Title: Management of HIV in children: Challenges and outcomes

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DECLARATION AND APPROVAL

This proposal is my own original work and has not been submitted for a degree in any other University.

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This dissertation is dedicated to children who were born with Human Immunodeficiency virus and their parents who care for them. May they find hope to live life to the fullest

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ABBREVIATIONS

ALHIV	Adolescents Living with HIV
ART	Antiretroviral Therapy
CALHIV	Children and Adolescents Living with HIV
CD	Cluster Differentiation
CDC	Centres for Disease Control and Prevention
CHER	Children with HIV Early Antiretroviral Therapy
CMV	Cytomegalovirus
DNA	Deoxyribonucleic Acid
DRM	Drug Resistance Mutation
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IeDEA	International Epidemiology Databases to Evaluate AIDS
JOOTRH	Jaramogi Oginga Odinga Teaching and Referral Hospital
KNH	Kenyatta National Hospital
MPI	Multiple Principal Investigator
NIH	National Institutes of Health
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
OPH	Optimizing Paediatric HIV-1 Therapy
PAD	Paediatric Adherence Study
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PITC	Provider Initiated Testing and Counselling
PMTCT	Prevention of Mother to Child Transmission
PUSH	Paediatric Urgent Start of HAART
RNA	Ribonucleic Acid
US	United States
WHO	World Health Organization
WHZ	Weight for Height Z-score

DEFINITION OF TERMS

Advanced Clinical Stage of HIV: Refers to WHO clinical stage 3 and/or 4 in which children and adolescents present with severe recurrent bacterial infections, AIDS-defining infections, major organ-system disorders or HIV-associated malignancy.[1]

Highly active antiretroviral therapy: A combination of at least 3 antiretroviral drugs from at least different classes given together as separate formulations or in fixed dose.[2] combinations.

Prevention of Mother to Child Transmission of HIV: Use of strategies to effect maternal viral suppression during pregnancy, labour/delivery and breastfeeding and infant prophylaxis.[2] using antiretroviral drugs.

Virologic suppression: Reduction of plasma HIV-1 RNA (viral load) level to below predetermined threshold (either < 50 or < 200 copies/mL) following at least 24 weeks of treatment. [2]

Virologic failure: Lack of reduction in plasma HIV-1 RNA (viral load) level to below predetermined threshold (either < 50 or < 200 copies/mL) following at least 24 weeks of treatment or rebound (rise) to higher levels after initial decrease. [2]

1. Adapted from: WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: World Health Organization; 2007. (www .who.int/hiv/pub/guidelines/HIVstaging150307.pdf).
2. Ministry of Health, National AIDS & STI Control Program. Kenya HIV Prevention and Treatment Guidelines, 2022 Edition. Nairobi, Kenya: NASCOP, Aug 2022. Print.

Chapter 1: General Introduction

1.1 List of Published Papers

This thesis uses the University of Nairobi alternative format for a PhD presentation and is based on the following published papers:

1. **Wamalwa DC**, Farquhar C, Obimbo EM, Selig S, Mbori-Ngacha DA, Richardson BA, Overbaugh J, Emery S, Wariua G, Gichuhi C, Bosire R, John-Stewart G. Early response to highly active antiretroviral therapy in HIV-1-infected Kenyan children. J Acquir Immune Defic Syndr. 2007 Jul 1;45(3):311-7
2. **Wamalwa DC**, Obimbo EM, Farquhar C, Richardson BA, Mbori-Ngacha DA, Inwani I, Benki-Nugent S, John-Stewart G. Predictors of mortality in HIV-1 infected children on antiretroviral therapy in Kenya: a prospective cohort. BMC Pediatr. 2010 May 18;10:33. PMID: 20482796
3. **Wamalwa D**, Lehman DA, Benki-Nugent S, Gasper M, Gichohi R, Maleche-Obimbo E, Farquhar C, John-Stewart G, Overbaugh J. Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy. J Acquir Immune Defic Syndr. 2013 Mar 62(3): 267-274
4. **Wamalwa D**, Benki-Nugent S, Langat A, Tapia K, Ngugi E, Slyker JA, Richardson BA, John-Stewart GC. Survival benefit of early infant antiretroviral therapy is compromised when diagnosis is delayed. Pediatr Infect Dis J. 2012 July; 31(7): 729-731.
5. **Wamalwa D**, Njuguna I, Maleche-Obimbo E, Begnel E, Chebet DJ, Onyango JA, Cranmer LM, Huang ML, Richardson BA, Boeckh M, John-Stewart G, Slyker J. Cytomegalovirus viraemia and clinical outcomes in Kenyan children diagnosed with Human Immunodeficiency Virus (HIV) in Hospital. Clin Infect Dis. 2022 Apr; 9;74(7):1237-1246.

1.2 ABSTRACT:

Background: An estimated 1.7 million children aged 0-14 years were living with HIV in 2021 globally with over 90% of them in Sub-Saharan Africa. Infants and children with HIV disproportionately contribute to mortality due to the aggressive nature of paediatric HIV, late presentation to care, suboptimal or unpalatable antiretroviral drug formulations and low levels of ART adherence. Gaps in the health system are responsible for preventable HIV infections in children as well as delayed linkage to care leading to poor outcomes. It is important to clearly characterize clinical and virologic outcomes and identify modifiable factors that undermine the effectiveness of antiretroviral therapy (ART) in infants and children including the impact of delayed diagnosis.

Objectives: To determine the impact of delayed diagnosis on survival benefit of early ART in infants with HIV, define early and long-term response to ART in children with HIV and determine factors associated with clinical outcomes in ART-treated children.

Methods: Three prospective studies involving HIV-1 infected infants and children were conducted between 2004 and 2016. In the Paediatric Adherence Study (PAD) an observational prospective study, 149 HIV-1 infected children aged 18 months to 12 years with advanced clinical disease were enrolled and initiated on ART at the Kenyatta National Hospital in 2004-2006. Children were followed prospectively for >15 years for clinical (morbidity, growth, mortality) immunologic (CD4 count and %) and virologic (HIV-1 plasma RNA, genotypic drug resistance mutations) outcomes. In the Optimizing HIV-1 Therapy- (OPH-03), a randomized controlled trial 99 HIV-1 infected infants aged between 0 and 5 months were enrolled and initiated on ART at the Kenyatta National Hospital between 2006 and 2008. In the pre-randomization phase of 24 months children were followed 3-monthly while on antiretroviral therapy and monitored for clinical parameters (mortality, morbidity, growth), immunologic (CD4) and virologic (plasma HIV-1 RNA, HIV-1 reservoir) outcomes. At 24 months children were randomized to either treatment interruption or continued ART. After a short interruption period (3 months) all children resumed ART. For this analysis only the 24-month pre-randomization phase is considered. In the Paediatric Urgent Start of HAART (PUSH), a randomized controlled trial 193 HIV-1 infected infants and children < 12 years of age hospitalized for severe illness were enrolled from 4 facilities (KNH and Mbagathi District Hospital, Kisumu County Hospital and JOOTRH) between 2013 and 2015 and randomized to urgent (within 48 hours) or post-stabilization (within 14 days) ART. Children were followed up prospectively for 2 years for clinical, immunologic and virologic monitoring. As part of this study we collected serial plasma samples for CMV viral load and used the entire population of children to determine the impact of high CMV viraemia on clinical outcomes. For this analysis we consider pooled analysis of children in both arms of the study in the first 12 months of follow-up.

Results: In the PAD study NNRTI-based ART introduced for children aged 18 months to 12 years with advanced HIV resulted in good early clinical, immunologic and virologic outcomes. After 6 months of ART in 52 children, the median height-for-age z-score rose from -2.54 to -2.17 ($p< 0.001$) and median weight-for-age z-score from -2.30 to -1.67 ($p = 0.001$). The median CD4 percentage rose from 5.8% before ART to 15.4 % ($p< 0.001$) and 67% of the children achieved viral suppression < 400 copies/mL. Hospitalization rates dropped from 58% in the 6 months before treatment to 17% in the first 6 months of ART ($p < 0.001$). The mortality rate in this cohort of children first 21 months was 13.4% and mortality was much higher in the first 4 months of ART (46 deaths /100 person years) compared to the period after 4 months (1.0

deaths per 100 person-years). Major causes of death were pneumonia, cardia failure from chronic lung disease or cardiomyopathy, and tuberculosis. Advanced WHO stage 4, low baseline weight-for-height scores < -2 and low haemoglobin (< 9 g/dL) predicted mortality. In this cohort 34% of 100 children followed for a median 5.5 years experienced virologic failure and at least two thirds of those failing ART had evidence of drug resistance. In a smaller group (n =14 children) that switched to protease inhibitor no major resistance developed to protease after 28 months of follow-up. In the OPH study 99 HIV-1 infected infants were enrolled at a median age of 3.7 months and initiated ART consisting AZT/3TC with either NVP or PIs. Nearly half (49%) had advanced clinical HIV disease (WHO stage 3-4), the median CD4 percent was 18%, 60% had hospitalization since birth and 58% were underweight (WAZ < -2). The mortality in infants who initiated ART was 14% with a 12-month probability of survival of 77% much lower than that found in the CHER study (96%). Predictors of mortality included WHO stage 3-4, underweight, wasting, microcephaly, low haemoglobin and gastroenteritis. In the PUSH study out of 163 children HIV-1 infected children aged 0-12 years initiating ART 54% had CMV viraemia at baseline, 32% has high levels of cytomegalovirus/CMV viraemia (> 1000 IU/mL) and 72% had CMV DNA detected at least once. Children with high levels of CMV viraemia (> 1000 IU/mL) had a 74% higher risk of a combined endpoint of prolonged hospital stay (> 15 days) or 6-month compared to those with levels of CMV viraemia ≤ 1000 IU/mL).

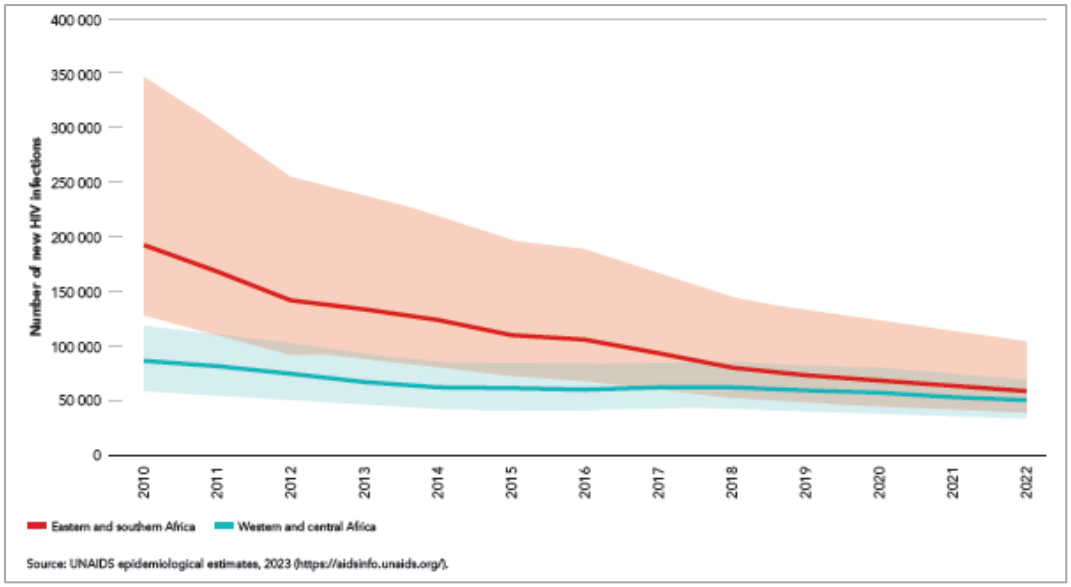
Conclusions and Recommendations: We demonstrated that it is of critical importance to identify both infants and children as early as possible in the course of HIV disease in order to optimize clinical outcomes. The key predictors of mortality in both infants and children are indicative of advanced HIV hence the need for earlier identification. Secondly, we identified cytomegalovirus viremia as an important modulator of response to HIV treatment which points to the need to explore CMV- suppressive therapy as a potential strategy to improve outcomes. Overall, at whichever point infants and children with HIV are identified prompt antiretroviral therapy still provides the only way to improve outcome. Finally, this work clearly showed that virologic monitoring cannot easily be replaced with clinical assessment which often underestimates true virologic failure. In out current setting we cannot afford to keep children on failing antiretroviral regimens which lead to accumulation of additional drug resistance and potentially jeopardizes their future options.

