70, 6.80)	4.40 (2.68, 7.23)
25th	3.48 (1.22, 9.91)	4.31 (1.44, 12.88)	0.75 (0.65, 0.87)	0.73 (0.62, 0.85)	2.75 (1.92, 3.95)	2.73 (1.85, 4.04)
33rd	2.28 (0.80, 6.50)	2.60 (0.87, 7.74)	0.75 (0.66, 0.86)	0.73 (0.63, 0.84)	2.39 (1.67, 3.41)	2.43 (1.66, 3.57)
50th	1.20 (0.42, 3.45)	1.11 (0.37, 3.31)	0.82 (0.73, 0.93)	0.81 (0.71, 0.92)	1.57 (1.07, 2.28)	1.57 (1.05, 2.34)

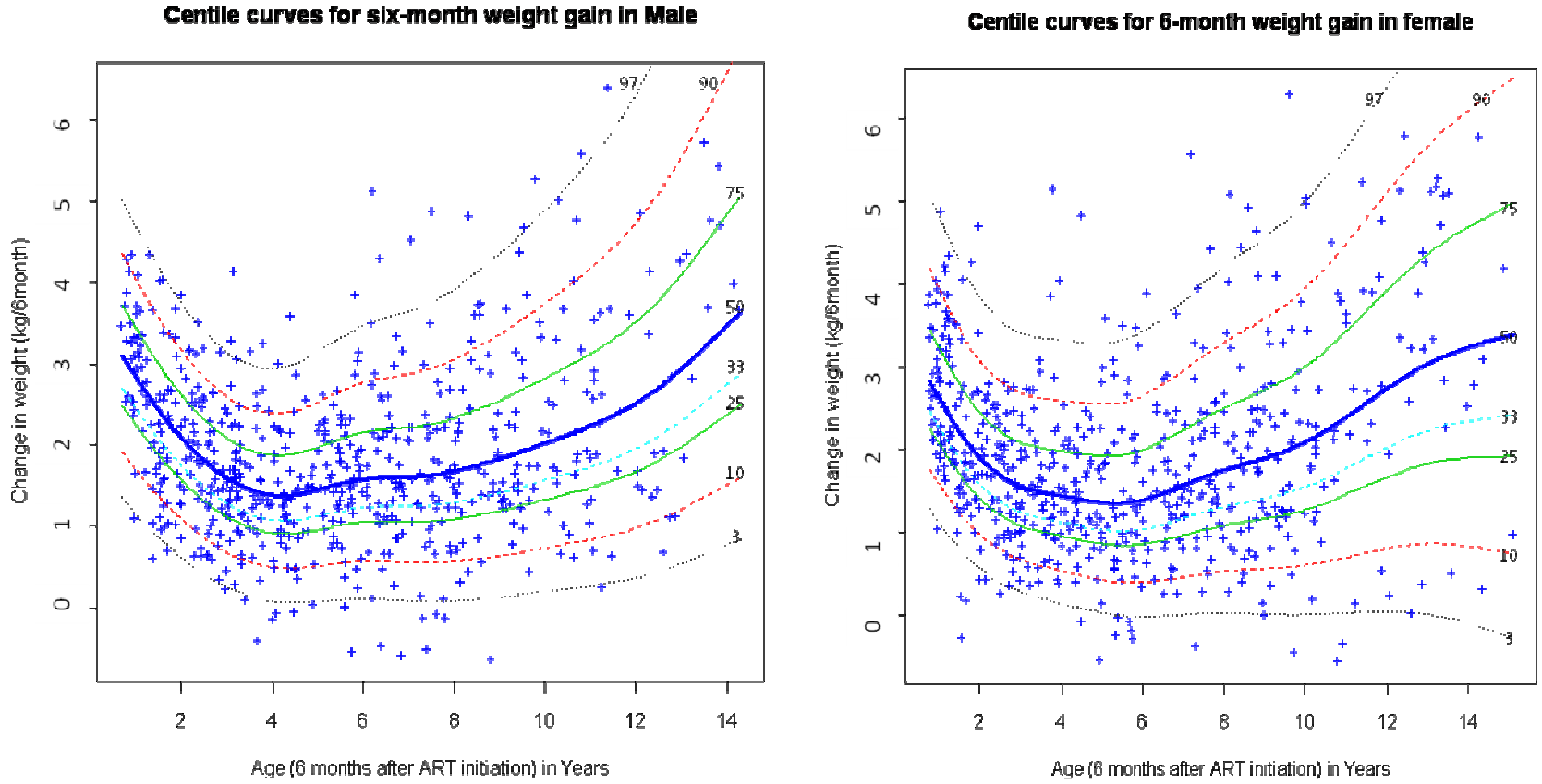
*Adjusted for Baseline WHO clinical stage, Baseline weight-for-age z score (WAZ), and TB treatment at ART initiation. †Adjusted for age for weight and height, baseline WHO clinical stage and age for CD4 count and CD4 percentage. ‡Adjusted for baseline WHO clinical stage and severity of immunosuppression for weight and height, baseline WHO clinical stage and WAZ for CD4 cell count and CD4%. **Viral load < 400 HIV RNA copies/ml, £2 viral load > 1000 after initial suppression or never suppress or > 1 year follow without viral suppression and regimen change

Table V-4. Association between lower percentile of attained weight- and height-for-age at 6 months of ART and time to death, virological suppression, and treatment failure in a cohort of 1394 HIV-infected children from Soweto, South Africa

	Time to Death after 6 months		Time to Viral suppression**		Time to Treatment Failure[£]	
	HR (95 Confidence Interval)		HR (95 Confidence Interval)		HR (95 Confidence Interval)	
	crude	Adjusted*	crude	adjusted†	crude	adjusted‡
Weight						
3rd	6.51 (1.82, 23.34)	4.63 (0.78, 27.42)	0.98 (0.87, 1.11)	0.98 (0.86, 1.11)	0.80 (0.55, 1.16)	0.74 (0.50, 1.10)
10th	2.87 (0.80, 10.28)	1.31 (0.25, 6.88)	0.99 (0.88, 1.11)	0.98 (0.86, 1.11)	1.02 (0.71, 1.47)	1.01 (0.69, 1.49)
25th	1.90 (0.43, 8.48)	0.81 (0.14, 4.61)	1.00 (0.87, 1.14)	0.97 (0.85, 1.12)	1.07 (0.70, 1.66)	1.04 (0.65, 1.65)
33rd	1.39 (0.31, 6.22)	0.65 (0.12, 3.58)	0.97 (0.84, 1.12)	0.93 (0.80, 1.09)	1.45 (0.84, 2.45)	1.37 (0.78, 2.41)
50th	-	-	0.94 (0.78, 1.14)	0.90 (0.73, 1.10)	0.92 (0.48, 1.75)	0.80 (0.40, 1.59)
Height						
3rd	2.93 (0.66, 13.08)	1.37 (0.24, 7.70)	0.95 (0.84, 1.07)	0.95 (0.84, 1.08)	1.00 (0.67, 1.49)	1.03 (0.67, 1.57)
10th	2.94 (0.39, 22.45)	1.45 (0.16, 13.20)	0.93 (0.81, 1.08)	0.93 (0.80, 1.08)	0.95 (0.58, 1.55)	1.02 (0.61, 1.72)
25th	0.96 (0.13, 7.37)	0.31 (0.03, 3.15)	0.81 (0.65, 1.01)	0.85 (0.67, 1.07)	1.30 (0.53, 3.19)	1.22 (0.50, 3.00)
33rd	0.61 (0.08, 4.65)	0.20 (0.02, 2.08)	0.82 (0.63, 1.08)	0.82 (0.61, 1.08)	0.90 (0.33, 2.45)	0.85 (0.31, 2.33)
50th	0.26 (0.03, 1.99)	0.08 (0,01, 0.84)	1.00 (0.68, 1.46)	0.98 (0.66, 1.47)	0.72 (1.18, 2.93)	0.74 (0.18, 3.01)

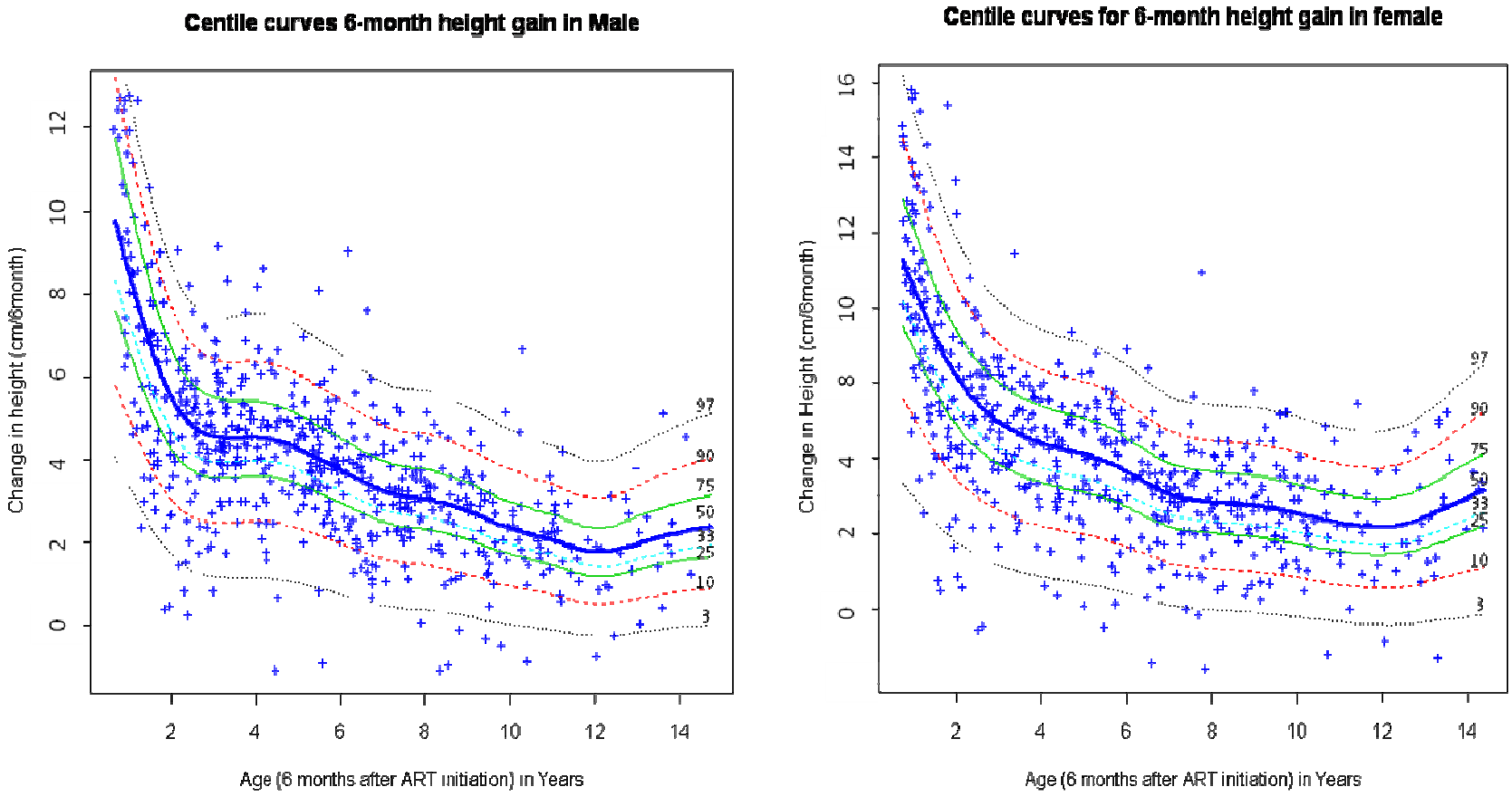
*Adjusted for Baseline WHO clinical stage, Baseline weight-for-age z score (WAZ), and TB treatment at ART initiation. †Adjusted for age . ‡Adjusted for baseline WHO clinical stage and severity of immunosuppression. **Viral load < 400 HIV RNA copies/ml, [£]2 viral load > 1000 after initial suppression or never suppress or > 1 year follow without viral suppression and regimen change

Figure V-1. Gender- and age-specific six-month weight gain reference curves in children who were initiated on ART at HSCC (Soweto) between April, 2004 and March, 2008