1.3 Background

Globally an estimated 1.7 million children aged 0-14 years and 1.68 million adolescents aged 10-19 years were living with HIV in 2021 with over 90% of them in Sub-Saharan Africa (1). Currently approximately 110,000 children age < 14 years and 91,000 adolescents are living with HIV in Kenya and nearly 15,000 new HIV infections occur annually in the age group 15 to 24 years (2). Although children and adolescents living with HIV (CALHIV) constitute 12% of the overall burden of HIV in the Kenyan population, they account for over 20% of the mortality. This is attributable to the aggressive nature of paediatric HIV, late presentation to care, suboptimal antiretroviral therapy (ART) formulations, unpalatable ART drug preparations and poor levels of ART adherence (3). The majority of children living with HIV acquire infection through mother-to-child transmission (also known as vertical transmission) while a smaller proportion of adolescents get infected through horizontal transmission, primarily sexual exposure (4). Timing of HIV acquisition is directly linked to outcome and infants who acquire HIV during pregnancy, labour and delivery have the lowest capacity to contain HIV and experience the highest rates of disease progression (5,6). Significant progress in prevention of vertical transmission has resulted in fewer numbers of infants acquiring HIV through vertical transmission, however gaps in the mother-baby HIV cascade continue to hinder elimination efforts (7-9).

Between 2000 and 2022 the number of new HIV infections in children fell by 75% (2023 (10). Figure 1 show the number of new HIV infections among children ages 0-14 years indicating the various regions. This fall in new infections has been achieved through identification of HIV in women pre-pregnancy or in early pregnancy coupled with provision of antiretroviral therapy as well as infant prophylaxis (11). Nonetheless, there are still new HIV infections children (0-14 years) in Kenya in 2022 were estimated to be 4000. The key reasons for ongoing infections despite availability of preventive measures are undiagnosed incident infections which are missed by early antenatal testing and non-adherence to antiretroviral therapy leading to maternal viremia through pregnancy, labour and/or breastfeeding (12). In addition, stock-outs in antiretroviral drugs used in infant prophylaxis may contribute to late infant HIV acquisition. This can be addressed through universal HIV retesting in late pregnancy and breastfeeding however there is variation in recommendations and level of uptake of retesting across different countries (13).

Figure 1. Trend of new HIV infections in children < 14 years



Although most children acquire HIV during a narrow window between late pregnancy and early infancy, diagnosis and linkage to care is often delayed. Consequently, up to 50% of children with HIV start ART in advanced disease stages which undermines the benefits of treatment (14). Scale-up of ART over the past two decades has resulted in improved survival of children but early deaths on ART remain a problem and it is important to identify modifiable factors that contribute to mortality (15). Rates of virologic failure following ART initiation are consistently highest in the youngest age especially those below 2 years due to limited choices of ARV formulations and challenges in adherence (16). Due to a relatively underdeveloped immune system infants with HIV face additional challenges from opportunistic infections such as cytomegalovirus whose contribution to outcomes requires further research (17). These challenges can only be addressed, and their effect mitigated through development of policies informed by targeted research conducted in local populations of infants and children living with HIV.

1.3.1 Problem statement

With an estimated 110,000 CLHIV (0-14 years), nearly 91,000 ALHIV (10-19 years) and 145,000 youth living with HIV (15-24 years) Kenya has the 8th highest number CALHIV globally. Increased efforts in the prevention of vertical transmission and scale-up of highly active antiretroviral therapy has significantly reduced the burden of HIV but important challenges remain. Gaps in diagnosis and linkage to prompt care persist and delayed diagnosis undermines the benefits known to accrue from early

ART. Response and outcomes in infants and children on ART are further compromised by co-infections, non-adherence and drug resistance and early mortality remains common. It is important to optimize diagnosis by ensuring the infants with HIV are identified as early as possible and ideally before becoming symptomatic. One way to advocate for early diagnosis is to clearly quantify the impact of delayed diagnosis on the survival of ART-treated infants in our setting. Characterization of outcomes in children on ART coupled with identification of co-factors for adverse outcomes provides opportunities to design interventions to improve the overall benefit treatment. Potential modifiable co-factors that require further study include co-infections such as cytomegalovirus and studies are required to better elucidate mechanisms by which outcomes in children with HIV are compromised. This doctoral thesis is based on a wide body of research conducted over the past two decades on infants and children living with HIV in Kenya to address questions in these domains. It addressed questions that could guide formulation of policy that mitigates the overall impact of HIV on infants and children in Kenya. The studies conducted in this thesis span over a period that saw substantial changes in availability and uptake of newer antiretroviral classes. Another major change in this period was the shift in policy to recommend antiretroviral treatment for all individuals with HIV as opposed to only those with clinical symptoms or immune deficiency (Figure 1)

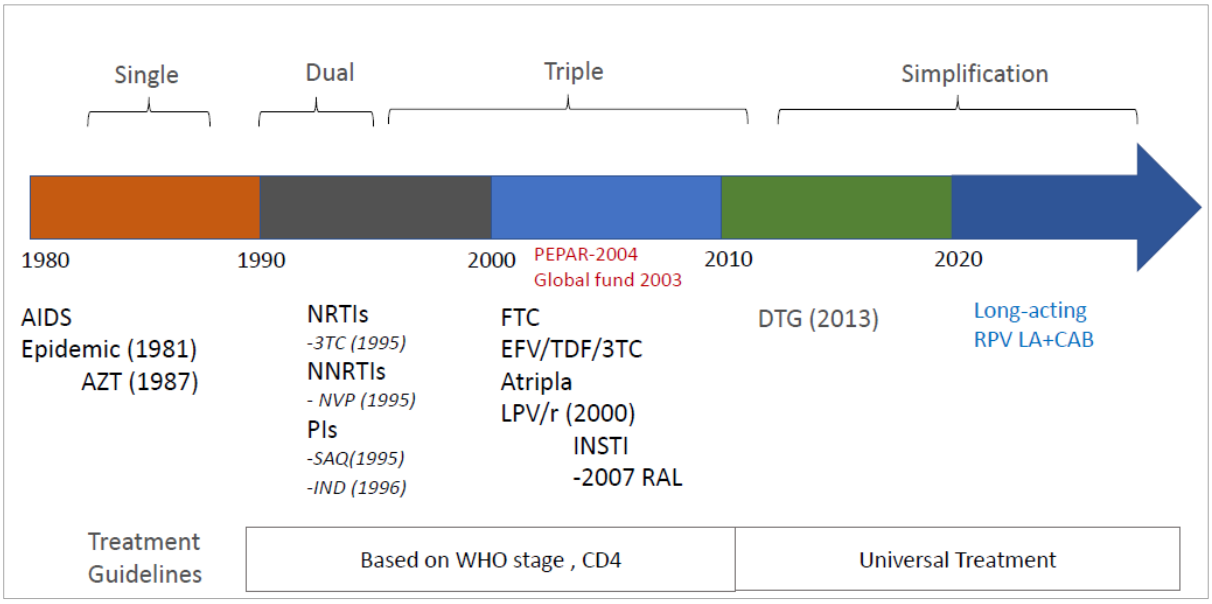


Figure 1. Antiretroviral drug timelines

1.3.2 Research Questions: Research questions are categorized under the following three broad themes summarized in table 1 below.

Table 1: Broad themes and over-arching research questions

Broad Theme	Research Question
Response to antiretroviral therapy in children living with HIV	How do children with HIV respond to recommended ART in the short and long-term?
Optimizing diagnosis of HIV in infants	What is the impact of delayed diagnosis on survival benefit of early ART in infants with HIV?
Modulators of clinical response to antiretroviral therapy in children living with HIV	What factors predict clinical outcomes in children with HIV treated with ART?

1.3.3 Study objectives:

The broad objectives as derived from the themes are listed below:

1. To evaluate outcomes in children with HIV initiated on antiretroviral therapy
2. To investigate the impact of delayed diagnosis on survival benefit of early ART in infants with HIV
3. To study factors associated with clinical outcomes in ART-treated children.

Table 2 shows the specific objective(s) under each broad objective.

Table 2. Themes, Broad and specific objectives

Theme	Broad objective	Specific objectives
Response to ART in children	To evaluate outcomes in children with HIV initiated on antiretroviral therapy	1. To describe early response in HIV-infected children initiated on highly active antiretroviral therapy. 2. To determine the long-term virologic outcomes and frequency & pattern of genotypic drug resistance in HIV-infected children on antiretroviral therapy.
Optimizing HIV diagnosis in infants	To investigate the impact of delayed diagnosis on survival benefit of early ART in infants with HIV	3. To determine the incidence and correlates of mortality in HIV-infected infants diagnosed at less than 5 months of age and initiated on antiretroviral therapy.
Modulators of clinical response to ART in children	To study factors associated with clinical outcomes in ART-treated children	4. To determine predictors of mortality in HIV-1 infected children treated with antiretroviral therapy. 5.To determine the impact of CMV viraemia on clinical outcomes in children diagnosed with HIV in hospital.

Each specific objective corresponds to a single published paper and have been arranged in the **following order**:

1. Early response to highly active antiretroviral therapy in HIV-1 infected Kenyan children. J Acquir Immune Defic Syndr. 2007;45:311-317… Chapter 2
2. Predictors of mortality in HIV-1 infected children on antiretroviral therapy in Kenya: a prospective cohort. BMC Pediatr. 2010:10:33 … Chapter 3
3. Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy. J Acquir Immune Defic Syndr. 2013;62:267-274 … Chapter 4
4. Survival benefit of early infant antiretroviral therapy is compromised when diagnosis is delayed. Pediatr Infect Dis J. 2013; 31:729-731… Chapter 5
5. Cytomegalovirus viraemia and clinical outcomes in Kenyan children diagnosed with human immunodeficiency virus in hospital. Clin Infect Dis. 2022;74(7): 1237- 46… Chapter 6

1.4.1 Optimizing diagnosis and care of HIV in children

***The timing of HIV acquisition in most infants is well understood**:*

Unlike adults, children predominantly acquire HIV-1 through vertical transmission during a well-defined window that includes late pregnancy (in-utero 5%), labour and delivery (intra-partum -20%) and breastfeeding (post-partum -15%) (4). In the landmark trial by Nduati *et. al*., an estimated 63% and 75% respectively of breast-feeding HIV-1 transmission occurred by 6 weeks and 6 months respectively suggesting that most transmission occurs early (18). Infants infected in-utero can be identified through a positive HIV-DNA PCR test taken at birth while those infected during labour, delivery and early breastfeeding can be identified by similar testing by the age of 6 weeks. It is therefore possible to identify the majority of HIV-1 infected infants within a few weeks of HIV acquisition.