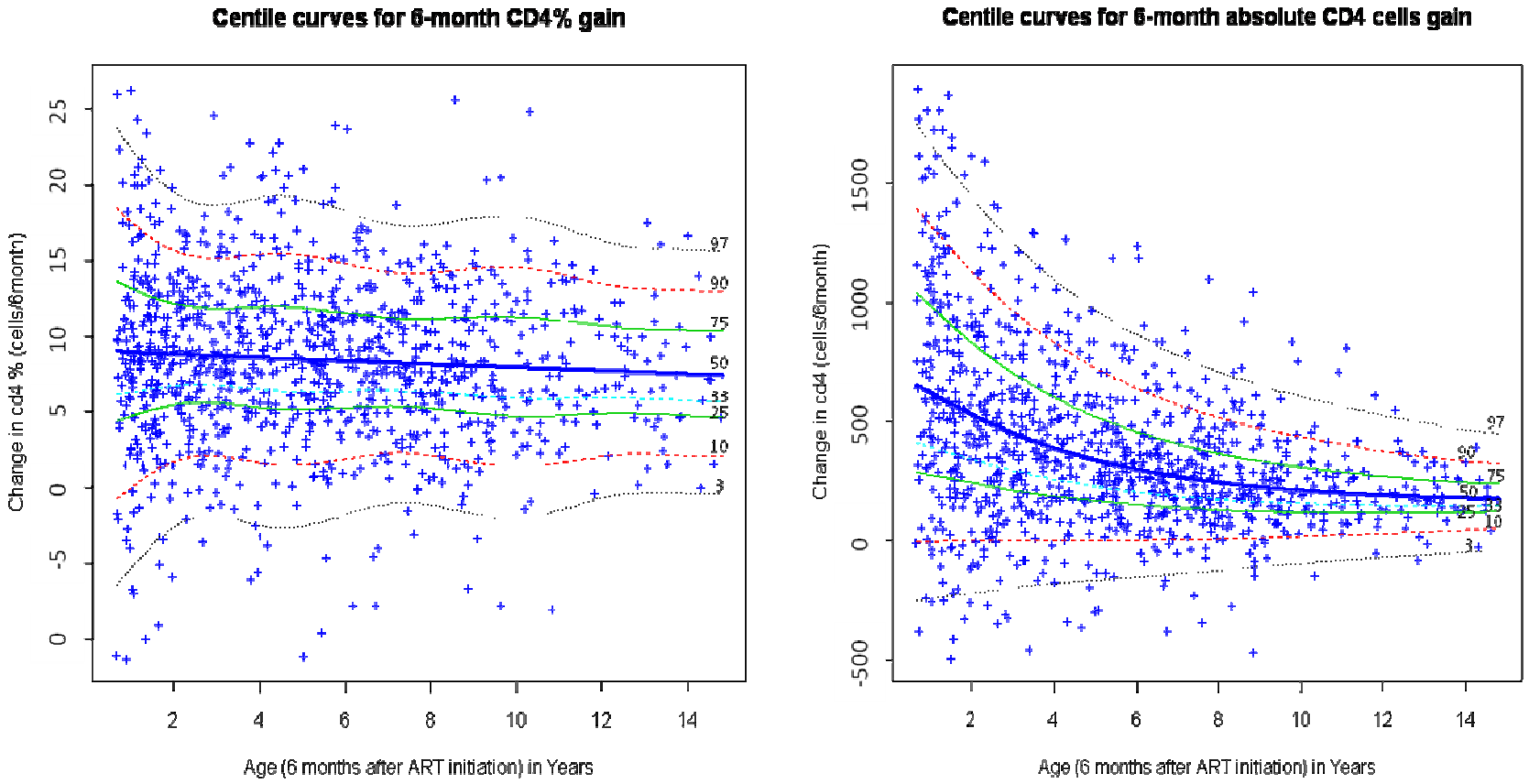
Curves were obtained using Box Cox power exponential distribution (BCPE) LMS and GAMLSS. HSCC: Harriet Shezi Children's Clinic
Model for male: BCPE (age, df(μ) = 4, df(σ) = 0.5, df(υ) =0, df(τ)=1). Model for female: BCPE (age, df(μ) = 5.3, df(σ) = 0, df(υ) =0, df(τ)=0)

Figure V-2. Gender- and age-specific six-month height gain reference curves among children who were initiated on ART at HSCC (Soweto) between April, 2004 and March, 2008



The curves were obtained using Box Cox power exponential distribution (BCPE) LMS and GAMLSS. HSCC: Harriet Shezi Children's Clinic
Model for male: BCPE (age, df(μ) =9.1 , df(σ) =0 , df(υ) =0, df(τ)=2). Model for female: BCPE (age, df(μ) =7, df(σ) =0, df(υ) =0, df(τ)=0).

Figure V-3. Age-specific six-month CD4 count and CD4% gain reference curves among children who were initiated on ART at HSCC (Soweto) between April, 2004 and March, 2008



The curves were obtained using Box Cox power exponential distribution (BCPE) LMS and GAMLSS. HSCC: Harriet Shezi Children's Clinic
Model for CD4 count: BCPE (age, df(μ) = 3, df(σ) = 0, df(υ) =0, df(τ)=1). Model for CD4%: BCPE (age, df(μ) = 0, df(σ) = 5, df(υ) =0, df(τ)=0).

VI. EFFECT OF DELAYING ANTIRETROVIRAL THERAPY INITIATION IN HIV/TB CO-INFECTED CHILDREN ON SURVIVAL AND VIROLOGICAL RESPONSE

A. INTRODUCTION

Worldwide, it is estimated that about 1500 new pediatric HIV infections occur daily and more than 90% of those new infections happen in sub-Saharan Africa [1, 2]. Children who are infected early in life progress more rapidly to acquired immuno-deficiency syndrome (AIDS) and death. Primary and reactivated tuberculosis (TB) is a major cause of morbidity and mortality among HIV-infected children in Africa [15]. Following TB infection, children (particularly young infants), have a higher risk of progression to disease (extra-pulmonary and pulmonary) and death irrespective of their HIV status [17]. However, HIV is a major risk factor for childhood TB [18]. The burden of TB in HIV-infected children is not well known because of the difficulties in diagnosing TB in these children [95], Reported prevalences of HIV among TB-infected children range from less than 5% in industrialized settings to over 50% in some high-burden African settings [8-10]. In HIV/TB co-infected children, diminished cell-mediated immunity increases the risk for disseminated TB disease, especially in advanced stages of HIV infection, resulting in high mortality [19, 20]. Restoration of cellular immunity with antiretroviral therapy (ART) reduces the susceptibility to tuberculosis [21].

Because of the high risk of disease progression, the World Health Organization (WHO) recommends initiation of TB treatment as soon as the diagnosis of TB is suspected in

an HIV-infected child. The management of HIV-infected children with TB is however complicated. Rifampicin, the backbone of any TB treatment regimen reduces the serum concentrations of most protease inhibitors by about 80% or more, and that of non-nuclease reverse transcriptase inhibitors by 20% to 60% [24]. In addition, TB and antiretroviral drugs have overlapping toxicities. To avoid drug interactions and to differentiate side effects for TB and antiretroviral drugs, WHO advises that initiation of ART be delayed for at least 2-8 weeks after TB treatment initiation [24, 30]. In South Africa (SA), the guidelines recommend completion of TB therapy before starting ART in children with clinical stage 3, CD4 percentage (CD4%) < 25% in children under 18 months of CD4% < 20% in older children or to delay ART for 4 to 8 weeks ,in children with severe immunosuppression.[96]. The potential effects of these delays on survival or virological response to ART are unknown.

In this study we aimed to estimate the effect of delaying ART initiation for at least 15, 30, or 60 days in HIV/TB co-infected children on virological suppression and survival. We hypothesized that children in whom ART is delayed will be at higher hazard of death but will be more likely to achieve viral suppression once they are initiated on ART.

B. METHODS

Data

We used information from a cohort of HIV-infected children, who sought care at the Harriet Shezi Children's Clinic, an outpatient pediatric HIV clinic at Chris Hanni Baragwanath Hospital in Soweto, SA, during the first four years (April 2004 to March 2008) of the government sponsored ART program [97]. TB was routinely diagnosed on clinical grounds including severe failure to thrive, prolonged (more than two weeks) cough, suspicious chest radiograph with or without a positive contact history. Bacteriological confirmation of clinical suspicion was attempted in older children who can produce a sputum sample. Children are treated according to national guidelines with a combination of rifampicin, isoniazid, and pyrazinamide for the initial 2 months followed by rifampicin + isoniazid for the remaining 4 months [59].

According to the SA National Guidelines, all children diagnosed with TB are eligible for ART. The first line ART regimen for children receiving TB treatment consists of stavudine, lamivudine, and ritonavir-boosted lopinavir (LPV/r) for children 3 years or younger; or stavudine, lamivudine and efavirenz for those over 3 years and over 10kg of weight.

Children initiated on ART were clinically reevaluated at 1 month, at 3 months, and every 3 months thereafter or as needed clinically. At each scheduled visit, children were assessed clinically and weight and height (children 2 years or older) or length (children < 2years) were recorded. Laboratory investigations (viral load, and CD4 cell count) were done at baseline and every 6 months or whenever indicated. Clinical and laboratory information were entered in an electronic database.

Children where included in this analysis if: their first visit in the clinic occurred between April 1st, 2004 and March 31st, 2008, they were initiated on TB treatment prior to ART initiation, and were 15 years or younger at the time of TB treatment initiation. Children who were already on TB treatment at first visit in the clinic were excluded. For analysis of time to viral suppression, eligible children had to also have at least one post-ART viral load measurement.

Two main outcomes were considered: (1) Time to death (survival) defined as time from TB treatment initiation to death. For 6 children whose exact dates of death were unknown, the last clinic visit date was used. (2) Time to viral suppression defined as time from ART initiation to the date of first viral load measure below 400 HIV RNA copies/ml.

Time from TB treatment initiation to ART initiation (Timing of ART) was categorized at 3 points: 15, 30, and 60 days for each of the 3 main exposures. The 15, 30, and 60 days cut-offs were chosen to approximate the minimum 2 to 8 weeks delay between TB treatment and ART initiation recommended by the WHO [24].

Additional covariables considered were: level of immunosuppression (CD4% below 25% for children younger than 1, or below 20% for children 1 to 2 years, or below 15% for children 3 to 15 years) [24], log viral load dichotomized at about the median (5 $\log_{10}$ copies/ml), age at TB initiation categorized to capture the average change in the rate of death in less than 18 months, 18-35 months, 36-59 months, 60 months or older, and weight-for-age Z score (WAZ) categorized in -2SD or greater, -2SD to -3SD, and less than - 3SD [49, 62].

Statistical analysis

Baseline characteristics (at time of TB treatment initiation), were compared using Wilcoxon Rank Sum test for continuous variables and Pearson Chi square for categorical variables. For the analysis of mortality, though per guidelines all children with TB were eligible for ART, the decision whether to initiate or delay ART for a given child was made at each visit based on clinical and immunological characteristics of the child at the given visit. Thus, children with more advanced disease and at higher risk of death were likely to be initiated on ART earlier than those who appeared relatively healthier. In addition, children in whom ART was delayed and whose follow-up time in the clinic was less than the cut-off point considered for each exposure could not be classified. To adjust for time-dependant confounding and account for the lead time, inverse-probability-of-treatment weighting (IPTW) of marginal structural models was used [76, 78]. IPTW estimator is an extension of the Horvitz & Thompson estimator for population means and totals in survey statistics [79]. But contrary to surveys where the probability of selection and therefore weights are known and fixed, for the IPTW, and in this analysis, the probability of staying in the study until a given time and initiating ART at that time has to be estimated from the observed sample and can be highly variable [78]. To limit the variability of estimated weights, stabilized weights are recommended [78]. To estimate the stabilized weights, time from TB treatment initiation to ART was treated as failure time and categorized as: less than 15 days, 15 to 30 days, 30 to 60 days, 2 to 3 months, 3-4, 4-5, 5-6, and 6 months or greater. These cut-offs were selected to reflect the categorization of the exposure (delay between initiation of TB treatment and ART) and to ensure that there were enough observations in each group to fit the models. For the numerator of stabilized weights, 2 pooled logistic models for the probabilities of initiating ART or remaining in care (censoring weights) in each time interval were specified with level of immunosuppression, log viral load, WAZ, and age at TB treatment initiation as fixed covariates. For the denominator, the same pooled logistic models above were specified with level of immunosuppression, log viral load, and WAZ as time-varying covariates in addition to age at TB initiation treated as fixed. Apart from the initial visit, if a patient had more than 1

measurement for a time-varying covariable in a time interval, only the last measurement was considered. The product of the stabilized weights for censoring and stabilized weights for ART initiation was used for the final weights. Assuming no misspecification of the models for weight estimation and provided that data are available on all time-varying and baseline joint predictors of ART initiation and mortality, the IPTW is a valid method to account for such confounding [76-78]. A necessary condition for correct model specification is that the stabilized weights have a mean of one [83]. The mean of the final weights was 1.00 and its values ranged from 0.44 to 15.76. Using the estimated weights, weighted Cox proportional models were fitted for mortality and each of the 3 exposures.

Children had to be on ART to be at risk of viral suppression. Hence, the only potential source of confounding that had to be adjusted for in the estimation of the effect of timing of ART initiation on viral suppression were the fixed baseline characteristics. Cox proportional models were used to obtain the crude and adjusted effect of the timing of ART initiation on virological suppression. All covariates included in the models were formerly assessed for the proportional hazard assumption using the Kolmogorov-type supremum test [75].