Early ART before immunosuppression and advanced clinical disease results in better survival

Optimal timing of ART in children remained a point of debate in the early ART era with two predominant views: US guidelines which advocated early aggressive ART from infancy and European and/or WHO guidelines which favoured symptom-based delayed ART (19,20). Following increased evidence that showed clear benefits of early treatment, international guidelines were revised in 2010 to recommend ART as soon as possible after HIV-1 diagnosis (21). In the landmark CHER randomized clinical trial which was instrumental in informing guideline change to early ART in children mortality in the early ART arm (median age of initiation 7.4 weeks) was 4% while that in the deferred ART arm (median age of ART initiation 40 weeks) was 16% (22). This represented a 76% reduction in mortality attributed to early ART. A close look at this study reveals the characteristics required for optimal benefit from early ART; a relatively intact immune system (median CD4 percentage 35%) and early or no clinical disease (94% mild disease-CDC N or A, or asymptomatic). The recruitment into the CHER study was done through active surveillance of PMTCT cohorts with close post-natal screening of all HIV-exposed infants to enable identification at the earliest time point.

The scenario in program settings, however, is substantially different for two primary reasons. Firstly, more than half of HIV-1 infected infants are not identified through PMTCT cohorts but as sick hospitalized infants and secondly the typical timing of early infant diagnosis through PCR testing (6 weeks, 6 and 12 months) with long time lags from testing to results does not favour very early identification of HIV-infected infants (23). These time-points serve well as surveillance for a largely HIV-exposed uninfected group of children but may be inappropriate for a significant proportion of perinatally infected children who become symptomatic with advanced HIV-1 as early as 6 -12 weeks of age (24).

Late diagnosis of infant HIV-1 remains common in treatment programs

Despite guideline change to early ART implementation has remained challenging largely due to delays in diagnosis and poor linkage to care and consequently most children living with HIV start ART at advanced disease stages (25,26). Even for infants and children who come into contact with the health system through routine immunization clinics, sick baby outpatient visits and hospitalization provider-initiated testing and counselling (PITC) is not universally done (27). Low rates of PITC have been widely reported with little evidence of improvement in trends (28,29). In an evaluation of health system gaps in the Mother-child HIV cascade, 38% of previously hospitalized HIV-infected children had never received an HIV test and nearly half (48%) of children with a previous HIV test had tested positive and were not on ART The final barrier is the poor linkage to care for infants and children who are diagnosed to have HIV which further contributes to late ART initiation (7). In the context of vertically HIV-1 infected infants where disease progression is most rapid the true impact of what may seem like a short delay in HIV diagnosis (e.g. few weeks) and linkage to treatment may easily be underestimated. Understanding the impact of delayed treatment is necessary to inform a policy shift that favours earlier and aggressive case finding and diagnosis of HIV-1 for infants in program settings.

1.4.2 Response and outcomes to Antiretroviral therapy in children

Early response to antiretroviral therapy in HIV-1 infected children

Despite the fact the most cases of paediatric HIV are due to vertical transmission many children are not diagnosed during their first year and will present in later years with

growth failure, recurrent bacterial and viral infections or organ-system such as cardiomyopathy, nephropathy, anaemia, encephalopathy or HIV- associated malignancies (30,31). In this group of children one of the first and consistent features of successful response to ART is restoration of growth. Early cohorts of ART-treated children demonstrated sustained increase in linear growth and weight gain in the subset that achieved viral suppression. Vreewal reported sustained growth following ART in a cohort of HIV-infected children whose pre-treatment weight and height z-scores were both less than -3.0 (32). Increase in growth was closely correlated with the magnitude of drop in HIV-1 RNA levels as well as rise in CD4 + T cells. Similar results were demonstrated by Nachman in a cohort of children whose height and weight was sub-normal at before ART. In this cohort weight normalized sooner (by 1 year of ART) than height (2 years of ART) and high viral loads correlated with poor growth recovery (33). Subsequent studies have confirmed the findings that growth increase is a reliable surrogate of favourable early response to antiretroviral therapy in children as long as adequate nutrition is provided (34).

Besides growth recovery ART initiation in ART-naïve is often associated with prompt increase in CD4+ T-cell count and percentage and decline in HIV-1 RNA. The rate of drop in HIV-1 viral load in children with good response to ART is approximately 1-2 $\log_{10}$ HIV RNA copies/mL within the first 3-6 months of effective ART. In one of the earliest paediatric ART-treated cohorts of children, Puthanakit reported an increase of CD4 percentage from 3.1 % at baseline to 21% after 72 weeks in children whose median age at ART start was 7,7 years (35). In this study which used stavudine/lamivudine and NNRTIs (efavirenz or nevirapine), 76% of the children achieved HIV-1 RNA levels < 50 copies/mL. A recent electronic review of records of paediatric HIV cohorts in East Africa consisting of 5927 children with a median age at ART initiation of 5.6 years reported 97% immunologic success. In this study, immunologic success was defined as achieving CD4 percentage of > 10% in children 2-5 years or > 100 cells /uL for those > 5 years) after 1 year of ART (36).

Finally, clinical response demonstrated by regression in WHO clinical stages has been demonstrated in the first few months following ART initiation in children. In the IeDEA East African cohort 89% of the children were judged to have clinical success after 24 weeks of ART (36). For families of children initiating ART the initial response in growth

and reduction of hospitalization is crucial and an important determinant of long-term success. Along with clinical response the safety of antiretroviral drugs in children is a valid concern for caregivers and a good understanding of the frequency of adverse drugs reactions based on local data is the most effective way of providing reassurance.

Long-term response and drug resistance to antiretroviral therapy in infants and children:

In the pre-ART era, the median survival of perinatally infected children was between 6 months and 2 years and an estimated 52% and 75% children died by 2 and 3 years respectively (37-38). Scale up of ART was mainly realized after 2010 following guidelines recommending universal ART in children and adolescents. While early response to ART in children is associated with growth, immune reconstitution and decline in opportunistic infections the most reliable measure of long-term success is sustained viral suppression (39-40). Such success should translate in a complete reversal of HIV clinical progression and enhanced survival. WHO has set targets of 95-95-95 which represent both the reach (95% of those infected identified) and uptake of ART (95% of those in care on ART) but also the response as determined by viral suppression (95% of those on ART virally suppressed) (41). Efforts to achieve these targets, especially the third target of 95% virally suppressed are particularly challenging in children and adolescents.

Studies consistently show that children achieve viral suppression at lower rates compared to adults (42-43). Non-adherence and use of drugs with low genetic barrier to resistance are important contributors to development of drug resistance mutations (44-45). A better understanding of the pattern of emerging drug resistance mutations in children is useful in informing sequencing ARV drugs which is a crucial part in the formulation and appraisal of HIV treatment guidelines. In view of the fact that children living with HIV will be receiving ART for a much longer duration than their adult counterparts they are more likely to require more than one ART regimen in the course of their lives. It is therefore important to define long-term virologic response to ART and the patterns of drug resistance at the point of virologic failure in order to inform ART sequencing for this population (46-47).

1.4.3 Factors associated with clinical outcomes in ART-treated children

Predictors of early mortality in HIV infected children receiving ART:

Following scale up of antiretroviral therapy (ART) a decline in HIV-related deaths in infants and children has been witnessed globally (44). Notwithstanding this, late presentation in advanced HIV disease remains common in Sub-Saharan Africa where the burden of paediatric HIV is highest. Such children presenting with severe immunosuppression and serious bacterial infection have mortality rates between 5-20% despite treatment (49, 50). It has become increasingly clear that although ART is broadly effective in improving outcomes early mortality in a sub-set of patients on treatment is a persistent challenge. Elucidating causes and factors associated with mortality is an important step in seeking interventions to prevent such mortality.

Overall earlier initiation of ART before advanced immunosuppression and clinical disease has resulted in better outcomes, but this is particularly challenging in children where disease progresses rapidly. Even in the context of ART initiation late in the disease course understanding risk factors for mortality and attrition remain important. This can inform efforts to address modifiable risk factors alongside advocacy for earlier and more effective ART. A few studies have sought to characterize factors associated with loss to follow-up and mortality in children treated with ART. In a large cohort of Zambian HIV-infected children on ART (1039 children median age 3.6 years) 71 (7%) died and 164(16%) were lost to follow-up. Age less than 1 year and WHO clinical stage 4 predicted death in this cohort (51). In a cohort of 344 HIV-infected Ethiopian children attrition (loss to follow-up and mortality) after a median 4.4 years was 6.6 per 100 person-years. Predictors of attrition were WHZ < -2, Hb < 10g/dl, WHO clinical stage 3/4 and non-use of cotrimoxazole preventive therapy (52). In Thailand out of 4618 children with HIV 9% died after a median follow-up of 4 years (rate of mortality 2.1 per 100 person years). In this cohort mortality was associated with lower CD4 count and age< 12 years (53).

In a cohort of 5 Asian countries (Cambodia, India, Thailand, Indonesia, Thailand) 371(6.3%) of 5918 children on ART died at a median age of 7 years (54). Leading causes of death were pneumonia, tuberculosis and sepsis and higher CD4 counts and better WAZ scores protected against death. Over time the risk factors may change as CLHIV initiate ART earlier and the contribution to mortality is evolving to include non-

communicable causes. Earlier cohorts on the other hand are able to characterise mortality and predictors in the context of advanced HIV. An evaluation of risk factors for mortality than considers both earlier cohorts (advanced HIV disease at ART initiation) and more recent cohorts is likely to provide a broad and comprehensive picture.

The role of Cytomegalovirus viraemia on clinical outcomes in HIV infected children:

Cytomegalovirus (CMV), a Herpes virus has been associated with poor outcomes as a co-infection in children and adults with HIV (55). Among CLHIV CMV is both an opportunistic infection as well as a driver of non-AIDS morbidity. CMV has been associated with multiple morbidity in CLHIV including retinitis, pneumonitis, hepatitis, enteritis and encephalopathy in sharp contrast to the scenario in healthy HIV-1 unexposed children in whom CMV infection is largely asymptomatic (56,57). The mechanisms by which CMV worsens outcomes in HIV infected children include enhancing immune activation, creating and maintaining a large HIV reservoir and producing sustained heightened inflammation through cytokine stimulation (58). CMV results in clonal expansion of activated T-cells which has been shown to diminish on institution of suppressive anti-viral therapy such as ganciclovir (59). In a South African cohort of HIV infected children higher plasma CMV viral load predicted a larger HIV reservoir (60).

Both HIV and CMV have been associated with increased ageing and frailty. Both a known to promote Chronic low-grade inflammatory phenotype (CLIP) directly or indirectly. This is associated broadly with changes seen in immunosenescence such as contraction of the immune repertoire, decrease in naïve lymphocytes, and accumulation of memory and senescent lymphocytes (61).

An important but not well studied aspect is the role of CMV viraemia in the related mortality. Limited data suggests that the presence of CMV viraemia may increase the risk of mortality in HIV infected children but there is no data on what cut off of CMV viral load is predictive of worse outcomes (62). If CMV viraemia is found to be a key driver of disease progression and mortality in sick HIV1- infected children, strategies for CMV viral control including viral suppressive drugs may be warranted. It is therefore important to define the role of high CMV viraemia on outcomes in children living with HIV as an initial measure.

1.4.4 Conceptual framework

The three domains highlighted in figure 2 form the subject of this doctoral thesis. A better understating of these key areas is pertinent to mitigating the impact of HIV on the paediatric population. Delay in diagnosis of HIV in infants directly affect survival and needs to be highlighted and it's on survival quantified. Optimization of diagnosis of HIV is urgently needed to ensure that children enter into care at the earliest possible time. Both early and long-term outcomes of children treated with ART need to be well characterised to inform successes and failures of treatment efforts in order to draw lessons for improvement. Predictors of outcome are important pointers of areas that need attention to enhance survival. Identifying modifiable factors that predict outcomes forms the basis for the search for interventions.