To assess if the effects of delaying ART on mortality or viral suppression vary by the level of immunosupression at baseline, each analysis was repeated in each stratum of level of immunosuppression when there were sufficient data. All analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC) and all tests were conducted using a 2-sided 0.05 significance level, without correction for multiple comparisons. All confidence intervals (CI) were estimated using robust variances [98].

C. RESULTS

Between April 2004 and March 2008, 3187 HIV-infected children received care in the clinic for the first time, 1341 of whom were on TB treatment at some point of follow-up at the clinic. In 909 children, TB treatment was initiated after their initial visit in the clinic. Of those 909, 8 were older than 15 years and 328 were already on ART at the time of TB treatment. The remaining 573 children were included in this analysis, of whom 461 (80.5%) were subsequently initiated on ART at a median time of 17 [Interquartile range (IQR): 0 to 49] days after TB treatment initiation. As of March 31st, 2008, 75 (13.0%) of those children had not been seen in the clinic for at least 6 months and their whereabouts were unknown (loss to follow-up): 38 of them prior to ART initiation and 37 while on ART. Children lost to follow-up did not differ from those still in care by any of the baseline characteristics or timing of ART initiation.

The 573 children were followed for a median time of 9.6 (IQR: 1.9 to 23.1) months. Follow-up time was administratively censored at 36 months. At baseline, the median CD4% was 11.9% (IQR: 6.6% to 18.3%), 75.2% of children were severely immunosuppressed, the median log viral load was 5.2 (IQR: 4.5 to 5.9) log copies/ml, 58.6% of children had viral load greater than 5 log copies/ml, the median WAZ was -2.3 (IQR: - 3.6 to -1.3) with 34.7% of children severely underweight-for-age (WAZ <-3SD), the median age was 3.5 (IQR: 1.4 to 6.8) years and 26.5% of children were younger than 1.5 years (table 1). Children who were initiated on ART earlier (within one month of start TB treatment) had lower CD4 count, lower WAZ, and higher viral load in general than those in whom ART was delayed (table 1).

Mortality

Of the 573 children eligible for the analysis, 37 died during follow-up; 30 among 461 children initiated on ART: 22, 25, 26 respectively among the 226, 288, 378 initiated on ART within 0 to 15, 0 to 30, 0 to 60 days of TB treatment. Children who died were more likely at baseline to be younger (median age 1.4 vs 3.7 years, $p < 0.01$), to be severely underweight for age (median WAZ – 3.8 vs -2.2, $p < 0.01$), and to have higher viral load (median 5.9 vs 5.2, $p <0.01$). The hazard ratio for death comparing children with severe immunosuppression to those without at baseline was 2.38 (95%CI: 0.85, 6.68) (table 2). Delaying ART for ≥15 or 30 days had no effect on mortality (adjusted hazard ratio (aHR): 0.90 , 95%CI: 0.30, 2.75 and 1.05, 95%CI: 0.29, 3.75, respectively). However, delaying ART initiation for ≥60 days after TB treatment initiation tended towards higher hazards of death, with aHR of 2.18 (95%CI: 0.64, 7.24). All hazard ratios account for the time-varying level of immunosuppression, log viral load, WAZ, and censoring prior to ART, and time fixed age at TB treatment initiation (table 3).

Full stratification of the analysis of mortality by level of immunosuppression was not possible because only 4 deaths occurred among the 125 children not severely immunosuppression at baseline (1 immediately after TB treatment initiation and before ART and 3 others after ART initiation). Restriction of the analysis to children with severe immunosuppression resulted in stronger and more précised effects. The aHRs for delaying ART for ≥15, ≥30, or ≥60 days were: 1.14 (95%CI: 0.65, 2.01), 1.36 (95%CI: 0.77, 2.41), and 3.40 (95%CI: 1.92, 6.02).

Virological suppression

Of the 461 children initiated on ART, 324 (70.3%) had at least one viral load measurement after ART initiation, of whom 291 (89.8%) ever achieved viral load below

400 copies/ml. Children who achieved virologic suppression were more likely at the time of TB treatment initiation to be older (median age 4.0 vs 1.6 years, $p < 0.01$), to have higher weight-for-age (median WAZ – 2.2 vs -3.1, $p < 0.02$), and to have lower viral load (median 5.2 vs 5.8, $p = 0.01$).

Delaying ART for ≥15 or 30 days had no effect on viral suppression (aHRs = 0.98, 95%CI: 0.76, 1.26, and 0.95, 95%CI: 0.73, 1.23, respectively), but delaying for 60 days or longer after TB treatment initiation tended towards lower hazards of achieving virological suppression: 0.84 (95%CI: 0.54, 1.10); i.e. children who initiated ART more than 60 days after start TB treatment were 16 % less likely to achieve viral suppression compared to those who started earlier . All HR adjusted for level of immunosuppression, log viral load, WAZ, and age at TB treatment initiation (table 3).

Full stratification of the analysis of viral suppression by level of immunosuppression was also not possible because of the 324 children included in the analysis for viral suppression, only 63 were not severely immunosuppressed at baseline and just 6 of them had not yet achieved virologival suppression at the time of this analysis (all but one with 6 months or less of follow-up on ART). Restricting the analysis to only severely immunosuppressed children did not change the results substantially. The aHRs were 0.96 (95%CI: 0.73, 1.26), 0.91 (95%CI: 0.67, 1.24), and 0.77 (95%CI: 0.50, 1.18).

D. Discussion

We sought to estimate the effect of delaying ART initiation in children receiving TB treatment on mortality and virologic response to ART. Our results showed that, a short delay (less than 30 days) between TB treatment and ART initiation had no effect on mortality and viral response to ART. The effect of delaying ART for 60 days or more tended towards higher higher hazards of death and lower hazards of viral suppression, but results were imprecise and not statistically significant. However, when the analysis was restricted to children severely immunosuppressed at TB treatment initiation, Delaying ART for 60 days or more, more than triple the hazard of death and was statistically significant (aHR 3.40, 95CI: 1.92, 6.02). ART is well known to improve survival in HIV infected children [37, 57, 61, 99] and early initiation of ART even in asymptomatic infants has been shown to substantially improve survival [100].

The principal justification for delaying ART in HIV/TB co-infected children is to allow differentiation between side effects from ART and TB drugs, and to improve the viral response by avoiding drug interactions [30]. The observation that children for whom ART was delayed for 60 days or more, appeared actually to be less likely to achieve viral suppression suggests that the current recommendation of delaying ART in children with TB has no benefit on virological response to ART.

Our analysis had several strengths. First, we used data from one of the largest pediatric HIV clinics in sub-Saharan Africa, resulting in a large sample size of 573 HIV-infected children with TB of whom 461 also initiated ART. Second, we used the most appropriate methods to measure the effect of delaying ART on mortality in an observational study, by controlling for a bias due to confounding by indication and lead time using the IPTW

method of marginal structure model. Third, by considering different cut-offs for the definition of delay, we were able to elicit a “dose-response” relationship, with a tendency of increasing negative effect on both mortality and viral response to ART with increasing length of delay. Fourth all children in our cohort came from a single clinic. This is important as initiation of TB treatment and assessment of ART involves some subjectivity; having the same set of clinicians making those decisions, limits the variability.

Our study also suffered from some limitations. First, this is a retrospective analysis of data from an observational clinical cohort. As a consequence, viral load results at TB treatment initiation were missing for 15% of children included in the analysis, and 13% were lost to follow up. The loss to follow-up was not associated with timing to ART or the baseline covariates, and more than half of loss to follow-up occurs prior to ART initiation which we accounted for by the censoring weights. Because of the high workload, reasons for initiation or delay of ART were not noted. Second, children receiving rifampin were treated with efavirenz (if older than 3 years) or ritonavir-boosted lopinavir (if 3 years or younger) to achieve optimal virological outcomes [101] [102, 103]. These regimens are often not available in most resource poor countries, limiting the generalizability of our results to other parts of sub-Saharan Africa. Third, we did not have adherence data to adjust for possible differential rates of adherence among children initiated on ART earlier and those in whom ART was delayed. In our cohort, children with more advanced HIV disease progression at TB initiation were more likely to be initiated on ART earlier than those in better clinical status. Compared to asymptomatic children, children in more advanced stage of HIV disease at ART

initiation are likely to be more adherent to treatment.[104]. Fourth, due to the small number of children not severely immunosuppressed at baseline and particularly the limited number of events among them, it was not possible to fully assess effect of delaying ART on mortality or viral suppression among those children, it is possible that the effect of delaying ART on mortality differs by the level of immunosuppression.

In conclusion, these results indicate that delaying ART initiation in children with TB for more than 60 days may be detrimental for survival and viral response to ART. These results need to be confirmed by a randomized controlled trial of when to start ART in children with TB, as results from ongoing adult trials [105] may not be generalizable to children.

Table VI-1. Characteristics of 573 HIV/TB children 15 years or younger who received care at Harriet Shezi Children Clinic in Soweto, South Africa between April 2004 and March 2008

Baseline characteristics	Overall	Initiation of ART		P-value*	Timing of ART initiation		P-value*
		No	Yes		< 1 month	>= 1 month	
CD4 cell count: median (IQR)	392 (165 to 773)	745 (436 to 1211)	327 (137 to 701)	<0.01	273 (98 to 604)	533 (238 to 868)	<0.01
CD4 cell percentage: Median (IQR)	11.90 (6.60 to 18.30)	19.55 (12.10 to 25.90)	10.10 (6.00 to 16.50)	<0.01	8.02 (4.62 to 13.60)	15.00 (9.82 to 21.20)	<0.01
Severity of immunosuppression							
Mild/not significant/advanced: n(%)	125 (24.80)	45 (53.57)	80 (19.05)		25 (9.62)	74 (38.74)	
Severe†: n(%)	379 (75.20)	39 (46.43)	340 (80.95)	<0.01	235 (90.38)	117 (61.26)	<0.01
Log viral load: median (IQR)	5.20 (4.54 to 5.88)	4.91 (4.32 to 5.85)	5.26 (4.62 to 5.88)	0.02	5.28 (4.64 to 5.98)	5.20 (4.52 to 5.80)	0.24
> 5: n(%)	284 (58.56)	33 (44.59)	251 (61.07)		154 (60.87)	108 (57.75)	
0-5: n(%)	201 (41.44)	41 (55.41)	160 (38.93)	0.01	99 (39.13)	79 (42.25)	0.53
Weight-for-age z score: Median (IQR)	-2.28 (-3.58 to -1.26)	-1.63 (-3.09 to -0.84)	-2.38 (-3.81 to -1.36)	<0.01	-2.71 (-4.11 to -1.60)	-1.92 (-2.92 to -0.83)	<0.01
>=-2SD: n(%)	241 (42.73)	63 (56.25)	178 (39.29)		96 (34.29)	106 (51.46)	
-2 to -3SD: n(%)	127 (22.52)	19 (16.96)	108 (23.84)		62 (22.14)	53 (25.73)	
< -3SD: n(%)	196 (34.75)	30 (26.79)	167 (36.87)	<0.01	122 (43.57)	47 (22.82)	<0.01
age at TB treatment initiation: median (IQE) years	3.47 (1.36 to 6.78)	2.48 (1.26 to 5.54)	3.65 (1.37 to 7.04)	0.20	3.50 (1.35 to 7.07)	3.53 (1.36 to 6.65)	0.45
0.0 – 1.4: n(%)	158 (27.57)	36 (32.14)	122 (26.46)		78 (27.08)	54 (26.21)	
1.5 – 2.9: n(%)	107 (18.67)	26 (23.21)	81 (17.57)		52 (18.06)	37 (17.96)	
3.0 – 4.9: n(%)	96 (16.75)	17 (15.18)	79 (17.14)		44 (15.28)	41 (19.90)	
5.0 – 15.0: n(%)	212 (37.00)	33 (29.46)	179 (38.83)	0.31	114 (39.58)	74 (35.92)	0.12
Time from TB to ART initiation: median (IQR) days	17 (0 to 49)	-	17 (0 to 49)		4 (0 to 14)	59 (42 to 130)	<0.01
Survival time after TB treatment initiation: median (IQR) months	9.64 (1.93 to 23.05)	0.00 (0.00 to 2.02)	14.23 (5.08 to 26.07)	<0.01	13.97 (2.89 to 26.39)	12.66 (5.74 to 24.20)	0.33

IQR = Interquartile range, SD = Standard Deviation, †CD4 percentage below 25% for children younger than 1, or below 20% for children 1 to 2 years, or below 15% for children 3 to 15 years. *From Wilcoxon Rank sum test for continuous variables and Pearson Chi Square test for categorical variables

Table VI-2. Characteristics associated with survival and virologic response of 573 HIV/TB co-infected ART-naïve children seen at Harriet Schezi children's clinic, Soweto, South Africa (April 2004, March 2008)

	Mortality			Virological suppression*		
Baseline characteristics	**Total eligible**	**Number of events n (%)**	**Hazard Ratio (95%CI)**	**Total eligible**	**Number of events n (%)**	**Hazard Ratio (95% CI)**
Severity of immunosuppression						
Mild/not significant/advanced	125	4 (3.20)	1	63	57 (90.48)	1
Severe[†]	379	33 (8.71)	2.38 (0.85, 6.68)	250	226 (90.40)	0.89 (0.68, 1.16)
Viral load (log copies/ml)						
> 5	284	28 (9.86)	1	186	165 (88.71)	1
0-5	201	8 (3.98)	0.42 (0.19, 0.93)	118	112 (94.92)	1.11 (0.87, 1.42)
Weight-for-age z score						
>=-2SD	241	5 (2.07)	1	133	125 (93.98)	1
-2 to -3SD	127	7 (5.51)	2.50 (0.74, 8.36)	75	68 (90.67)	0.70 (0.52, 0.93)
< -3SD	196	24 (12.24)	6.22 (2.21, 17.54)	112	95 (84.82)	0.79 (0.58, 1.06)
age at TB treatment initiation: years						
0.0 – 1.4	158	21 (13.29)	1	78	62 (79.49)	1
1.5 – 2.9	107	5 (4.67)	0.32 (0.12, 0.88)	61	56 (91.80)	1.12 (0.76, 1.65)
3.0 – 4.9	96	4 (4.17)	0.28 (0.09, 0.83)	58	54 (93.10)	1.42 (0.93, 2.16)
5.0 – 15.0	212	7 (3.30)	0.22 (0.09, 0.51)	127	119 (93.70)	1.41 (1.00, 1.98)

CI = Confidence Interval: estimated using robust variance, SD = Standard Deviation, *first viral load measure below 400 HIV RNA copies/ml. [†]CD4 percentage below 25% for children younger than 1, or below 20% for children 1 to 2 years, or below 15% for children 3 to 15 years. All estimates were obtained using the weighted Cox proportional hazard.

Table VI-3. Effect of Delaying ART initiation on survival and virologic response among a cohort of 483 HIV/TB co-infected ART-naïve children seen at Harriet Schezi children's clinic, Soweto, South Africa (April 2004, March 2008)

Model & Cut-off used for exposure	Total eligible[‡†]	Number of events[‡†] n (%)	Hazard Ratio (95%CI) Crude	Adjusted for baseline characteristics[‡]	Weighted**
Time from TB to ART initiation					
			Mortality[†]		
			Overall		
<=15 days	226	22 (9.73)	1	1	1
> 15 days	275	13 (4.73)	0.44 (0.22, 0.88)	0.73 (0.34, 1.55)	0.90 (0.30, 2.75)
<= 30 days	288	25 (8.68)	1	1	1
> 30 days	206	9 (4.37)	0.46 (0.22, 0.98)	0.72 (0.32, 1.61)	1.05 (0.29, 3.75)
<= 60 days	378	26 (6.88)	1	1	
> 60 days	111	8 (7.21)	0.96 (0.44, 2.12)	1.76 (0.71, 4.40)	2.18 (0.64, 7.48)
			Restricted to severely immunosuppressed children at baseline		
<=15 days	184	21 (11.41)	1	1	1
> 15 days	170	11 (6.47)	0.53 (0.26, 1.09)	0.71 (0.32, 1.59)	1.14 (0.65, 2.01)
<= 30 days	235	24 (10.21)	1	1	1
> 30 days	117	7 (5.98)	0.56 (0.24, 1.28)	0.68 (0.27, 1.71)	1.36 (0.77, 2.41)
<= 60 days	298	24 (8.05)	1	1	
> 60 days	54	7 (12.96)	1.61 (0.71, 3.67)	2.04 (0.77, 5.44)	3.40 (1.92, 6.02)
			Virological suppression*		
			Overall		
<=15 days	152	136 (89.47)	1	1	
> 15 days	172	155 (90.12)	1.07 (0.86, 1.35)	0.98 (0.76, 1.26)	-
<= 30 days	197	179 (90.86)	1	1	
> 30 days	127	112 (88.19)	1.03 (0.81, 1.30)	0.95 (0.73, 1.23)	-
<= 60 days	265	238 (89.81)	1	1	
> 60 days	59	53 (89.83)	0.96 (0.72, 1.27)	0.84 (0.61, 1.15)	-
			Restricted to severely immunosuppressed children at baseline		
<=15 days	133	120 (90.23)	1	1	
> 15 days	117	106 (90.60)	1.06 (0.82, 1.37)	0.96 (0.73, 1.26)	-
<= 30 days	173	158 (91.33)	1		
> 30 days	77	68 (88.31)	0.96 (0.72, 1.28)	0.91 (0.67, 1.24)	-
<= 60 days	222	201 (90.54)	1		
> 60 days	28	25 (89.29)	0.84 (0.57, 1.23)	0.77 (0.50, 1.18)	-

CI = Confidence Interval: estimated using robust variance, *First viral load measure below 400 HIV RNA copies/ml. **Weighted with the inverse probability-of-ART-initiation that accounts for time-varying confounding by indication. [‡]Unweighted model that includes severity of immunosuppression (CD4 percentage below 25% for children younger than 1, or below 20% for children 1 to 2 years, or below 15% for children 3 to 15 years), viral load, weight-for-age Z score, and age at TB treatment initiation as covariates. [‡†]For each cut-off of timing to ART, children who are yet to be initiated on ART and whose follow-up time is less than the cut-off were excluded.

VII. CONCLUSIONS

A. Overall findings

Sub-Saharan Africa is home to 9 out of every 10 children living worldwide with HIV. In many countries in the region, the sustained declines of child mortality observed since the independence have been halted or reversed. In South Africa for example, where more than 5% of children 5 years or younger are HIV-infected [106], HIV accounts for 57% of all deaths among children under 5 years of age [6]. TB is a major cause of death among HIV-infected children in the region. Prevalence of HIV as high as 60% had been reported in some of the pediatric TB clinics [8]. Although ART has revolutionized the treatment of HIV worldwide and despite the ambitious goal of universal access to ART by 2010 [2, 107], access to ART in sub-Saharan Africa in general lags behind that of the rest of world and access for children substantially lags behind that of adults and tends to be clustered in urban areas [2, 38].

The major barrier for expanding ART into some of the poorest settings is the lack of health care infrastructures including trained health personnel [84]. For example, while frequent measurements of viral load and CD4 is the standard for monitoring ART in developed countries, the required technologies are not available or sustainable in those settings with no electricity and running water. WHO recommends that in settings, somatic growth (change in weight and height) be used. However, contrary to viral load that has a clear cut-point (below detection limit) clear cut-points for discriminating

children that are failing ART from those who are responding are yet to be defined for post-ART gains in weight, height, and CD4 count.

The first aim of this study was to construct reference charts for gains in weight, height, absolute CD4 count, and CD4% in the first 6 months of ART, and to assess the value of the 3rd, 10th, 25th, 33rd, and 50th percentiles as predictors of subsequent death, virological suppression, or treatment failure. We found that children with weight gain below the 33rd percentile at 6 months of ART had a four fold hazard of death, 1.6 fold hazard of treatment failure and were less likely (adjusted HR 0.8) to achieve viral suppression compared to children with weight gains above the 33rd percentile. Similar strengths of associations were observed for CD4 cell gain (both absolute count and CD4%), but no association was observed between height gain and treatment outcomes. These findings suggest that if the usual -2SD (or 3rd percentile) was to be used as cut-off for identifying children who are failing to gain appropriate weight or CD4 and therefore are failing ART, a substantial number of children at higher risk of death or treatment failure will be missed. Our results also show that children in our cohort gain substantially more weight than non-HIV infected North American children of the Fels Institute, indicating that reference distributions of weight gain from normal children are inappropriate for HIV-infected children on ART. In fact, it has been shown that weight and height velocity of children in the Soweto area might differ from that of children in the Fels Cohort [93]. Moreover, one should have expected that the grow velocity in children initiated on ART be higher than that of normal children. Following ART initiation there is a “catch-up” period of about 12 months during which the weight, height, and CD4 count

values of those immuno-suppressed children who were failing to thrive get back close to normal values albeit not completely [37].

Another major challenge for the roll out of pediatric ART in sub-Saharan Africa is the high burden of TB/HIV co-infection. Because of the substantial drug interactions and common side-effects between anti-TB and ART it is generally recommended to delay ART in HIV/TB co-infected children for at least 2-8 weeks. However, the optimal timing of ART in those patients is not known. In the aim # 2 of this study, we sought to determine the effect of delaying ART for at least 15, 30, or 60 days in HIV/TB co-infected children on virological suppression and survival. We found that delaying ART for less than 30 days after TB treatment initiation had no effect on survival or virological response. However, delays for 60 days or longer, though not statistically significant tended to increase the hazard of death and decrease that of achieving virologic suppression, suggesting that the current recommendation to delay ART in TB infected children should be revaluated.

A. Strengths

In addition to strengths discussed in chapters V and VI additional, other strengths of this study include:

1) The innovative statistical and epidemiological methods.

For aim one, we used the GAMLSS and BCPE distribution to construct the reference distribution of age-specific six-month gains in weight, height, CD4, and CD4%. GAMLSS is a semi-parametric method and because of the smoothed curves they produced, semi-parametric methods are the methods of choice for the construction of the growth curves are [65, 86]. Moreover, previous studies that used semi-parametric

methods to construct growth curves assumed a skewed normal distribution for the outcome variable [55, 66] and used the Box Cox power transformation to correct for potential skewness [67]. However, though kurtosis is not a major contribution to non-normality, presence of important kurtosis that is not accounted for has the potential to bias the estimated centiles curves. In our analyses, important kurtosis was present for the distribution of six-month weight and height gains for male, and CD4 gain. BPCE and GAMLSS are also the methods adopted by the WHO for the construction of growth reference [69].

For study aim #2, the major methodological challenges were to account for the time-varying confounding by indication introduced by the fact that sicker children who were at greater risk for death were more likely to be initiated on ART earlier while treatment was delayed for healthier ones. There was also a lead time that resulted from the fact that children in whom ART was delayed could die before ART initiation or before they could be classified for exposure. Special techniques were required to adjust for those two potential sources of bias. The IPTW method of marginal structural modelling is a valid method to account for those sources of bias assuming that the models for estimating the stabilized weights were not misspecified and that there were no unmeasured confounders [78]. With the IPTW we were able to account for substantial amount of bias in our estimate as evidenced by the difference between the results from multivariate Cox models and marginal structural models.

2) Careful definition of exposure for each of the aim and multiple outcomes

Whether for aim 1 or 2, we considered multiple cut-offs for the definition of exposures. For aim 1, previous studies that examined the association of growth velocity with the

prognosis of HIV [55] or response to ART [50], treated the normalized six-month weight and height gains as continuous and estimated the effect associated with 1SD change in six-month somatic growth. No specific cut-point was explored [55]. In the latter study after converting the weight and height measurements in Z score using the CDC database, they used +1SD (~90th percentile) as cut [50]. The WHO recommendation refers to children who are failing to thrive, which correspond to children falling below -2SD or ~3rd percentile. By considering multiple cut-points, we were able to show that -2SD is a too conservative cut-point because even children whose six-month weight gains were above the 33rd percentile were still at higher risk of treatment failure and death and needed closer follow-up.

For aim 1 as well as aim 2, with multiple cut-offs for the exposure, we were able to elicit a sort of “dose response” response relationship between exposure and outcomes that served as a proxy for the consistency of our results. In addition to multiple cut-points for the exposure, our consistent results across multiple outcomes could also be viewed as another indirect element of the internal validity of our study.

B. Limitations

Beyond the limitations discussed in chapter V and VI, for aim 1, we only examined the six-month gains in weight, height, CD4 and CD4%. Six months is the interval that has been used in the previous two similar studies [50, 55] and corresponds to the interval recommended for repeating viral load and CD4 in the clinic [57]. However, for some children, there might be a need for monitoring at closer intervals. Moreover, monitoring of ART does not stop after the first six months. Reference distributions for shorter intervals and beyond the first six months of ART are needed. Moreover, as we touched

on in the manuscript of paper #1, we used information from children treated in one single clinic in a poor urban neighborhood in South Africa. It is hard to speculate how results from this single cohort are generalizable or not to all HIV-infected children in South Africa or to the rest of the continent and beyond. However, results from the WHO multicenter growth study suggest that, when their health and nutrition needs are met, non HIV-infected children from diverse region of the world experience similar patterns of growth [69].

For aim 2, almost half of eligible children were excluded from the analysis because they were already on TB treatment at the first visit in the clinic and we were unable to determine how long they had been on it. Our study thus had a small sample size and our estimates had wide confidence intervals. Finally, the main assumption for interpreting our estimates as causal is that there were no unmeasured confounders. This is a strong assumption for an observational clinical cohort. Some indicators of ART such as hospitalization or social factors were not available in the dataset, and particular reasons for why a child was initiated on ART or delayed at a given visit were not recorded. Moreover for viral suppression, data on adherence was also not available. However, we do not believe that the residual bias alone, could explain our results.