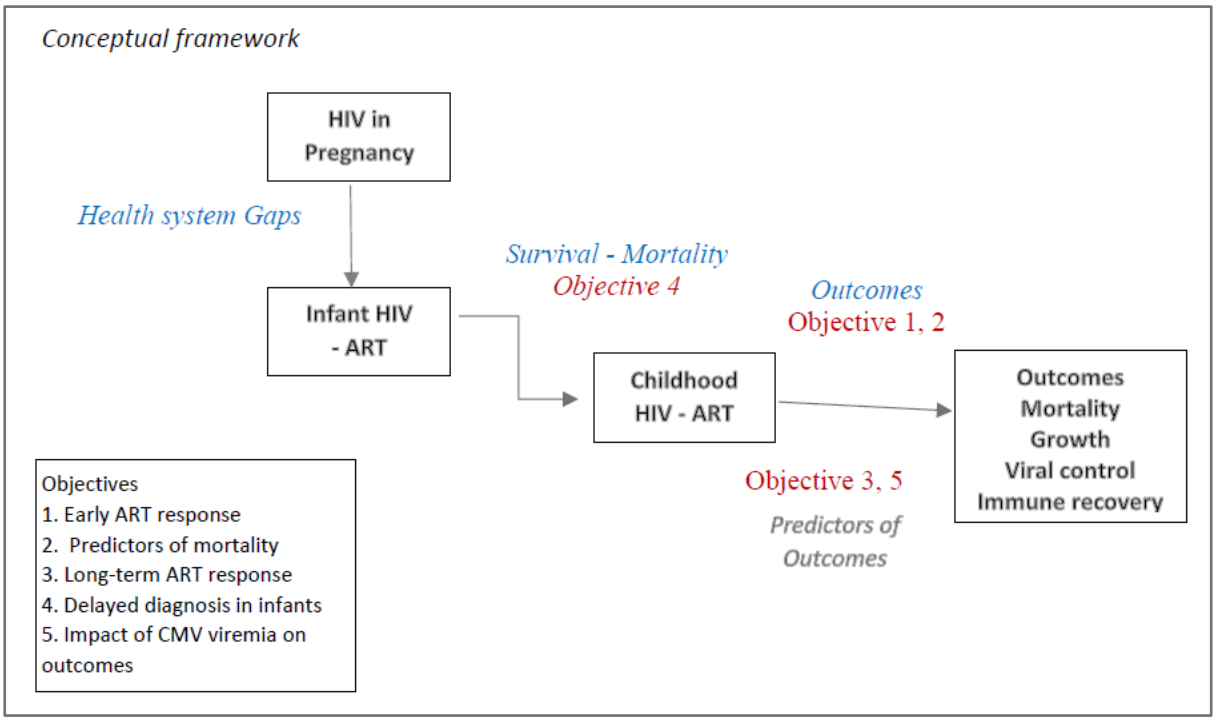


Figure 2. Conceptual framework (broad themes)

1.5 Summary of Methods and Materials

1.5.1 Studies conducted and setting

This thesis will be based on five (5) publications which correspond to the 5 specific objectives. The publications are based on data from 3 studies conducted among HIV-1 infected infants and children between 2004 and 2016. The studies were conducted

in Nairobi (Kenyatta National Hospital and/or Mbagathi County Hospital) and/or Kisumu (Jaramogi Oginga Odinga Teaching and Referral Hospital, and Kisumu County Hospital). Table 3 summarizes the main characteristics the studies which were all funded by the US National Institutes of Health (NIH) funding.

Table 3. Summary of design and methods for relevant studies

Study Title Dates of enrolment Site	**Design (Sample size)**	**Population characteristics**	**Intervention** (If applicable)	**Outcome(s)**
Paediatric Adherence (PAD) 2004-2006 KNH	Prospective observational cohort (n = 149)	HIV +VE children Age:1.5 - 12 years Median 4.9 years ARV naïve Advanced disease	N/A All initiated ART	Mortality, growth Viral suppression, Drug resistance Immune recovery
Optimizing Paediatric HIV-1 Therapy (OPH) 2007-2009 KNH, Mbagathi, KCH	Randomized controlled trial (24 months pre-randomization phase) (n = 99)	HIV +VE infants Age: 1- 5 months Median age 3.7 months ARV naïve	Treatment interruption after 24 months of ART	Mortality, growth Viral suppression. Drug resistance Development Immune recovery
Paediatric Urgent Start of HAART (PUSH) 2013-2015 KNH, KCH, JOOTRH, Mbagathi	Randomized controlled trial (n = 183) Pooled analysis used entire cohort	HIV +VE 0-12 years ARV naïve Hospitalized for severe illness	Urgent < 48 hrs. Post-stabilization 7-14 days ART (n : 90, 93)	Mortality at 6 months Growth IRIS Viral suppression Immune recovery All-cause SAEs

KNH- Kenyatta National Hospital; KCH- Kisumu County Hospital. JOOTRH- Jaramogi Oginga Odinga Teaching & Referral Hospital

The following section provides further description of each of the studies and lists the specific objective answered by the study:

1.5.2 Paediatric Adherence Study (PAD):

Objectives addressed: Data from this study was used for publications that address the following three specific objectives:

1. To describe early response in HIV-infected children initiated on highly active antiretroviral therapy.
2. To determine predictors of mortality in HIV-1 infected children treated with antiretroviral therapy

3. To determine the long-term virologic outcomes and frequency & pattern of genotypic drug resistance in HIV-infected children on antiretroviral therapy.

Design and Methods: The Paediatric Adherence Study was one of the first HIV treatment cohorts to be established for research in Kenya. Established in 2004 and with a subset child (now young adults) still in active follow-up it is likely has the longest duration of follow-up. In this observational prospective study 149 ARV-naïve HIV-1 infected children aged 18 months to 12 years with advanced clinical disease were enrolled and initiated on 1st line NASCOP recommended ART at the Kenyatta National Hospital in 2004-2006. Socio-demographic and clinical parameters were collected at baseline. Plasma HIV-1 RNA, CD4 count & %, blood chemistry and haematology were measured at baseline. Children were then followed prospectively for >15 years with serial observation for clinical parameters (mortality, growth, morbidity), adherence to ART and immunologic (CD4) and virologic (HIV-1 plasma RNA, genotypic drug resistance mutations) assays. For the first 5 years the frequency of follow-up was 3-monthly and thereafter 6-monthly follow-up.

Given the urgent need for local data to inform policy (specifically the effectiveness of NNRTI-containing ART in children) it was important to perform interim analysis and provide this data for policy makers to work with. Thus, as soon as a sizeable number of children (67 out of the 149 enrolled) achieved their 6-month follow-up on ART we analysed their response and published this in the first manuscript on Early response to antiretroviral therapy (2007). In subsequent analysis on mortality and predictors of mortality we utilized the entire cohort since this gave us a true representation of deaths including those occurring very early. Finally, in the analysis on long-term virologic outcomes in the PAD cohort we utilized only children who had baseline viral loads available and returned for at least one scheduled follow-up visit. Out of the 149 children enrolled and started on ART, 14 did not have a baseline viral load sample available and 35 children either died or were lost to follow-up before the next scheduled appointment leaving 100 available for analysis.

The publications based on this study are listed below:

i. **Wamalwa DC**, Farquhar C, Obimbo EM, Selig S, Mbori-Ngacha DA, Richardson BA, Overbaugh J, Emery S, Wariua G, Gichuhi C, Bosire R, John-Stewart G. Early

response to highly active antiretroviral therapy in HIV-1-infected Kenyan children. J Acquir Immune Defic Syndr. 2007 Jul 1;45(3):311-7

ii. **Wamalwa DC**, Obimbo EM, Farquhar C, Richardson BA, Mbori-Ngacha DA, Inwani I, Benki-Nugent S, John-Stewart G. Predictors of mortality in HIV-1 infected children on antiretroviral therapy in Kenya: a prospective cohort. BMC Pediatr. 2010 May 18;10:33. PMID: 20482796

iii. **Wamalwa D**, Lehman DA, Benki-Nugent S, Gasper M, Gichohi R, Maleche-Obimbo E, Farquhar C, John-Stewart G, Overbaugh J. Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy. J Acquir Immune Defic Syndr. 2013 Mar 62(3): 267-274

Optimizing HIV-1 Therapy- (OPH-03):

Objectives addressed: Data from this study was used for the publication that address the following specific objective:

1.To determine the incidence and correlates of mortality in HIV-infected infants diagnosed at less than 5 months of age and initiated on antiretroviral therapy.

Design and Methods: In this randomized controlled trial 99 ARV-naïve HIV-1 infected infants aged between 0 and 5 months were enrolled and initiated on ART at the Kenyatta National Hospital between 2006 and 2008. At baseline we collected sociodemographic characteristics and measured clinical (history, anthropometry, physical examination) and laboratory (HIV-1RNA, CD4, blood chemistry and haematology). Children were then followed prospectively for with serial clinical evaluation (mortality, growth, morbidity), adherence to ART and immunologic (CD4) and virologic (HIV-1 plasma RNA, genotypic drug resistance mutations) assays ad HIV-1 reservoir. There was a pre-randomization phase of 24 months on antiretroviral therapy before children were randomized to treatment interruption versus continued therapy. After the short phase of treatment interruption (3-6 months) all children resumed antiretroviral therapy and have been followed longitudinally. For this analysis we consider only the pre-randomization phase of follow-up (effectively prospective observational component). The publication based on this study is listed below:

i. **Wamalwa D**, Benki-Nugent S, Langat A, Tapia K, Ngugi E, Slyker JA, Richardson BA, John-Stewart GC. Survival benefit of early infant antiretroviral therapy is compromised when diagnosis is delayed. Pediatr Infect Dis J. 2012 July; 31(7): 729-731.

1.5.3 Paediatric Urgent Start of HAART (PUSH):

Objectives addressed: Data from this study was used for the publication that address the following specific objective:

1. To determine the impact of CMV viraemia on clinical outcomes in children diagnosed with HIV in hospital.

Design and Methods: In this randomized controlled trial 183 ARV-naïve HIV-1 infected infants and children < 12 years of age and hospitalized for severe illness were enrolled from 4 facilities and randomized to urgent (within 48 hours) versus post-stabilization (within 14 days) ART. Two facilities were drawn from Nairobi (KNH and Mbagathi District Hospital) and two from Kisumu (Jaramogi Oginga Odinga Teaching and Referral Hospital and Kisumu County Hospital). Study enrolment started in April 2013 and the last follow-up visit was completed November 2015. Children were followed up prospectively and clinical, immunologic and virologic outcomes measured. We collected plasma samples CMV viral load at baseline (pre-treatment) and serially during follow-up. For this analysis we used children in both arms of the study (urgent as well as post-stabilization ART) to determine the impact of high CMV viraemia on clinical outcomes in the first 12 months of follow-up. The publication based on this study is:

i. **Wamalwa D**, Njuguna I, Maleche-Obimbo E, Begnel E, Chebet DJ, Onyango JA, Cranmer LM, Huang ML, Richardson BA, Boeckh M, John-Stewart G, Slyker J. Cytomegalovirus viraemia and clinical outcomes in Kenyan children diagnosed with Human Immunodeficiency Virus (HIV) in hospital. Clin Infect Dis. 2022 Apr; 9;74(7):1237-1246.

1.5.5 Contribution to the submitted publications

These publications are based on a research conducted through a long-standing collaboration between the University of Nairobi and the University of Washington. I was the lead Kenyan scientist in the design and conduct of the research studies. I played a key role in securing grant funding to support the projects. I provided scientific and administrative leadership to the projects that led to the publications and gave guidance to the clinical teams. I directly participated in the authorship of all the publications on these papers either as first author or senior author mentoring the first author in accordance with best authorship practice. In summary I was directly involved in the work leading to the publications as follows:

1. Design of the studies and setting up the cohorts of children
2. Application for funding to conduct the studies (PI or MPI on the grant proposals)
3. Directing conduct of the studies, analysis of findings
4. Manuscript development as either first author, senior author or member of the team of authors

1.5.6 Ethics and human subjects: All the publications to be submitted derive from studies conducted with grant funding from the US National Institutes of Health (NIH) and received ethics approval from the University of Nairobi- Kenyatta National Ethics and Research Committee. Approvals are attached as appendices to this proposal.