C. Future directions

For public health: Our reference curves provide practitioners in settings where they have to use weight or CD4 as sole tools for monitoring the response to ART in HIV-infected children, effective and simple screening tools for discriminating those who are responding from those who are not responding well to ART. However, reference

distributions for shorter intervals and for longer follow-up period need to be constructed and the tools have to be introduced to clinicians in the field.

Despite the lack of statistical significance for the results of aim 2, they provide some evidence to clinicians and policy makers to reconsider the actual recommendation of delaying ART initiation in HIV/TB co-infected children. If the need to separate side effects of anti-TB from that of ART is deemed too important to justify the delay, it should not be more than 2 weeks.

For future research: For growth curves, in addition to obtaining more samples that will enable generalizing more broadly, future studies should consider shorter intervals and should go beyond the first 6 months of ART.

For timing of ART in HIV/TB co-infected children, in the absence of a randomized control trial, future studies should at least be prospective and appropriately powered.

Methodological considerations: GAMLSS is a new analytic technique and despite the flexibility it provides relative to the generalized linear model, it is yet to be introduced in analysis of epidemiological data. Potential areas of application might include for example overdispersion with count poisson data, parametric modeling of survival time, and other continuous or ordinal variable where simple logarithmic transformations fail to normalize it.

VIII. APPENDICES

A. Q-statistics and its interpretation

The main assumption of the Box-cox power exponential regression model is that after the appropriate transformation, the distribution of outcome is normally distributed independently of age and residuals should have a normal distribution and this can be evaluated using the residuals plots. However, the residual plots are not power to detect age related departure from the model (i.e. a poor fit of the model at given age point). To this end, Q-test have recently been proposed [72]. For the test, age is categorized in groups of approximate equal size and the Q-test statistics which are sensitive to age dependency are computed for the first four moments, z1, z2, z3, z4. Values larger than 2 indicate misfit of respectively the mean, variance, skewness or kurtosis.

B. Worm Plot and interpretation

The worm plot is a collection of detrended Q-Q plots, each of which applies to one of successive age groups. The data plot in each plot form a worm-like string. The shape of the worm indicate how the data differ from the assumed underlying distribution [73]. A flat worm indicates a good fit. The table below summarizes the interpretation of different patterns of the worm plot.

Table VIII-1. Interpretation of various patterns in the worm plot.

Shape	Moment	If the	Then the
Intercept	Mean	Worm passes above the origin	Fitted mean is too small
		Worm passes below the origin	Fitted mean is too large
Slope	Variance	Worm has a positive slope,	Fitted variance is too small
		Worm has a negative slope,	Fitted variance is too large
Parabola	Skewness	Worm has a U-shape,	Fitted distribution is too skew to the left.
		Worm has an inverted U-shape	Fitted distribution is too skew to the right.
S-curve	Kurtosis	Worm has an S-shape on the left bent down,	Tails of the fitted distribution are too light.
		Worm has an S-shape on the left bent up,	Tails of the fitted distribution are too heavy

Source: [73]

C. GAMLSS Models diagnostics

Overall, the fit of our models was relatively good. But for all our models the fit at extreme lower value for age was quite as good, particularly for the model for weight in female and absolute CD4 count. Transformation of age did improve the fit in those regions. But given that we are not focusing on extreme centile which would be much affected but those violations, and for the simplicity and interpretability of the constructed curve we proceeded with our models as presented.

Note: when df(υ) =0 (υ=1) and df(τ)=0 (τ =2), the BCPE distribution simplifies to the Box-Cox Cole and Green LMS distribution.

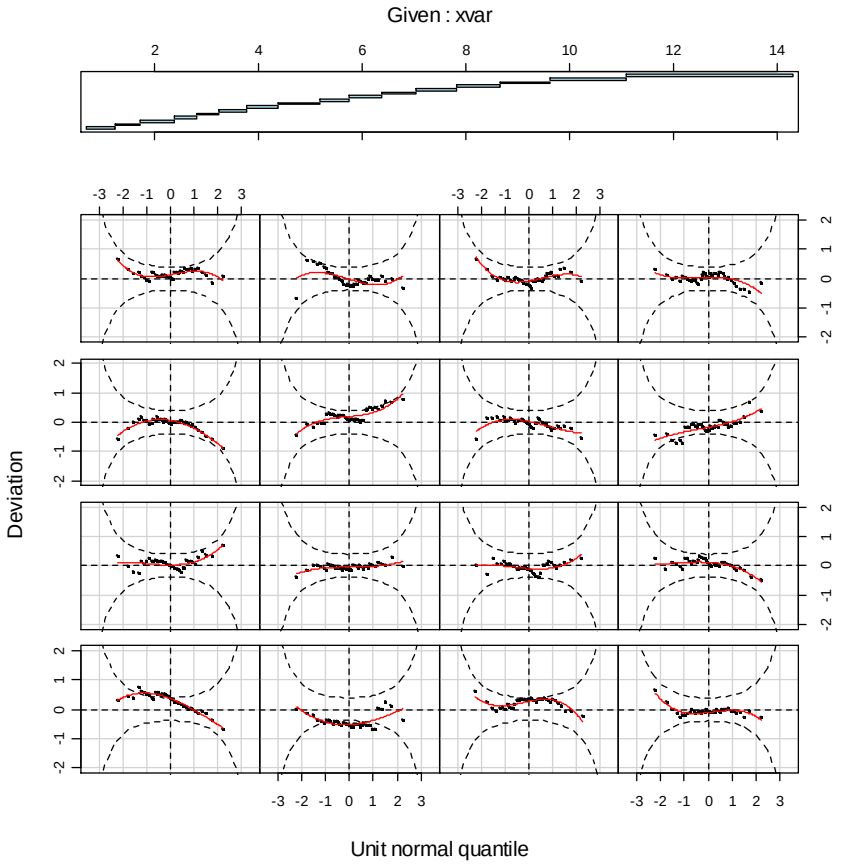
Weight gain male

Model: BCPE (age, df(μ) = 4, df(σ) = 0.5, df(υ) =0, df(τ)=1)

Table VIII-1. Q-statistics for the goodness-of-fit of finial model for weight gain in males

	Z1	Z2	Z3	Z4	AgostinoK2	N
0.68035 to 1.56468	0.753343	-0.59917	-1.14248	0.463149	1.519774	63
1.56468 to 2.42436	0.640133	0.1941	0.044676	-1.55722	2.426925	62
2.42436 to 3.18822	-0.67988	0.424044	1.201748	0.266181	1.515051	63
3.18822 to 4.08076	-0.65597	0.371518	-0.30841	0.14874	0.117237	63
4.08076 to 5.19507	0.237683	-0.91227	0.486766	-0.17307	0.266894	63
5.19507 to 6.12320	0.108389	-0.75613	-1.05729	0.668627	1.564917	62
6.12320 to 7.26488	0.64693	1.223694	-0.00754	0.655597	0.429864	63
7.26488 to 8.55715	-0.29321	1.13767	-0.29022	-0.35274	0.208653	63
8.55715 to 10.2600	0.234524	-0.54883	-0.03799	1.252063	1.569105	62
10.2600 to 14.2874	0.023547	0.159488	0.435311	-0.75528	0.759951	63
TOTAL Q stats	2.498107	5.236869	4.476593	5.901777	10.37837	627
df for Q stats	3.999227	8.249898	7.97512	7.000358	14.97548	0
p-val for Q stats	0.644857	0.75417	0.809858	0.551305	0.793911	0

Figure VIII-1. Worm plots for the final model for weight gain among males

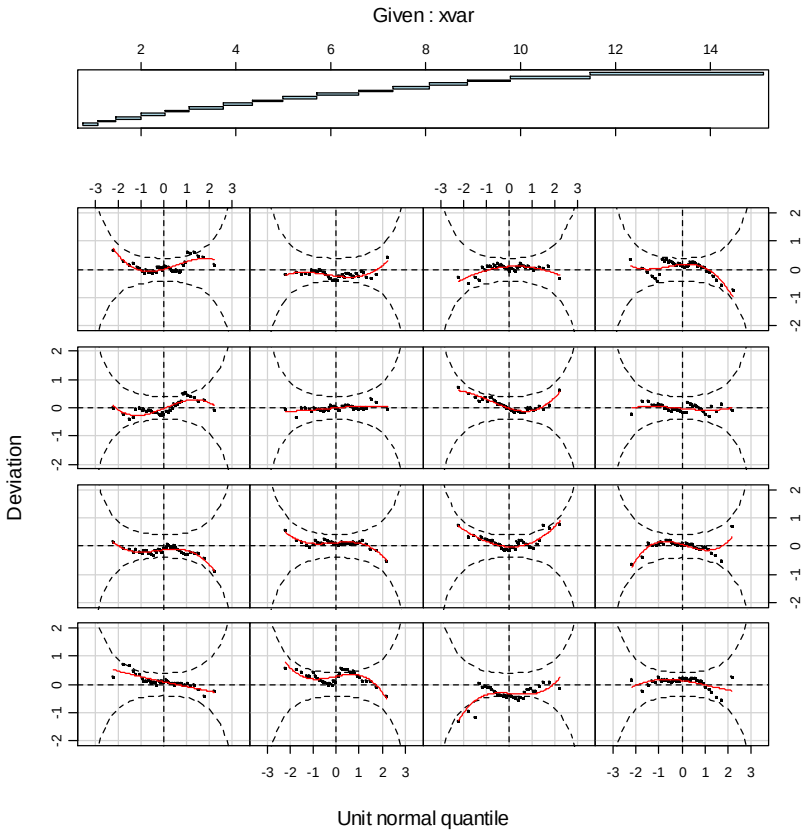

Given : xvar
2
4
6
8
10
12
14
Deviation
Unit normal quantile
-3 -2 -1 0 1 2 3
-2 -1 0 1 2

Weight gain female
Model: BCPE (age, df(μ) = 5.3, df(σ) = 0, df(υ) =0, df(τ)=0)

Table VIII-2. Q-statistics for the goodness-of-fit of finial model for weight gain in females

	Z1	Z2	Z3	Z4	AgostinoK2	N
0.75154 to 1.28542	2.007267	-1.27786	0.328482	-1.10459	1.328023	60
1.28542 to 2.04928	-2.05858	1.745161	-0.35878	1.724085	3.10119	60
2.04928 to 2.94455	-0.40893	-1.70453	-1.62114	-0.58662	2.972218	61
2.94455 to 3.93292	0.450993	0.300249	2.319167	1.566385	7.8321	61
3.93292 to 4.99520	0.087439	-0.28438	0.913401	2.651014	7.862178	59
4.99520 to 6.12046	-0.36081	1.637159	0.528791	-0.88927	1.070419	60
6.12046 to 7.44558	0.399437	-0.91208	1.095213	1.757966	4.289935	60
7.44558 to 8.81451	0.389042	-0.34063	1.756777	-0.35594	3.212959	61
8.81451 to 10.2765	-0.3326	-0.43225	0.408673	0.665923	0.610467	60
10.2765 to 15.1088	-0.03571	0.336292	-2.59883	0.162253	6.780231	60
TOTAL Q stats	9.198117	11.68308	20.56385	18.49587	39.05972	602
df for Q stats	3.868961	8.492491	9	10	19	0
p-val for Q stats	0.051616	0.197033	0.014734	0.047153	0.004339	0

Figure VIII-2. Worm plots for the final model for weight gain among males

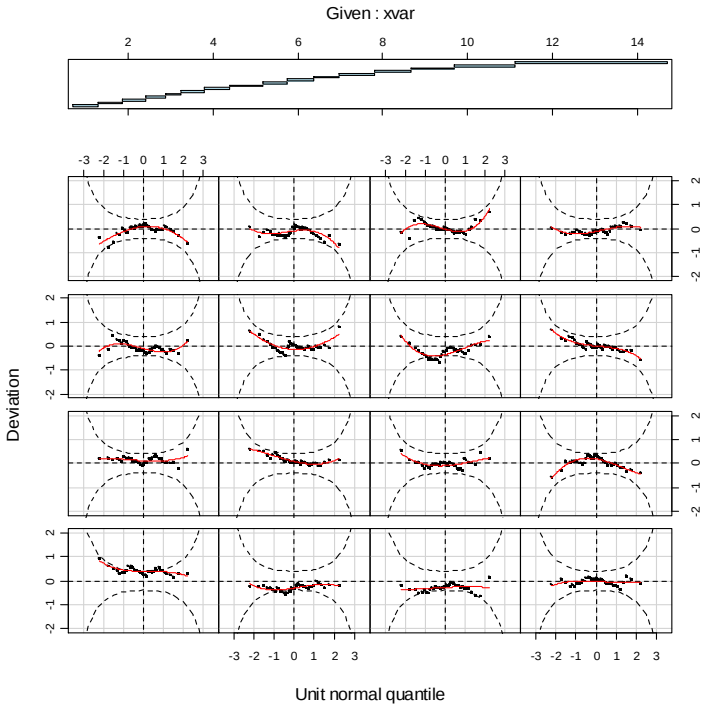


Height gain male
Model: BCPE (age, df(μ) =9.1 , df(σ) =0 , df(υ) =0, df(τ)=2)