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Chapter 2:

Early response to highly active antiretroviral therapy in HIV-1 infected Kenyan children

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Title

Early Response to Highly Active Antiretroviral Therapy in HIV-1–Infected Kenyan Children

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Abstract

Objectives: To describe the early response to World Health Organization (WHO)–recommended nonnucleoside reverse transcriptase inhibitor (NNRTI)–based first-line highly active antiretroviral therapy (HAART) in HIV-1–infected Kenyan children unexposed to nevirapine.

Design: Observational prospective cohort.

Methods: HIV-1 RNA level, CD4 lymphocyte count, weight for age z score, and height for age z score were measured before the initiation of HAART and every 3 to 6 months thereafter. Children received no nutritional supplements.

Results: Sixty-seven HIV-1–infected children were followed for a median of 9 months between August 2004 and November 2005. Forty-seven (70%) used zidovudine, lamivudine (3TC), and an NNRTI (nevirapine or efavirenz), whereas 25% used stavudine (d4T), 3TC, and an NNRTI. Nevirapine was used as the NNRTI by 46 (69%) children, and individual antiretroviral drug formulations were used by 63 (94%), with only 4 (6%) using a fixed-dose combination of d4T, 3TC, and nevirapine (Triomune; Cipla, Mumbai, India). In 52 children, the median height for age z score and weight for age z score rose from −2.54 to −2.17 ($p < 0.001$) and from −2.30 to −1.67 ($p = 0.001$), respectively, after 6 months of HAART. Hospitalization rates were significantly reduced after 6 months of HAART (17% vs. 58%; $p < 0.001$). The median absolute CD4 count increased from 326 to 536 cells/μL ($p < 0.001$), the median CD4 lymphocyte percentage rose from 5.8% before treatment to 15.4% ($p < 0.001$), and the median viral load fell from 5.9 to 2.2 log10 copies/mL after 6 months of HAART ($p < 0.001$). Among 43 infants, 47% and 67% achieved viral suppression to less than 100 copies/mL and 400 copies/mL, respectively, after 6 months of HAART.

Conclusion: Good early clinical and virologic response to NNRTI-based HAART was observed in HIV-1–infected Kenyan children with advanced HIV-1 disease.

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Background

As programs providing highly active antiretroviral therapy (HAART) scale up in Africa, survival of HIV-1–infected children is expected to improve, based on what has been observed in Western settings (1). In a recent study in Cote d'Ivoire, where the effects of protease inhibitor– or efavirenz-based HAART regimens were evaluated, 50% of children showed suppression of their HIV-1 plasma viral load to less than 250 copies/mL at 2 years (2). In Kenya, there are an estimated 100,000 HIV-1–infected children, most of whom require antiretroviral therapy (3). Most HIV-1–infected children in Kenya and other resource-poor settings present at an advanced stage of HIV disease, with marked immunosuppression because of delays in diagnosis and limited access to treatment (2). This may potentially limit clinical benefits from HAART, given that in the Cote d'Ivoire study, children with a baseline CD4 lymphocyte percentage (CD4%) <5% had higher mortality than their counterparts with a CD4% >5% (2).

The first-line HAART regimens recommended by the World Health Organization (WHO) and Kenyan national guidelines are nonnucleoside reverse transcriptase inhibitor (NNRTI) based (4-6). For HIV-1–infected Kenyan children aged <3 years, nevirapine-containing HAART is recommended as first-line therapy, and for children aged >3 years, nevirapine or efavirenz is recommended. Nevirapine is first-line therapy, in part, because of its stability at room temperature in liquid form, favourable pharmacokinetic characteristics allowing for twice-daily dosing, lack of interaction with food, and relatively lower cost (7). There is concern that nevirapine-based therapy may result in suboptimal viral suppression for children with extremely high viral loads, however.7The first-line regimens in resource-poor settings typically do not include protease inhibitors, for which there are extensive data showing reduction in mortality in Western settings (1,4-6)..

The virologic efficacy of nevirapine-containing triple therapy in early European and US studies ranged from 17% to 50% (8-11). For example, in a study in the United Kingdom, only 17% of children on recommended doses of nevirapine achieved virologic suppression to <400 copies/mL at 96 weeks (11). In these studies, children initiated HAART before the CD4% dropped to levels less than 10%, which is unlike most African children currently starting therapy. In a large US clinical trial, more than two thirds of the children had a CD4% ≥25% at enrollment, further highlighting the

differences in timing of HAART initiation between the 2 settings (12). However, more recently, better response nevirapine based HAART has been reported from the Thai national treatment program, with 71% of the children achieving viral suppression to less than 50 copies/mL at 72 weeks (13). The Thai children had a median CD4% of 3% before the start of HAART.

In the pre-HAART era, African children experienced rapid and more aggressive HIV disease with higher mortality (14). This observation, coupled with late presentation of children in advanced stages of HIV, underscores the need to define the efficacy of NNRTI-based and, in particular, nevirapine-containing HAART regimens in HIV-1 infected African children. We undertook the present study to define the efficacy of first line NNRTI-containing HAART in HIV-1–infected Kenyan children. This information should better inform treatment guidelines, with a view to maximize benefits from HAART as more HIV-infected children access therapy.

Methods

Study Design and Subjects

We conducted a prospective cohort study at the Kenyatta National Hospital (KNH), beginning in August 2004, of HIV-1–infected Kenyan children aged 18 months to 12 years. Eligibility criteria included living in Nairobi, being antiretroviral drug naive, and presenting with advanced disease (WHO clinical stage 3–4) or having a CD4% <15% if in an earlier stage of disease. Children were recruited from the KNH paediatric wards and HIV Care Clinic and were enrolled after receiving written informed consent from their legal guardians.

Study Procedures

At baseline, medical information was obtained by interview and from hospital records. A complete physical examination was performed, and blood samples were collected for baseline hematologic assays, biochemical tests, CD4 cell profile, and plasma HIV-1 RNA viral load. Parents or legal guardians were counselled on the importance of drug adherence and on how to recognize common adverse drug reactions associated with antiretroviral drugs. The primary caregiver was identified as the person

responsible for giving the child medications and bringing the child in for clinic appointments. Caregivers underwent 3 sessions of adherence counselling over a 2-week period before initiation of HAART, during which the importance of giving all antiretroviral drug doses was emphasized. Caregivers were advised to repeat dosing if the child vomited within 30 minutes of administering the antiretroviral drugs.

Antiretroviral Drugs

Antiretroviral therapy was initiated in accordance with Kenyan Ministry of Health guidelines.[5] The first-line anti-retroviral drug regimen consisted of zidovudine (AZT) and lamivudine (3TC) in combination with nevirapine or efavirenz. Nevirapine was prescribed for children aged less than 3 years, whereas older children received nevirapine or efavirenz. At the start of the study, some children were initiated on fixed-dose formulations of stavudine (d4T), 3TC, and nevirapine (Triomune; Cipla, Mumbai, India). Subsequently, individual antiretroviral drug formulations became available through the US President's Emergency Program for AIDS Relief (PEP-FAR), and most children used those. AZT (Retrovir; Glaxo-SmithKline, Parsippany, NJ) was given at a dose of 200 mg/m^2 twice daily, and 3TC (Epivir; GlaxoSmithKline) was given at a dose of 4 mg/m^2 twice daily. Nevirapine (Viramune; Boehringer Ingelheim, Ingelheim, Germany) was given at a lead dose of 120 mg/m^2 once daily for 2 weeks, which was subsequently increased to 200 mg/m2 twice daily. Efavirenz (Stocrin; Merck, Whitehouse Station, NJ) was administered according to the manufacturer's insert based on the child's weight. Triple-nucleoside therapy, including abacavir (Ziagen; GlaxoSmith-Kline), was used for children less than 3 years of age who were on antituberculous treatment. Abacavir was administered at a dose of 8 mg/kg twice daily. AZT was replaced by d4T for children with a haemoglobin count less than 8 g/ dL. d4T (Zerit; Bristol-Myers Squibb, Princeton, NJ) was given at a dose of 1 mg/kg twice daily.

Follow-Up

Children were followed at 2 weeks, at 1 month, and then at monthly intervals after HAART initiation for the first 9 months and once per quarter thereafter. At every appointment, a complete physical examination, including measurement of weight and height, was performed. Information regarding adherence, adverse drug effects, and intercurrent illness was obtained by interview. Follow-up haematologic and

biochemical tests for liver function were performed 1 month after HAART initiation and quarterly thereafter. The CD4 cell profile was determined every 6 months, and the plasma HIV-1 RNA level was assessed quarterly. All children received daily cotrimoxazole prophylaxis against Pneumocystis pneumonia, according to the WHO/United Nations Program on HIV/AIDS (UNAIDS) recommendations (15).

Adherence was assessed at each visit by self-report, where the caregiver was asked whether the child had missed any dose of antiretroviral drugs in the preceding 3 days and 2 weeks, respectively. If a child had missed any doses, further details were obtained as to the exact number of doses missed since the last clinic visit.

Clinical response to antiretroviral therapy was assessed by gain in weight and height and decreased frequency of hospitalization. Immunologic response was measured by change in $CD4^+$ T-lymphocyte count and CD4%, whereas virologic response was assessed by change in plasma HIV-1 RNA levels from baseline. Changes in total lymphocyte counts (TLCs) after HAART initiation were assessed as a potential surrogate marker for CD4 cell counts. An undetectable viral load was defined as <100 copies/mL. The analysis was repeated with a higher viral load cut-off of 400 copies/mL.

Laboratory Methods

HIV-1 was diagnosed using 2 rapid immunoassays, Determine (Abbott Laboratories, Abbott Park, IL) and Uni-Gold (Trinity Biotech, Dublin, Ireland), in a parallel testing algorithm. Plasma HIV-1 RNA assays were performed in Seattle using a transcription-mediated amplification (TMA) method developed by Gen-Probe (San Diego, CA) (16-17). This method has been tested and has shown high sensitivity for detection of Kenyan HIV-1 subtypes A, C, and D.[14] T-cell lymphocyte subsets were measured using FACScan (Becton Dickinson, Franklin Lakes, NJ).

Statistical Methods

The primary outcomes of this study were changes from baseline in clinical, immunologic, and virologic parameters measured after initiating HAART. Height and weight measurements were converted into z scores using the nutrition module of Epi Info 3.2 (Centers for Disease Control and Prevention [CDC], Atlanta, GA). Body mass

index z scores were also computed using Epi Info's nutrition module. This software sets a z score of 0 to correspond to the median score, whereas a score of −2 means 2 SDs less than the median. Reported hospitalization in the 6 months before initiating HAART was compared with that observed within the first 6 months of HAART by the McNemar test. We compared z scores, serum albumin levels, $CD4^+$ lymphocyte counts, CD4%, TLC, and plasma HIV-1 RNA levels before and after treatment using the Wilcoxon signed rank test. We also determined the proportion of children achieving undetectable plasma HIV-1 RNA (<100 copies/mL) after 3, 6, and 9 months of HAART, respectively. This analysis was repeated using a cut-off of 400 copies/mL to define undetectable viral load. Logistic regression was used to determine factors associated with viral suppression to less than 100 copies/mL after 9 months of HAART. Differences in baseline viral load and $CD4^+$ lymphocyte counts and CD4% between children who died versus those who survived were assessed using Mann- Whitney U tests. STATA version 8 (Stata Corporation, College Station, TX) was used for the analyses.

Results

Description of Study Subjects

Between August 2004 and November 2005, we enrolled 67 HIV-1–infected children, of whom 61 (91%) were followed for at least 3 months after HAART initiation (median = 9 months, range: 3–15 months). Six (9%) children died within 3 months of initiating HAART. Forty-six (69%) children were recruited as inpatients, whereas 21 (31%) were from outpatient settings. Only 1 child had received nevirapine for prevention of mother-to-child HIV-1 transmission. The median age at initiation of antiretroviral therapy was 4.4 years (range: 18 months to 12 years), and approximately half (51%) of the cohort was male. For 45 (67%) children, the biologic mother was the primary caregiver, whereas the biologic father was the primary caregiver for 11 (16%) of the children (Table 1).

Antiretroviral Therapy Regimens

Forty-seven (70%) children were initiated on a combination of AZT and 3TC and an NNRTI, whereas 17 (25%) were started on d4T and 3TC in combination with an

NNRTI. Nevirapine was the most frequently prescribed NNRTI, and it was used for 46 (69%); efavirenz was prescribed for 18 (27%) children (Table 2). Two children on concomitant antituberculous therapy were started on triple-nucleoside therapy (AZT, 3TC, and abacavir and d4T, 3TC, and abacavir, respectively). The 1 child who had failed nevirapine perinatal prophylaxis was initiated on AZT, 3TC, and nelfinavir. Individual drug formulations were used by 63 (94%) children, whereas 4 (6%) used a fixed-dose combination of d4T, 3TC, and nevirapine (Triomune). These 4 children were later switched to individual drugs when they became available through the PEPFAR.

Response to HAART

Clinical Response: Anthropometric scores, serum albumin results, hospitalization history, and CD4 cell data were available at baseline and after 6 months for 52 children. After 6 months of HAART, there was a significant increase in the median height for age z score (−2.54 to −2.17; p <0.001) and weight for age z score (−2.30 to −1.67; p = 0.001) (Table 3). In addition, serum albumin, a marker of macronutrient status, increased from a median of 33 g/L at baseline to 41 g/L after 6 months of HAART (P < 0.001).

In this subset of 52 children, 30 (58%) had been hospitalized at least once in the 6 months preceding initiation of HAART compared with only 9 (17%) in the first 6 months after HAART initiation (p < 0.001; see Table 3). Hospitalizations were attributable to infectious disease, including pneumonia, pulmonary tuberculosis, diarrhoea, and severe failure to thrive.

Immunologic Response: In the 52 children with CD4 cell results available at baseline and after 6 months of HAART, the absolute CD4 cell count increased from a median of 326 cells/μL at baseline to 536 cells/μL at 6 months after HAART initiation (p < 0.001). Similarly, the median CD4% increased from 5.8% at baseline to 15.4% at 6 months after initiation of HAART (p < 0.001; see Table 3). Among children with follow-up to 15 months (n = 31), the median absolute CD4 count rose from 286 to 682 cells/μL (p < 0.001). For this group, the CD4% increased from 5.4% to 18.1% (p < 0.001). Overall, the CD4% increased by a median of 7.4% within 6 months of receiving HAART, and in the subset of 31 children with longer follow-up, the CD4% rose by a median of 11.3% (interquartile range [IQR]: 3.4–16.2) after 15 months of therapy.

Although we observed a modest increase in TLC after HAART initiation (median TLC from 3849 cells/mm3 at baseline to 4025 cells/mm3 after 6 months for 52 children), this change was not statistically significant (p = 0.28). After 15 months of HAART, the median TLC had risen to 4116 cells/mm3 (p = 0.59 for change from baseline to 15 months).

Virologic Response: Viral load results were available at baseline and 3 months after HAART initiation for 50 children, at baseline and 6 months after HAART initiation for 43 children, and at baseline and 9 months after HAART initiation for 28 children. At baseline, children aged 3 years or less had a significantly higher viral load than those older than 3 years of age (median: 6.4 vs. 5.8 $\log_{10}$ copies/mL; p = 0.007; see Table 1).

Among children with a viral load available at baseline and 3 months (n = 50), the median plasma HIV-1 RNA load decreased from 6.0 $\log_{10}$ copies/mL at baseline to 2.5 $\log_{10}$ copies/ mL 3 months after HAART initiation (p < 0.001). Viral load was suppressed to less than 100 copies/mL in 15 (30%) and to less than 400 copies/mL in 27 (54%) of these 50 children within 3 months of starting HAART (Table 4).

For children with a viral load available at baseline and 6 months (n = 43), the median viral load decreased from 5.9 $\log_{10}$ copies/mL at baseline to 2.2 log10 copies/mL at 6 months after HAART initiation (p <0.001). Viral load was suppressed to less than 100 copies/mL in 20 (47%) and to less than 400 copies/mL in 29 (67%) of these 43 children within 6 months of starting HAART.

For the subset of children with a viral load available at baseline and 9 months after starting HAART (n = 28), the median viral load decreased from 5.9 $\log_{10}$ copies/mL at baseline to 2.1 $\log_{10}$ copies/mL after 9 months (P < 0.001). Eleven (39%) and 19 (68%) of the 28 children had viral suppression to less than 100 copies/mL and 400 copies/mL, respectively, 9 months after HAART initiation (see Table 4).

Correlates of Virologic Response: Children with a higher viral load at baseline were less likely to have a viral load less than 100 copies/mL after 9 months of HAART (odds ratio [OR] = 0.16 per log10-copies/mL increase in baseline viral load, 95% confidence

interval [CI]: 0.03 to 0.80; p = 0.025; Table 5), and this effect was independent of the age of the child. There was a trend toward children whose parent(s) had undergone HIV testing before the child's enrollment being more likely to have a viral load less than 100 copies/mL after 9 months of HAART (60% vs. 20%, OR = 6.0, 95% CI: 0.93 to 38; p = 0.06). There was no association between self-reported adherence, baseline CD4%, or type of antiretroviral drug regimen used (nevirapine containing vs. efavirenz containing) and virologic response (see Table 5).

Mortality: Six (9%) children died after initiating antiretroviral therapy, all within the first 3 months of HAART. The median time to death was 57 days (range: 23–90 days). The children who died had a median age of 4.6 years, and 4 were male. The baseline HIV viral load was not significantly different between children who died early and those who survived beyond 3 months of treatment (median baseline viral load: 6.4 vs. 6.1 $\log_{10}$ copies/mL, respectively; p = 0.23). Although the baseline CD4 lymphocyte count and CD4% were lower in children who died early (median absolute CD4 count: 135 vs. 364 cells/μL, median CD4%: 5.1% vs. 6.3%), the differences did not reach statistical significance. There was also no significant difference in age or self-reported adherence between children who died and those who survived. The causes of death included cor pulmonale (n = 3), disseminated tuberculosis (n = 1), sepsis (n = 1), and severe pneumonia (n = 1). The 3 children who died of cor pulmonale had presented initially with predominantly right-sided congestive heart failure with severe digital clubbing. The child who died of suspected disseminated tuberculosis presented with multiple abdominal masses and marked wasting and had radiographic evidence of hilar adenopathy. The child with sepsis presented with multiple sites of infection, including septic arthritis and skin abscesses, and had *Staphylococcus aureus* subspecies recovered from blood cultures.

Adherence to and Tolerance of Antiretroviral Drugs

Over the duration of follow-up, 43 (64%) caregivers reported never missing administering any antiretroviral drug dose, whereas 24 (36%) reported at least 1 missed dose. Of the 24 who reported a missed dose, 13 (54%) did so once, 8 (33%) reported missing between 2 and 5 doses, and 3 (13%) reported 6 or more missed doses since the last clinic visit. Ten (15%) children changed at least 1 antiretroviral drug, of whom 7 did so because of severe adverse effects, 2 did so after treatment

failure, and the remaining 1 did so to prevent drug interactions with the antituberculous drug rifampicin. Serious side effects included grade 2 to 3 nevirapine-associated rash in 4 children, AZT-associated anaemia in 2 children, and abacavir hypersensitivity in 1 child. Minor side effects included grade 1 skin rash in 15 (22%) children and gastrointestinal effects, including nausea and vomiting, in 14 (21%) children. Of the children who experienced gastrointestinal effects, the symptoms subsided after 1 month of therapy in 11 (79%).

Discussion

In this cohort of antiretroviral-naive HIV-1–infected Kenyan children with advanced immunosuppression, we observed good early clinical benefit and virologic response to the WHO first-line NNRTI-based HAART regimen. Seventy percent of the children in our cohort were treated with nevirapine-containing regimens, whose efficacy in African children is not well described.

The children experienced a good immunologic response, with a large proportion moving from the severe to moderate suppression category within the first 6 months of treatment (4,18). The immune reconstitution was accompanied by a significant decline in hospitalization rates, which confirms the social and economic benefits of HAART that have been demonstrated in HIV-1–infected children receiving HAART in other settings (19,20). By reducing the frequency of hospitalization, the cost to caregivers is lowered, given that they often have to pay out for pocket for such episodes.

We further observed significant early increases in anthropometric measures, including weight for age, height for age, and body mass index. Such positive impacts on a child's growth are key indicators of good response after initiation of antiretroviral therapy, as reported consistently across paediatric studies (2,13,19). Our cohort consisted of children from poor settings, where malnutrition is highly prevalent. It is therefore possible that with nutritional supplementation, better weight gain may be achievable after HAART initiation in similar settings. Weight and height are simple clinical measurements that do not require costly sophisticated equipment and can be undertaken at all health facilities. Our findings strengthen the WHO's recommendation that where resources are limited, early response to HAART in children can be

evaluated by regular weight measurements. As part of the scaleup of HAART to peripheral health facilities, children who show lack of early weight gain after initiation of therapy should be promptly referred for detailed evaluation and investigations in regional centres.

The virologic efficacy in our study (68% with HIV-1 RNA plasma viral load <400 copies/ mL at 9 months after HAART initiation) is higher than that reported in children treated with nevirapine-containing HAART in early European and US studies, where efficacy ranged from 25% to 50% (7,11). In contrast, 71% of children on nevirapine containing HAART in the Thai national program achieved viral suppression to less than 50 copies/mL after 72 weeks, which is higher than the 39% with levels less than 100 copies/mL at 9 months in our cohort.[13] Possible reasons for the superior efficacy in the Thai cohort may include longer duration of follow-up, higher levels of adherence, having older children with less viral burden at the start of therapy, or different viral strains.

Although other studies have found TLC to be a useful tool in monitoring the response to HAART in adults, our study did not demonstrate significant increases in the TLC in the short term (21,22). This may be attributable, in part, to the small numbers of children and limited duration of follow-up.

The steady rise in serum albumin after initiation of HAART is evidence of macronutrient improvement experienced by the children. A recent study in Kenyan women found low serum albumin to be predictive of low selenium, which, in turn, has been associated with advanced HIV disease (23,24).

All deaths in the study occurred early in the course of treatment. Of the 6 children who died, 3 succumbed to cor pulmonale resulting from recurrent chest infections in the pre-HAART period. It is likely that HAART was initiated too late in these children when the immune system had already been severely damaged or irreversible organ damage had occurred. There is growing evidence that children who start HAART with severe immunosuppression at a CD4% less than 5% may have higher mortality and may be unable to achieve optimal immune recovery, despite good virologic response in the long term (2,25,26).

Ten percent of the children experienced serious side effects that necessitated withdrawal of the drugs; however, there was no drug-related mortality. This is a significant proportion of children and should raise concern for improved monitoring for serious toxicity as programs scale up in sub-Saharan Africa. Laboratory networks that transport samples collected from smaller peripheral sites to regional centres for assays and send results back should be encouraged (2).

In the early analysis (6 months post-ART initiation), two thirds of the caregivers reported full adherence (self-report of no missed doses in the past 3 days and 2 weeks respectively) while one third reported some non-adherence. We found not statistical association between self-reported adherence and mortality. In a subsequent analysis following longer term prospective follow-up, the level of self-reported adherence did not predict virologic outcomes. This illustrates the weakness of self-reported adherence which is prone to recall bias and social desirability bias (27).

In summary, our study provides evidence that the NNRTI-based first-line antiretroviral regimen currently being scaled up for African children is highly efficacious and well tolerated in the short term. There is a need for long-term trials involving larger numbers to obtain further information on the prolonged use of HAART in African children.