Table VIII-3. Q-statistics for the goodness-of-fit of finial model for Height gain in males

	Z1	Z2	Z3	Z4	AgostinoK2	N
0.68035 to 1.38672	1.27526	0.381458	-0.0689	-0.63693	0.410428	44
1.38672 to 2.01368	-1.56072	0.281042	-0.2351	-0.47343	0.279407	44
2.01368 to 2.61054	-0.47297	0.875998	-0.24158	-0.15528	0.082471	43
2.61054 to 3.11704	0.415657	0.342519	0.435325	0.244075	0.24908	44
3.11704 to 3.67282	-0.02562	-2.4071	1.610587	1.467083	4.746324	44
3.67282 to 4.34633	0.33205	0.207718	0.937359	-0.44964	1.080819	44
4.34633 to 5.18138	0.528128	-0.39264	-2.12795	1.220003	6.016582	43
5.18138 to 5.79466	-0.5351	-0.54854	0.142027	1.156836	1.358442	44
5.79466 to 6.54757	-0.33341	-0.31166	1.763032	1.306524	4.815286	44
6.54757 to 7.42094	-0.26791	0.939945	0.900098	-1.15452	2.143097	44
7.42094 to 8.22313	0.257151	-1.90244	-0.79888	0.178359	0.670015	44
8.22313 to 9.41409	-0.82758	0.392116	-1.48084	-0.13978	2.21242	43
9.41409 to 11.0184	-1.33601	0.665045	1.544616	1.466037	4.535104	44
11.0184 to 14.7091	0.08955	0.299359	-0.1474	-0.64602	0.439071	44
TOTAL Q stats	7.861671	12.68709	17.48598	11.55257	29.03855	613
df for Q stats	2.89827	12.49148	11.98122	10.00051	21.98173	0
p-val for Q stats	0.045174	0.431477	0.131427	0.316157	0.143161	0

Figure VIII-3. Worm plots for the final model for height gain among males

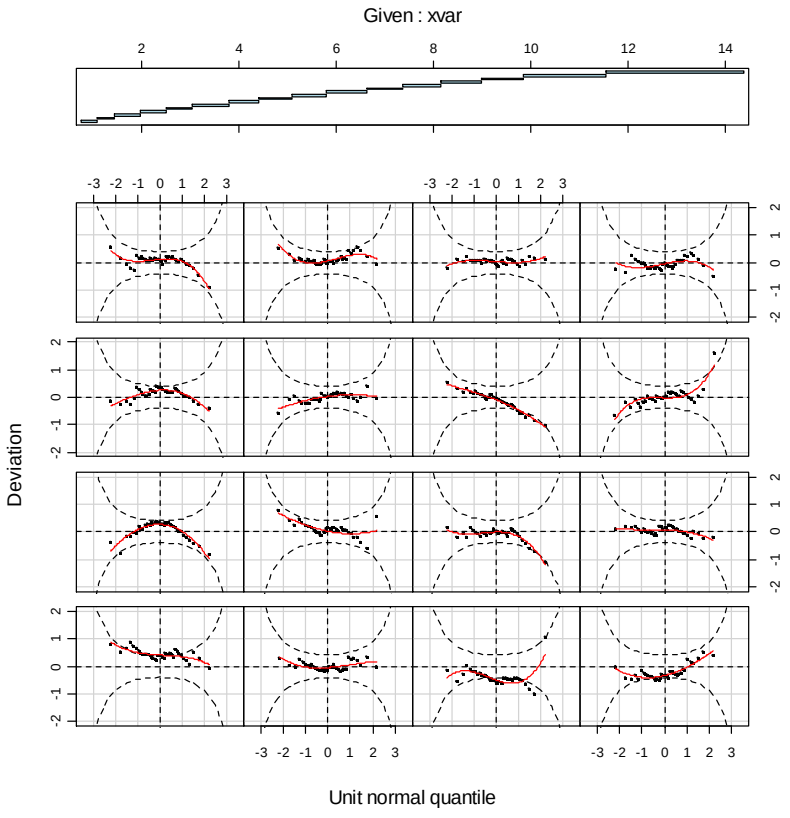


Height gain female
Model: BCPE (age, df(μ) =7, df(σ) =0, df(υ) =0, df(τ)=0)

Table VIII-4. Q-statistics for the goodness-of-fit of finial model for weight gain in females

	Z1	Z2	Z3	Z4	AgostinoK2	N
0.75154 to 1.24982	2.524756	-0.92259	0.403968	-0.59655	0.519058	54
1.24982 to 1.91512	-2.21731	0.484323	2.321226	1.946641	9.177505	54
1.91512 to 2.72005	-1.00522	1.573861	-0.21604	-0.01901	0.047035	54
2.72005 to 3.64544	0.316918	-1.21314	0.722675	0.530918	0.804133	54
3.64544 to 4.61464	-0.05207	-1.72777	-1.54618	-0.40157	2.551919	54
4.61464 to 5.64681	0.603876	-0.60191	-1.1207	0.124588	1.271497	54
5.64681 to 6.72826	-0.32348	0.779021	-0.84604	-0.3555	0.842171	54
6.72826 to 7.81245	-0.16848	0.447394	2.255154	2.445696	11.06715	54
7.81245 to 9.01437	-0.08185	-0.55527	-1.25779	0.973282	2.529316	54
9.01437 to 10.4654	0.462762	-0.70317	0.698815	-1.28756	2.146155	54
10.4654 to 14.3805	-0.05482	1.010861	-0.05086	-0.3248	0.108083	54
TOTAL Q stats	13.12603	11.01362	17.64133	13.42269	31.06402	594
df for Q stats	2.201042	9.493401	8.985375	8.985916	17.97129	0
p-val for Q stats	0.001818	0.314328	0.039301	0.143669	0.028015	0

Figure VIII-4 Worm plots for the final model for height gain among females

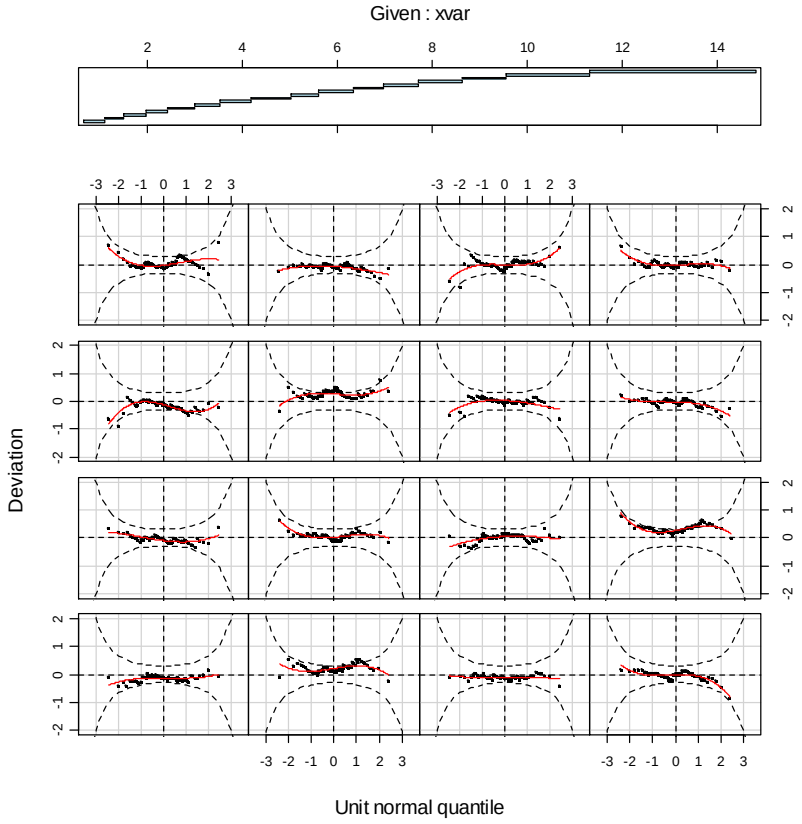


CD4 percentage gain
Model : BCPE (age, df(μ) = 0, df(σ) = 5, df(υ) =0, df(τ)=0)

Table VIII-5. Q-statistics for the goodness-of-fit of finial model for CD4% gain

	Z1	Z2	Z3	Z4	AgostinoK2	N
0.65571 to 1.34565	-0.20463	1.309294	0.191018	-0.27737	0.113421	106
1.34565 to 2.06297	-0.85258	-0.1745	-0.82565	0.278797	0.759429	106
2.06297 to 2.85694	-0.21539	-2.1985	-0.22455	-2.57753	6.694096	106
2.85694 to 3.80424	0.247904	-0.48251	1.098682	0.642409	1.619792	106
3.80424 to 5.02532	2.124456	1.284236	-0.08319	-0.89854	0.814293	106
5.02532 to 5.99999	-0.15714	0.31015	-0.78325	1.549324	3.013884	105
5.99999 to 7.17727	0.385877	-0.1298	-1.24144	1.201899	2.985743	106
7.17727 to 8.43668	-0.65773	-0.97501	0.393026	-2.41913	6.006639	106
8.43668 to 10.2600	-0.03182	0.473693	0.22752	0.896653	0.855752	106
10.2600 to 14.7994	0.510112	0.186189	0.73189	0.127458	0.551908	106
TOTAL Q stats	6.257357	9.7829	4.879195	18.53576	23.41496	1059
df for Q stats	7.914749	6.999646	9	9	18	0
p-val for Q stats	0.609623	0.201188	0.844709	0.029443	0.175144	0

Figure VIII-5. Worm plots from the model for CD4% gain

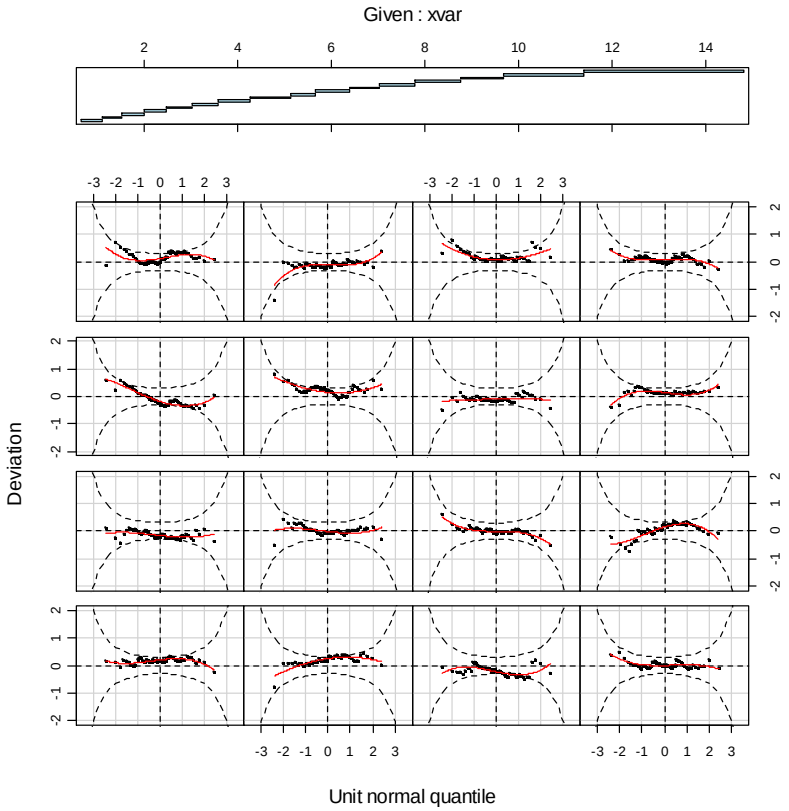


Absolute CD4 count
Model: BCPE (age, df(μ) = 3, df(σ) = 0, df(υ) =0, df(τ)=1)

TableVIII-6. Q-statistics for the goodness-of-fit of finial model for absolute CD4 gain

	Z1	Z2	Z3	Z4	AgostinoK2	N
0.65571 to 1.36755	1.947251	0.71608	-0.5052	-1.70078	3.147884	106
1.36755 to 2.09856	-0.89405	0.253593	-0.26231	1.218888	1.554497	105
2.09856 to 2.90622	-1.07904	-0.46138	0.618735	0.0352	0.384072	105
2.90622 to 3.86995	-0.3166	-1.06151	0.26402	0.722429	0.59161	106
3.86995 to 5.12936	0.663851	0.791278	-1.6178	-0.43472	2.806248	106
5.12936 to 6.12320	0.895421	-1.83715	2.599934	1.326597	8.519515	105
6.12320 to 7.26488	-0.38414	0.037088	0.344691	-0.09243	0.127355	105
7.26488 to 8.55715	1.028	0.193066	-0.83233	0.291286	0.777627	107
8.55715 to 10.2874	0.064079	1.2119	0.205509	1.722284	3.008495	105
10.2874 to 14.7994	0.956308	-0.39573	0.567051	-0.57468	0.6518	105
TOTAL Q stats	9.221123	7.581947	11.32887	10.24023	21.5691	1055
df for Q stats	4.99944	8.46427	7.953775	6.999704	14.95348	0
p-val for Q stats	0.100533	0.523177	0.180768	0.175344	0.117995	0