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Tables

Table 1

Baseline Characteristics of HIV-1–Infected Children and Their Caregivers Before Initiating HAART

Characteristics	Median or Number (N = 67)	(IQR) or Percent
Child		
Age (y)	4.4	(2.4–6.0)
Male	34	51%
Clinical		
Weight for age z score	−2.45	(−4.3 to −1.54)
Weight for height z score	−1.34	(−2.85 to 0.09)
Height for age z score	−2.00	(−3.32 to −1.04)
Immunologic		
CD4 count, cells/μL	288	(101 to 560)
CD4%	6.2	(3.6 to 10.3)
CD4% <15%	55	82%
Virologic		
Log_{10} HIV-1 RNA copies/mL		
Age 18 months to 3 years	6.4	(6.0 to 6.6)*
Age >3 years	5.8	(5.3 to 6.3)*
Caregiver		
Age (y)	30	(26 to 37)
Education		
Primary	29	43%
Secondary	27	40%
College	7	10%
Married	42	63%
Relationship to child		
Mother	45	67%
Father	11	16%
Grandmother	6	10%
Other	5	7%

Table 2

Antiretroviral Drug Regimens at Initiation

	Nevirapine	Efavirenz	Abacavir	Nelfinavir
AZT and 3TC	36	11	1*	1†
d4T and 3TC	10‡	7	1*	—

Children had concomitant antituberculous treatment, including rifampicin.

Child had been exposed to nevirapine as part of prevention of mother-to-child transmission.

Four of these used a fixed-dose formulation of Triomune (Cipla, Mumbai, India).

Table 3

Clinical and Immunologic Parameters Before and After 6 Months of HAART (n = 52*)

Characteristic	Before HAART	After HAART	*P*
	Median (IQR) or Number (%)		
Weight for age z score	−2.30 (−3.49 to −1.01)	−1.67 (−2.43 to −0.47)	0.001
Height for age z score	−2.54 (−3.56 to −1.44)	−2.17 (−2.86 to −1.13)	<0.001
Body mass index z score	−1.26 (−2.73 to −0.17)	−0.09 (−1.23 to 0.47)	0.01
Serum albumin, g/dL	33 (28 to 37)	41 (37 to 45)	<0.001
Hospitalized	30 (58%)	9 (17%)	<0.001
CD4 count, cells/μL	326 (86 to 540)	536 (273 to 841)	<0.001
CD4%	5.8 (3.1 to 9.7)	15.4 (9.8 to 21)	<0.001

Subset of children who had all variables available at baseline and 6 months after HAART initiation.

Table 4

Time From HAART Initiation (mo)	No. Children	Median HIV-1 RNA Viral Load log copies/ml (IQR)	VL <100 Copies/mL Number (%)	VL <400 Copies/mL Number (%)
0	65	6.1 (5.5 to 6.5)	1 (2)	1 (2)
3	50	2.5 (1.8 to 3.0)	15 (30)	27 (54)
6	43	2.2 (1.4 to 2.9)	20 (47)	29 (67)
9	28	2.1 (1.6 to 2.9)	11 (39)	19 (68

Attrition in numbers is attributable to loss through death and children still in follow-up. VL indicates HIV-1 RNA plasma viral load.

Table 5

Predictors of Viral Suppression to Less Than 100 Copies/mL at 9 Months of Treatment (n = 28)

Characteristic	OR (95% CI)	*P*
Univariate		
Female gender	1.6 (0.33 to 7.26)	0.58
Age (per year increase)	1.18 (0.83 to 1.69)	0.36
Plasma HIV-1 RNA (per $\log_{10}$ increase)	0.16 (0.03 to 0.80)	0.025
CD4% (per unit)*	0.99 (0.90 to 1.00)	0.32
Self-reported adherence problem	0.83 (0.17 to 4.01)	0.82
Nevirapine use (vs. efavirenz)	2.27 (0.36 to 14.5)	0.38
Parent tested for HIV before enrollment	6.0 (0.93 to 38)	0.06
Multivariate		
Plasma HIV-1 RNA (per $\log_{10}$ increase)	0.15 (0.03 to 0.87)	0.035

Measured at baseline.

Controlled for child's age.

Chapter 3:

Predictors of mortality in HIV-1 infected children on antiretroviral therapy in Kenya: a prospective cohort

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Title:

Predictors of mortality in HIV-1 infected children on antiretroviral therapy in Kenya: a prospective cohort

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Abstract

Background: Among children, early mortality following highly active antiretroviral therapy (HAART) remains high. It is important to define correlates of mortality in order to improve outcome.

Methods: HIV-1-infected children aged 18 months-12 years were followed up at Kenyatta National Hospital, Nairobi after initiating NNRTI-based HAART. Cofactors for mortality were determined using multivariate Cox regression models.

Results: Between August 2004 and November 2008, 149 children were initiated on HAART of whom 135 were followed for a total of 238 child-years (median 21 months) after HAART initiation. Baseline median CD4% was 6.8% and median HIV-1-RNA was 5.98-log10 copies/mL. Twenty children (13.4%) died at a median of 35 days post-HAART initiation. Mortality during the entire follow-up period was 8.4 deaths per 100 child-years (46 deaths/100 child-years in first 4 months and 1.0 deaths/100 child-years after 4 months post-HAART initiation). On univariate Cox regression, baseline haemoglobin (Hb) <9 g/dL, weight-for-height z-score (WHZ) < -2, and WHO clinical stage 4 were associated with increased risk of death (Hb <9 g/dL HR 3.00 [95% C.I. 1.21-7.39], p = 0.02, WHZ < -2 HR 3.41 [95% C.I. 1.28-9.08], p = 0.01, and WHO clinical stage 4, HR 3.08 [1.17-8.12], p = 0.02). On multivariate analysis Hb < 9 g/dl remained predictive of mortality after controlling for age, baseline CD4%, WHO clinical stage and weight-for-height z-score (HR 2.95 (95% C.I. 1.04-8.35) p = 0.04).

Conclusion: High early mortality was observed in this cohort of Kenyan children receiving HAART, and low baseline haemoglobin was an independent risk factor for death.

Abstract word count: 251

Background

Sub-Saharan Africa carries the highest burden of paediatric HIV-1 with an estimated 1.8 million children < 15years infected which represents 90% of all children living with HIV worldwide [1]. In Kenya there are approximately 150,000 HIV-1 infected children, out of whom nearly 60,000 are in need of antiretroviral therapy and about 25,000 are currently accessing treatment [2]. There is a concerted effort to raise the number of children on antiretroviral therapy through increased availability of early infant diagnosis and strengthening provider-initiated counselling and testing in health facilities. As a result, survival of HIV-1 infected children in Kenya and similar settings has dramatically improved as more children access highly active antiretroviral therapy [3-7]. However, mortality within the first few months of starting antiretroviral therapy remains high with various studies reporting between 8% and 15% and most deaths attributable to infections and failure to thrive [3-9]. This level of mortality is substantially higher than what is observed for children initiating HAART in developed nations [10-13]. There is limited but increasing published literature on predictors of early mortality following initiation of HAART and few studies have involved African children. Children with advanced HIV disease manifesting as low weight-for-height, as well as those with very low CD4% have been found to be at highest risk of early mortality following HAART initiation in Cote d'Ivoire, Malawi, and Zambia [4,8,9]. In a large Zambian cohort of children followed up for a limited period of time, younger age and low haemoglobin levels were additional factors associated with higher likelihood of early death following HAART initiation [8]. Following the results of the Children with HIV Early Antiretroviral Therapy (CHER) Trial in which early treatment reduced early infant mortality by 76%, international guidelines were modified to recommend initiation of HAART for infants below 18 months of age upon HIV diagnosis [14-16]. Initiation of antiretroviral therapy for older children is still dependent on clinical and immunologic staging [15]. Ideally, a child would be diagnosed and treated early, however, in spite of increased efforts to diagnose children early, some don't present for care till they are older. Therefore, it remains important to better define factors that impact survival of such children in local settings with a view to maximize benefits by addressing any modifiable cofactors. We describe mortality in a cohort of HIV-1 infected children receiving antiretroviral therapy who have been followed up prospectively since 2004.

Methods

The Pediatric Adherence Study was a 5-year prospective observational study which started enrolling children in August 2004 as previously described [6]. Children were drawn from the Pediatric wards and HIV-1 Clinic of the Kenyatta National Hospital (KNH) in Nairobi Kenya. The study enrolled children aged 18 months - 12 years who had advanced clinical and/or immunological HIV disease (WHO clinical stage 3-4 or WHO clinical stage 2 with CD4 <15%) and were antiretroviral drug-naïve. After counselling and informed consent, sociodemographic information was obtained, and a physical examination performed. Samples were taken for baseline laboratory investigations including full haemogram, T-cell lymphocyte subsets (CD4), plasma HIV-1 RNA, and liver function tests, and a return appointment given for initiation of antiretroviral therapy. Children were initiated on a first line antiretroviral drug combination recommended by the Kenya national guidelines which consists of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) [16]. Follow-up visits were scheduled after 2 weeks, monthly for the first year, and quarterly thereafter. At each follow-up visit, children had a physical examination and information regarding adherence, adverse drug effects, and intercurrent illness was obtained. Hematologic and biochemical tests for toxicity monitoring and plasma HIV-1 RNA were performed 3-monthly during the first year and 6-monthly thereafter, while CD4 counts were obtained every 6 months. Information on the cause of death was abstracted from medical records for children who died in hospital, while for those who died at home, verbal autopsy was used. The caregiver was invited to the clinic to give details of the conditions preceding the child's death and a presumptive diagnosis was reached. In cases where the caregiver was unable to come to the research clinic, the study staff paid a home visit to conduct the verbal autopsy. Written informed consent was obtained from all study participants. Verbal assent was obtained from children between ages 7 and 12 years. This study received ethical approval from the Institutional Review Boards of the University of Washington and the Kenyatta National Hospital-University of Nairobi.

Statistical methods

Stata version 8 (Stata Corp, College Station Texas) was used for all analyses. The probability of survival was estimated using the Kaplan-Meier method. Cox proportional hazards models were used to determine baseline characteristics associated with mortality. Factors found to be significant predictors of mortality on univariate Cox

proportional Hazards model ($p < 0.05$) were entered into a multivariate model. We computed z-scores for anthropometric measures (weight-for-age, height-forage, and weight-for-height) using the Epi-Info (version 3.2, Centers for Disease Control, Atlanta Georgia) using CDC 2000 reference population. We analysed weight-for-height z-score as a categorical variable with a cut off value of -2 which is the WHO upper limit for children with moderate protein energy malnutrition [17]. Similarly, we categorized haemoglobin at a cut off value of 9 g/dL, below which children in low-income countries are considered to have significant iron deficiency anaemia [18].

Results

Description of study subjects

One hundred and forty-nine children initiated HAART between August 2004 and December 2006. Baseline characteristics of the children who initiated HAART are shown in Table 1.

Follow-up and outcomes

Figure 1 provides a summary of follow-up and mortality among these children. Out of the 149 children who initiated HAART, 135 returned for at least one scheduled follow-up visit two weeks after initiation of treatment. Of the 14 children who did not complete any clinic follow up, one child died 2 days after HAART initiation while for 13 (9%) children there was no follow-up information available after initiating antiretroviral therapy. By October 2008, median follow-up time on antiretroviral therapy for the 135 who returned to clinic was 21 months (IQR 6, 33) equivalent to 238 child-years. While on follow-up, six (4%) children were transferred to other medical facilities on request of caregivers and 20 (13%) children died. Eleven children (7%) were lost to follow-up after completing at least one clinic visit after HAART initiation.