Figure VIII-6. Worm plots from the model for absolute CD4 count gain



IX. References

1. UNAIDS, *2007 AIDS epidemic update*. 2007: Geneva, Switzerland.
2. World Health Organization, *Towards universal access: Scaling up priority HIV/AIDS interventions in the health sector - Progress report 2008*. 2008, WHO: Geneva, Switzerland.
3. De Cock, K.M.,M.G. Fowler,E. Mercier,I. de Vincenzi,J. Saba,E. Hoff*et al.*, *Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice.* Jama, 2000. **283**(9): p. 1175-82.
4. Newell, M.L.,H. Coovadia,M. Cortina-Borja,N. Rollins,P. Gaillard, and F. Dabis, *Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis.* Lancet, 2004. **364**(9441): p. 1236-43.
5. Shisana O,Rehle T,Simbayi LC,Parker W,Zuma K,Bhana A*et al.*, *South African National HIV Prevalence, HIV Incidence, Behaviour and Communication Survey, 2005* 2005, Human Research Council: Cape Town, South Africa.
6. World Health Organization, *World Health Statistics 2008*. 2008, World Health Organization: Geneva, Switzerland.
7. Corbett, E.L.,C.J. Watt,N. Walker,D. Maher,B.G. Williams,M.C. Raviglione*et al.*, *The growing burden of tuberculosis: global trends and interactions with the HIV epidemic.* Arch Intern Med, 2003. **163**(9): p. 1009-21.

8. Coovadia, H.M.,P. Jeena, and D. Wilkinson, *Childhood human immunodeficiency virus and tuberculosis co-infections: reconciling conflicting data.* Int J Tuberc Lung Dis, 1998. **2**(10): p. 844-51.

9. Nelson, L.J.,E. Schneider,C.D. Wells, and M. Moore, *Epidemiology of childhood tuberculosis in the United States, 1993-2001: the need for continued vigilance.* Pediatrics, 2004. **114**(2): p. 333-41.

10. Nelson, L.J., and C.D. Wells, *Global epidemiology of childhood tuberculosis.* Int J Tuberc Lung Dis, 2004. **8**(5): p. 636-47.

11. Frieden, T.R.,T.R. Sterling,S.S. Munsiff,C.J. Watt, and C. Dye, *Tuberculosis.* Lancet, 2003. **362**(9387): p. 887-99.

12. Small, P.M., and P.I. Fujiwara, *Management of tuberculosis in the United States.* N Engl J Med, 2001. **345**(3): p. 189-200.

13. Fauci, A.S., *The human immunodeficiency virus: infectivity and mechanisms of pathogenesis.* Science, 1988. **239**(4840): p. 617-22.

14. Sattentau, Q.J., and R.A. Weiss, *The CD4 antigen: physiological ligand and HIV receptor.* Cell, 1988. **52**(5): p. 631-3.

15. Chakraborty, R., *HIV-1 infection in children: a clinical and immunologic overview.* Curr HIV Res, 2005. **3**(1): p. 31-41.

16. *Natural history of vertically acquired human immunodeficiency virus-1 infection. The European Collaborative Study.* Pediatrics, 1994. **94**(6 Pt 1): p. 815-9.

17. Newton, S.M.,A.J. Brent,S. Anderson,E. Whittaker, and B. Kampmann, *Paediatric tuberculosis.* Lancet Infect Dis, 2008. **8**(8): p. 498-510.

18. Mukadi, Y.D.,S.Z. Wiktor,I.M. Coulibaly,D. Coulibaly,A. Mbengue,A.M. Folquet*et al.*, *Impact of HIV infection on the development, clinical presentation, and outcome of tuberculosis among children in Abidjan, Cote d'Ivoire.* Aids, 1997. **11**(9): p. 1151-8.

19. Palme, I.B.,B. Gudetta,J. Bruchfeld,L. Muhe, and J. Giesecke, *Impact of human immunodeficiency virus 1 infection on clinical presentation, treatment outcome and survival in a cohort of Ethiopian children with tuberculosis.* Pediatr Infect Dis J, 2002. **21**(11): p. 1053-61.

20. Elenga, N.,K.A. Kouakoussui,D. Bonard,P. Fassinou,M.F. Anaky,M.L. Wemin*et al.*, *Diagnosed tuberculosis during the follow-up of a cohort of human immunodeficiency virus-infected children in Abidjan, Cote d'Ivoire: ANRS 1278 study.* Pediatr Infect Dis J, 2005. **24**(12): p. 1077-82.

21. Walters, E.,M.F. Cotton,H. Rabie,H.S. Schaaf,L.O. Walters, and B.J. Marais, *Clinical presentation and outcome of tuberculosis in human immunodeficiency virus infected children on anti-retroviral therapy.* BMC Pediatr, 2008. **8**: p. 1.

22. *Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection - February 28, 2008*. 2008 [cited 2008 March 21].

23. Food and Drugs Administration. *significant declines in HIV-related morbidity andhospitalizations in childrenhave been observed inthe United States and Europe over the same time period*. 2007 [cited 2007 March 21,]; Available from: http://www.fda.gov/oashi/aids/pedlbl.html.

24. World Health Organization, *Antiretroviral Therapy of HIV Infection in Infants and Children in Resource-Limited Settings: Towards Universal Access*, W.H. Organization, Editor. 2006, World Health Organization: Geneva, Switzerland.

25. Bellibas, S.E.,Z. Siddique,A. Dorr,A. Bertasso,P. Sista,S.J. Koliset *al.*, *Pharmacokinetics of enfuvirtide in pediatric human immunodeficiency virus 1-infected patients receiving combination therapy.* Pediatr Infect Dis J, 2004. **23**(12): p. 1137-41.

26. Church, J.A.,M. Hughes,J. Chen,P. Palumbo,L.M. Mofenson,P. Deloraet *al.*, *Long term tolerability and safety of enfuvirtide for human immunodeficiency virus 1-infected children.* Pediatr Infect Dis J, 2004. **23**(8): p. 713-8.

27. Soy, D.,F.T. Aweeka,J.A. Church,C.K. Cunningham,P. Palumbo,B.W. Koselet *al.*, *Population pharmacokinetics of enfuvirtide in pediatric patients with human immunodeficiency virus: searching for exposure-response relationships.* Clin Pharmacol Ther, 2003. **74**(6): p. 569-80.

28. Wiznia, A.,J. Church,P. Emmanuel,S. Eppes,L. Rowell,C. Evanset *al.*, *Safety and efficacy of enfuvirtide for 48 weeks as part of an optimized antiretroviral regimen in pediatric human immunodeficiency virus 1-infected patients.* Pediatr Infect Dis J, 2007. **26**(9): p. 799-805.

29. Zhang, X.,T. Lin,A. Bertasso,C. Evans,A. Dorr,S.J. Koliset *al.*, *Population pharmacokinetics of enfuvirtide in HIV-1-infected pediatric patients over 48 weeks of treatment.* J Clin Pharmacol, 2007. **47**(4): p. 510-7.

30. Mofenson, L.M.,J. Oleske,L. Serchuck,R. Van Dyke, and C. Wilfert, *Treating opportunistic infections among HIV-exposed and infected children: recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America.* MMWR Recomm Rep, 2004. **53**(RR-14): p. 1-92.

31. Eley, B.,J. Nuttall,M.A. Davies,L. Smith,C. Cowburn,H. Buys*et al., Initial experience of a public sector antiretroviral treatment programme for HIV-infected children and their infected parents.* S Afr Med J, 2004. **94**(8): p. 643-6.

32. George, E.,F. Noel,G. Bois,R. Cassagnol,L. Estavien,M. Rouzier Pde*et al., Antiretroviral therapy for HIV-1-infected children in Haiti.* J Infect Dis, 2007. **195**(10): p. 1411-8.

33. Hammer, S.M.,K.E. Squires,M.D. Hughes,J.M. Grimes,L.M. Demeter,J.S. Currier*et al., A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team.* N Engl J Med, 1997. **337**(11): p. 725-33.

34. Puthanakit, T.,L. Aurpibul,P. Oberdorfer,N. Akarathum,S. Kanjanavanit,P. Wannarit*et al., Sustained immunologic and virologic efficacy after four years of highly active antiretroviral therapy in human immunodeficiency virus infected children in Thailand.* Pediatr Infect Dis J, 2007. **26**(10): p. 953-6.

35. Reddi, A.,S.C. Leeper,A.C. Grobler,R. Geddes,K.H. France,G.L. Dorse*et al., Preliminary outcomes of a paediatric highly active antiretroviral therapy cohort from KwaZulu-Natal, South Africa.* BMC Pediatr, 2007. **7**: p. 13.

36. Eley, B.,M.A. Davies,P. Apolles,C. Cowburn,H. Buys,M. Zampoli*et al., Antiretroviral treatment for children.* S Afr Med J, 2006. **96**(9 Pt 2): p. 988-93.

37. Moultrie, H.,M. Yotebieng,L. Kuhn, and T. Meyers. *Mortality and Virological Outcomes of 2105 HIV-infected Children Receiving ART in Soweto, South Africa*. in *16th Conference on Retroviruses and Oportunistic Infections*. 2009. Montreal, Canada.

38. Sutcliffe, C.G.,J.H. van Dijk,C. Bolton,D. Persaud, and W.J. Moss, *Effectiveness of antiretroviral therapy among HIV-infected children in sub-Saharan Africa.* Lancet Infect Dis, 2008. **8**(8): p. 477-89.

39. Wamalwa, D.C.,C. Farquhar,E.M. Obimbo,S. Selig,D.A. Mbori-Ngacha,B.A. Richardson*et al.*, *Early response to highly active antiretroviral therapy in HIV-1-infected Kenyan children.* J Acquir Immune Defic Syndr, 2007. **45**(3): p. 311-7.

40. McKinney, R.E., Jr., and J.W. Robertson, *Effect of human immunodeficiency virus infection on the growth of young children. Duke Pediatric AIDS Clinical Trials Unit.* J Pediatr, 1993. **123**(4): p. 579-82.

41. Moye, J., Jr.,K.C. Rich,L.A. Kalish,A.R. Sheon,C. Diaz,E.R. Cooper*et al.*, *Natural history of somatic growth in infants born to women infected by human immunodeficiency virus. Women and Infants Transmission Study Group.* J Pediatr, 1996. **128**(1): p. 58-69.

42. Arpadi, S.M.,P.A. Cuff,D.P. Kotler,J. Wang,M. Bamji,M. Lange*et al.*, *Growth velocity, fat-free mass and energy intake are inversely related to viral load in HIV-infected children.* J Nutr, 2000. **130**(10): p. 2498-502.

43. Newell, M.L.,M.C. Borja, and C. Peckham, *Height, weight, and growth in children born to mothers with HIV-1 infection in Europe.* Pediatrics, 2003. **111**(1): p. e52-60.

44. Nachman, S.A.,J.C. Lindsey,J. Moye,K.E. Stanley,G.M. Johnson,P.A. Krogstad*et al.*, *Growth of human immunodeficiency virus-infected children receiving highly active antiretroviral therapy.* Pediatr Infect Dis J, 2005. **24**(4): p. 352-7.

45. Guillen, S.,J.T. Ramos,R. Resino,J.M. Bellon, and M.A. Munoz, *Impact on weight and height with the use of HAART in HIV-infected children.* Pediatr Infect Dis J, 2007. **26**(4): p. 334-8.

46. Kabue, M.M.,A. Kekitiinwa,A. Maganda,J.M. Risser,W. Chan, and M.W. Kline, *Growth in HIV-Infected Children Receiving Antiretroviral Therapy at a Pediatric Infectious Diseases Clinic in Uganda.* AIDS Patient Care STDS, 2008.

47. Buchacz, K.,J.S. Cervia,J.C. Lindsey,M.D. Hughes,G.R. Seage, 3rd,W.M. Dankner*et al.*, *Impact of protease inhibitor-containing combination antiretroviral therapies on height and weight growth in HIV-infected children.* Pediatrics, 2001. **108**(4): p. E72.

48. CDC. *A SAS program for CDC growth charts*. 2000 May 22, 2007 [cited 2008 December 31]; Available from: http://www.cdc.gov/NCCDPHP/dnpa/growthcharts/resources/sas.htm.

49. WHO Multicentre Growth Reference Study Group, *WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height, and Body Mass Index-for-Age: Methods and Development.*, W. Press, Editor. 2006, WHO: Geneva, Switzerland.

50. Benjamin, D.K., Jr.,W.C. Miller,R.W. Ryder,D.J. Weber,E. Walter, and R.E. McKinney, Jr., *Growth patterns reflect response to antiretroviral therapy in HIV-positive infants: potential utility in resource-poor settings.* AIDS Patient Care STDS, 2004. **18**(1): p. 35-43.

51. Berhane, R.,D. Bagenda,L. Marum,E. Aceng,C. Ndugwa,R.J. Bosch*et al.*, *Growth failure as a prognostic indicator of mortality in pediatric HIV infection.* Pediatrics, 1997. **100**(1): p. E7.

52. Brettler, D.B.,A. Forsberg,E. Bolivar,F. Brewster, and J. Sullivan, *Growth failure as a prognostic indicator for progression to acquired immunodeficiency syndrome in children with hemophilia.* J Pediatr, 1990. **117**(4): p. 584-8.

53. Clarick, R.H.,W.A. Hanekom,R. Yogev, and E.G. Chadwick, *Megestrol acetate treatment of growth failure in children infected with human immunodeficiency virus.* Pediatrics, 1997. **99**(3): p. 354-7.

54. McKinney, R.E., Jr., and C. Wilfert, *Growth as a prognostic indicator in children with human immunodeficiency virus infection treated with zidovudine. AIDS Clinical Trials Group Protocol 043 Study Group.* J Pediatr, 1994. **125**(5 Pt 1): p. 728-33.

55. Carey, V.J.,F.H. Yong,L.M. Frenkel, and R.E. McKinney, Jr., *Pediatric AIDS prognosis using somatic growth velocity.* Aids, 1998. **12**(11): p. 1361-9.

56. Baumgartner, R.N.,A.F. Roche, and J.H. Himes, *Incremental growth tables: supplementary to previously published charts.* Am J Clin Nutr, 1986. **43**(5): p. 711-22.

57. Meyers, T.,B. Eley, and W. Leoning, *Guidelines for the management of HIV-infected children - 2005*, Health, Editor. 2005, Jacana Media.

58. Department of Health South Africa, *Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa*. 2003.

59. *National Tuberculosis Control Programme Practical Guidelines*, Health, Editor. 2000.

60. van Belle, G.,L. Fisher,P. Heagerty, and L. T., *Biostatistics: A Methodology for the Health Sciences*. 2nd ed. 2004, Hoboken, NewJersey.: John Wiley&Sons, Inc. 883.

61. Bolton-Moore, C.,M. Mubiana-Mbewe,R.A. Cantrell,N. Chintu,E.M. Stringer,B.H. Chi*et al.*, *Clinical outcomes and CD4 cell response in children receiving antiretroviral therapy at primary health care facilities in Zambia.* Jama, 2007. **298**(16): p. 1888-99.

62. Centers for Disease Control and Prevention. *A SAS program for CDC growth charts*. [cited October 10, 2007]; Available from: http://www.cdc.gov/nccdphp/dnpa/growthcharts/sas.htm.

63. Collett D, *Modelling Survival data in Medical Research*. 2nd ed. 2003, Florida: Chapman&Hall/CDC.

64. Cleveland, W.,S. Devlin, and E. Grosse, *Regression by local fitting.* Journal of Econometrics, 1988. **37**: p. 87-114.

65. Wei, Y.,A. Pere,R. Koenker, and X. He, *Quantile regression methods for reference growth charts.* Stat Med, 2006. **25**(8): p. 1369-82.

66. Cole, T.J., and P.J. Green, *Smoothing reference centile curves: the LMS method and penalized likelihood.* Stat Med, 1992. **11**(10): p. 1305-19.

67. Box, G.E.P., and D.R. Cox, *An analysis of transformations.* Journal of the Royal Statistical Society, Series B (Methodological), 1964. **26**: p. 211-243.

68. Rigby, R.A., and D.M. Stasinopoulos, *Smooth centile curves for skew and kurtotic data modelled using the Box-Cox power exponential distribution.* Stat Med, 2004. **23**(19): p. 3053-76.

69. WHO Multicentre Growth Reference Study Group, *WHO Child Growth Standards: Growth velocity based on weight, length and head circumference: Methods and development.* 2009, World Health Organization: Geneva. p. 242.

70. Stasinopoulos, D., and R. Rigby, *Generalized additive models for location scale and shape (GAMLSS) in R.* Journal of Statistical Software, 2007. **23**(7).

71. Stasinopoulos, D.,R. Rigby, and C. Akantziliotou, *Instructions on how to use the gamlss package in R*. Second ed. 2008.

72. Royston, P., and E.M. Wright, *Goodness-of-fit statistics for age-specific reference intervals.* Stat Med, 2000. **19**(21): p. 2943-62.

73. van Buuren, S., and M. Fredriks, *Worm plot: a simple diagnostic device for modelling growth reference curves.* Stat Med, 2001. **20**(8): p. 1259-77.

74. Cox, D.R., *Regression Models and Life-Tables.* Journal of the Royal Statistical Society. Series B (Methodological), 1972. **34**(2): p. 187-220.

75. Lin, D.Y.,L.J. Wei, and Z. Ying, *Checking the Cox Model with Cumulative Sums of Martingale-Based Residuals.* Biometrika, 1993. **80**(3): p. 557-572.

76. Hernan, M.A.,B. Brumback, and J.M. Robins, *Marginal structural models to estimate the causal effect of zidovudine on the survival of HIV-positive men.* Epidemiology, 2000. **11**(5): p. 561-70.

77. Hernan, M.A.,E. Lanoy,D. Costagliola, and J.M. Robins, *Comparison of dynamic treatment regimes via inverse probability weighting.* Basic Clin Pharmacol Toxicol, 2006. **98**(3): p. 237-42.

78. Robins, J.M.,M.A. Hernan, and B. Brumback, *Marginal structural models and causal inference in epidemiology.* Epidemiology, 2000. **11**(5): p. 550-60.

79. Horvitz, D.G., and D.J. Thompson, *A Generalization of Sampling Without Replacement From a Finite Universe* Journal of the American Statistical Association, 1952. **47**(260): p. 663-685

80. Little, R.J., *Inference with Survey Weights.* Journal of Official Statistics, 1991. **7**(4): p. 405-424.

81. Hernan, M.A., and J.M. Robins, *Estimating causal effects from epidemiological data.* J Epidemiol Community Health, 2006. **60**(7): p. 578-86.

82. Rosenbaum, P.R., and D.B. Rubin, *The central role of the propensity score in observational studies for causal effects.* Biometrika, 1983. **70**: p. 41-55.

83. Cole, S.R., and M.A. Hernan, *Constructing inverse probability weights for marginal structural models.* Am J Epidemiol, 2008. **168**(6): p. 656-64.

84. Kober, K., and W. Van Damme, *Scaling up access to antiretroviral treatment in southern Africa: who will do the job?* Lancet, 2004. **364**(9428): p. 103-7.

85. World Health Organization. *Task shifting: rational redistribution of tasks among health workforce teams, global recommendations and guidelines*. 2008 [cited 2009 January 4].

86. Carey, V.J.,F.H. Yong,L.M. Frenkel, and R.M. McKinney, *Growth velocity assessment in paediatric AIDS: smoothing, penalized quantile regression and the definition of growth failure.* Stat Med, 2004. **23**(3): p. 509-26.

87. Rigby, R.A., and D.M. stasinopoulos, *Generalized additive models for location, scale and shape.* Journal of the Royal Statistical Society: Series C (Applied Statistics), 2005. **54**(3): p. 507 - 554.

88. Bakshi, S.S.,P. Britto,E. Capparelli,L. Mofenson,M.G. Fowler,S. Rasheed*et al.*, *Evaluation of pharmacokinetics, safety, tolerance, and activity of combination of zalcitabine and zidovudine in stable, zidovudine-treated pediatric patients with human immunodeficiency virus infection. AIDS Clinical Trials Group Protocol 190 Team.* J Infect Dis, 1997. **175**(5): p. 1039-50.

89. Englund, J.A.,C.J. Baker,C. Raskino,R.E. McKinney,B. Petrie,M.G. Fowler*et al.*, *Zidovudine, didanosine, or both as the initial treatment for symptomatic HIV-infected children. AIDS Clinical Trials Group (ACTG) Study 152 Team.* N Engl J Med, 1997. **336**(24): p. 1704-12.

90. Kline, M.W.,R.B. Van Dyke,J.C. Lindsey,M. Gwynne,M. Culnane,R.E. McKinney, Jr.*et al.*, *A randomized comparative trial of stavudine (d4T) versus zidovudine (ZDV, AZT) in*

children with human immunodeficiency virus infection. AIDS Clinical Trials Group 240 Team. Pediatrics, 1998. **101**(2): p. 214-20.

91. McKinney, R.E., Jr.,G.M. Johnson,K. Stanley,F.H. Yong,A. Keller,K.J. O'Donnell*et al.*, *A randomized study of combined zidovudine-lamivudine versus didanosine monotherapy in children with symptomatic therapy-naive HIV-1 infection. The Pediatric AIDS Clinical Trials Group Protocol 300 Study Team.* J Pediatr, 1998. **133**(4): p. 500-8.
92. Roche, A.F., and J.H. Himes, *Incremental growth charts.* Am J Clin Nutr, 1980. **33**(9): p. 2041-52.
93. Cameron, N.,T. De Wet,G. Ellison, and B. Bogin, *Growth in height and weight of South African urban infants from birth to five years: The Birth to Ten Study.* Am. J. Hum. Biol. , 1998. **10**(4): p. 495-504.
94. Voss, L.D.,T.J. Wilkin,B.J. Bailey, and P.R. Betts, *The reliability of height and height velocity in the assessment of growth (the Wessex Growth Study).* Arch Dis Child, 1991. **66**(7): p. 833-7.
95. Marais, B.J.,S.M. Graham,M.F. Cotton, and N. Beyers, *Diagnostic and management challenges for childhood tuberculosis in the era of HIV.* J Infect Dis, 2007. **196 Suppl 1**: p. S76-85.
96. *South African National Tuberculosis Guidelines*. 2008.
97. Department of Health. *Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa*. 2003 19 November 2003 [cited 2009 April 2].
98. Lin, D.Y., and L.J. Wei, *The Robust Inference for the Proportional Hazards Model.* Journal of the American Statistical Association, 1989. **84**: p. 1074-1078.

99. Callens, S.F.,N. Shabani,J. Lusiama,P. Lelo,F. Kitetele,R. Colebunders*et al.*, *Mortality and Associated Factors After Initiation of Pediatric Antiretroviral Treatment in the Democratic Republic of the Congo.* Pediatr Infect Dis J, 2009. **28**(1): p. 35-40.

100. Violari, A.,M.F. Cotton,D.M. Gibb,A.G. Babiker,J. Steyn,S.A. Madhi*et al.*, *Early antiretroviral therapy and mortality among HIV-infected infants.* N Engl J Med, 2008. **359**(21): p. 2233-44.

101. Manosuthi, W.,S. Sungkanuparph,A. Thakkinstian,A. Vibhagool,S. Kiertiburanakul,S. Rattanasiri*et al.*, *Efavirenz levels and 24-week efficacy in HIV-infected patients with tuberculosis receiving highly active antiretroviral therapy and rifampicin.* Aids, 2005. **19**(14): p. 1481-6.

102. la Porte, C.J.,E.P. Colbers,R. Bertz,D.S. Voncken,K. Wikstrom,M.J. Boeree*et al.*, *Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers.* Antimicrob Agents Chemother, 2004. **48**(5): p. 1553-60.

103. Ren, Y.,J.J. Nuttall,C. Egbers,B.S. Eley,T.M. Meyers,P.J. Smith*et al.*, *Effect of rifampicin on lopinavir pharmacokinetics in HIV-infected children with tuberculosis.* J Acquir Immune Defic Syndr, 2008. **47**(5): p. 566-9.

104. Gibb, D.M.,R.L. Goodall,V. Giacomet,L. McGee,A. Compagnucci, and H. Lyall, *Adherence to prescribed antiretroviral therapy in human immunodeficiency virus-infected children in the PENTA 5 trial.* Pediatr Infect Dis J, 2003. **22**(1): p. 56-62.

105. Blanc, F.X.,D.V. Havlir,P.C. Onyebujoh,S. Thim,A.E. Goldfeld, and J.F. Delfraissy, *Treatment strategies for HIV-infected patients with tuberculosis: ongoing and planned clinical trials.* J Infect Dis, 2007. **196 Suppl 1**: p. S46-51.

106. Shisana, O.,T. Rehle,L. Simbayi, C,W. Parker,K. Zuma,A. Bhana*et al.*, *South African National HIV Prevalence, HIV Incidence, Behaviour and Communication Survey*. 2005, Human Research Council: Cape Town, South Africa.

107. World Health Organization, *Universal Access: scaling up priority HIV/AIDS interventions in the health sector. Progress Report April 2007*. 2007: Geneva, Switzerland.