Mortality

Twenty children died at a median of 35 days post HAART initiation (IQR 13 - 99 days), of whom 18 (90%) died in the first 120 days. The mortality rate over the entire follow-up period was 8.4 deaths per 100 child-years (20 deaths over 238 child-years). Mortality in the first 4 months of follow-up was 46 deaths per 100 child-years (18 deaths over 39 child-years) but this dropped to 1.0 death per 100 child-years between 4 months and 2 years post-HAART (2 deaths over 199 child-years). The cumulative

survival was 95% after 1 month of HAART, 89% 3 months and 85% after 6 and 12 months respectively. The cumulative survival after 25 months was 84% (Figure 2).

Correlates of mortality

On univariate analysis, low baseline haemoglobin level (<9 g/dL), low baseline weight-for-height z-scores < -2, and WHO stage 4 at baseline were significantly associated with mortality (Table 2). Trends for association with mortality were also observed for prior hospitalization, baseline total lymphocyte count, baseline CD4 count, and serum albumin

Causes of mortality

Nineteen of the 20 (95%) children who died had been hospitalized at least once since birth and 18 of them were recruited into the study from the Kenyatta National Hospital paediatric wards. Nine of 20 (45%) children died in health facilities while 11 (55%) died at home. Of those dying in health facilities, 8 died in Kenyatta National Hospital while 1 child died in the local district hospital. Eleven (55%) children had evidence of pneumonia either from hospital records or information given through verbal autopsy at the time of death. Five children (25%) had congestive heart failure with ventricular dysfunction secondary to possible HIV associated cardiac disease. One of these children had dilated cardiomyopathy on echocardiography. Tuberculosis contributed to death in 4 children (20%) and one child died from each of the following conditions: acute diarrhoea, Non-Hodgkin's lymphoma, and anaemia. The primary cause of death could not be established for one child who died at home. Additional file 1, Table S3 summarizes the characteristics of the 20 children who died after initiation of antiretroviral therapy.

Discussion

In this cohort of HIV-1 infected children who initiated highly active antiretroviral therapy at advanced immune suppression we observed high early mortality. The first 4 months after HAART initiation were associated with highest mortality, and children who survived this period were less likely to die in the subsequent 2 years of follow-up. In our cohort pre-treatment haemoglobin <9 g/dL, weight-for-height z-scores < -2, and WHO clinical stage 4 were associated with mortality on univariate analysis. Only haemoglobin < 9 g/dL remained significantly predictive of mortality on multivariate

analysis. However, with 20 events, the power for assessing multiple covariates was limited and trends remained for weight-for-height z-score < -2 and WHO clinical stage 4.

Thirteen percent of the children died over the entire period of follow-up, 12% within the first 4 months of starting HAART. This falls within the range of mortality observed in similar African cohorts, but significantly higher than death rates among HAART-treated children in industrialized countries [3, 4, 8-12]. A recent review comparing mortality in HIV-1 infected children receiving HAART between resource-rich and resource-limited settings found mean mortality rates of 2.9 vs. 9.0 deaths per 100 person years, respectively, or 4.4% vs. 8.1% with higher death rates in poor countries [13]. The main differences identified between children initiating HAART in the two settings were higher baseline CD4 levels (24% vs. 11%) and lower baseline viral loads (4.87 log vs. 5.57 log copies/mL) in resource-rich countries compared to resource-poor settings, respectively. These differences indicate that children in resource-rich settings generally initiated HAART earlier in their illness and suggest that early initiation of HAART will most likely result in fewer deaths. The difference in mortality rates could also be due to the limited supportive care available to children in resource-poor settings at the most crucial period before they immune reconstitute to a level that reduces their vulnerability to potentially fatal opportunistic infections. Finally, the disparate mortality rates may reflect a difference in the rates of Immune reconstitution inflammatory syndrome (IRIS) between the two settings. One child in our study died from probable TB - IRIS and it is possible that IRIS contributed to other deaths.

The pattern of mortality we observed is typical of what has been described in other cohorts of HIV-1 infected African children receiving HAART [8,9]. The death rate dropped from a high of 46 deaths per 100 person-years to 1 death per 100 person years, a remarkable fall after the 4-month post-HAART initiation point. The consistent pattern of high mortality in the first 3-6 months before a dramatic and sustained decrease in death rate suggests that children with severe immune deficiency require some time before the benefits of HAART are fully realized. It appears that with the current approach to therapy it may be difficult to salvage some children with extremely advanced HIV and the number of such children increases as HAART is initiated much later in the disease course.

Although there are no data for age-specific mortality figures for Kenyan children above 5 years, it is notable that the mortality in our study, (13%) approximates the current under-5 child mortality in Kenya (128 per 1000 live births) [19]. In contrast, mortality approaching 50% was reported in an untreated cohort of HIV-1 infected Kenyan children [20]. Thus the use of HAART has substantially reduced mortality to rates that are at least as high as those observed in the general population compared to the pre-HAART period when mortality was about 3 times higher in HIV infected children.

Our finding of anaemia as a predictor of mortality is consistent with reports from few paediatric and adult African cohorts [8,9]. It is notable that the levels of anaemia in question (Hb < 9 g/dL) are modest and would not normally lead to rapid clinical deterioration. It is more likely that anaemia is a surrogate marker for advanced HIV [21, 22]. In addition, anaemia is a well-recognized component of severe protein-energy malnutrition which exists in 5-10% of Kenyan children [23]. We also found a trend of association between low weight-for-height and mortality. Growth failure (measured as low weight-for-height or low weight-for-age) has been found to independently predict mortality in several studies [8, 9, 24]. In the context of our study, in which 89% children were classified in the severe immune category, weight-for-height z-score < -2 may represent additional nutritional deprivation. The effect of severe protein energy malnutrition on both humoral and cell-mediated immunity has been previously described [25-27]. It is conceivable that children with both conditions, advanced HIV -1 disease and severe protein energy malnutrition will have limited capacity for immune recovery and are especially prone to life-threatening microbial infections.

Unlike several paediatric studies, we did not demonstrate a significant association between the baseline CD4% or viral load and mortality [7, 8, 28]. This may be due to the small sample size and generally low CD4 counts, thus limiting power to detect differences between various CD4 thresholds.

Infectious conditions, specifically pneumonia, contributed to the greatest number of deaths followed by underlying cardiac conditions and tuberculosis. Although infections remain an important cause of mortality in HAART-treated children in Western cohorts, the proportion and overall burden of infectious illness is less [12]. Given such a high

burden of infectious illness in this cohort, it is also possible that IRIS played a role in some of the deaths. A large proportion of the children had known risk factors for IRIS such as low weight-for-age and severe immune suppression [29]. Cardiac-related causes contributed to approximately a quarter of the deaths in our study. HIV-associated cardiac involvement presenting as myocarditis, left ventricular dysfunction, and dilated cardiomyopathy has been described in patients with advanced disease with low CD4 counts such as the majority of the children in our cohort [30]. The mechanisms for cardiac disease include direct effect of HIV, other cardiotropic viruses, cytokines and opportunistic infections [31, 32].

Strengths of our study include long follow-up of children starting HAART with data on many concurrently important predictors of mortality (including viral load, CD4% and nutritional parameters). Limitations of our study include the relatively small sample size, the lack of post-mortem studies, and for children who died at home, reliance on verbal autopsy to determine the cause of death. This may lead to inaccurate labelling of the cause of death in such cases. In addition, the substantial number of children lost to follow-up may include children who died and thereby our study may underestimate mortality.

The importance of our findings relates to building evidence that it may be possible to identify the children at highest risk of early death following initiation of HAART in resource-poor settings. Efforts aimed at prompt identification of HIV infected infants early in the disease course should be prioritized to enable timely initiation of HAART in order for children to realize maximal benefits from expanding treatment programs. It is also important to investigate targeted interventions that intensify support for children during the first 4-6 months following HAART initiation with particular attention given to those presenting with anaemia and low weight-for-height.

Conclusion

Weight-for-height z-score < -2, Haemoglobin <9 g/dL and WHO stage IV were predictive of mortality on univariate analysis. Only Haemoglobin <9 g/dL at baseline remained independently predictive of mortality. Besides efforts to initiate HAART earlier in the course of illness, HIV infected children presenting with anaemia and severe wasting should be especially prioritized for care post- HAART initiation.

Targeted research on the role of nutritional support and correction of anaemia should be considered.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors read and approved the final manuscript. DW was the lead author who led the design and conduct of the study as well as manuscript development. EO provided clinical expertise and epidemiological input in manuscript development. CF provided epidemiological support for manuscript development. BAR provided guidance on study design and led statistical analysis. DMN gave input on clinical and epidemiological aspects of the study and manuscript development. II provided clinical care of the children in study and gave input on clinical aspects of manuscript development. B provided statistical support while GJS provided mentorship in the overall design and epidemiological aspects of the study and manuscript development.

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Figures

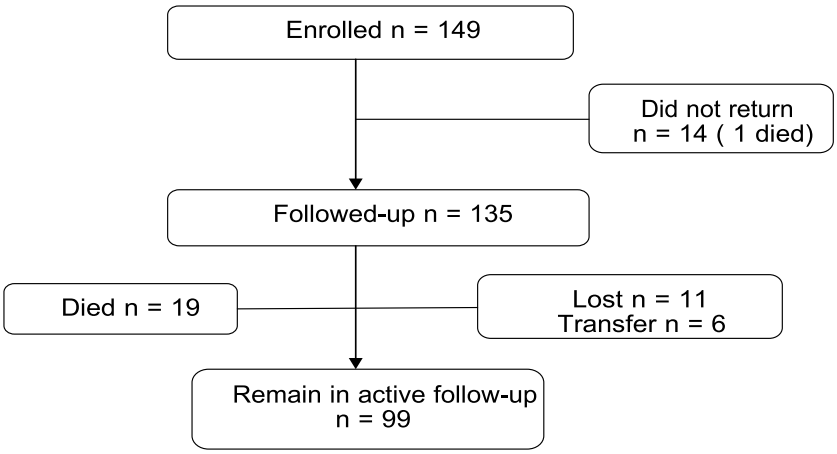

Figure 1 Follow-up and retention in the cohort .

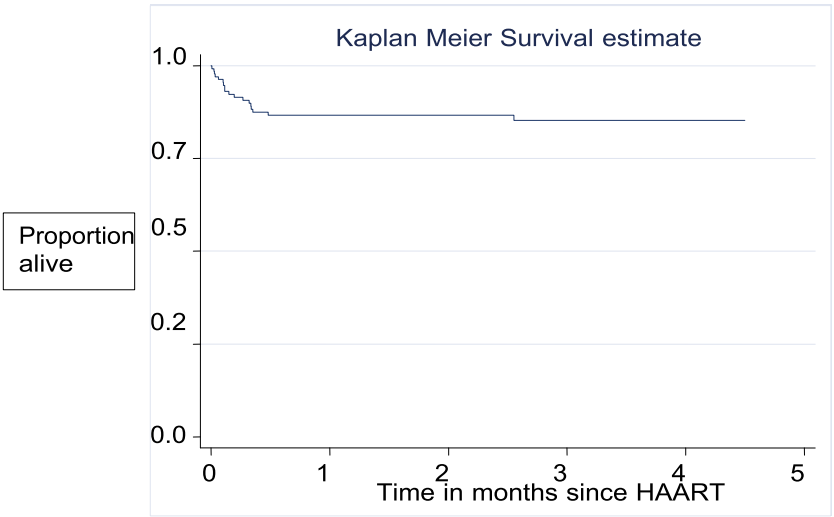

Figure 2 Probability of survival among children receiving HAART .

Tables

Table 1: Baseline characteristics in children who initiated highly active antiretroviral therapy.

Characteristics	Number	Median (IQR) or N (%) (N = 149)
Socio-demographic		
Age (years)	147	4.9 (2.6, 6.7)
Female	149	74 (50)
Orphaned Lost one parent	149	44 (30)
Lost both parents	149	10 (7)
Clinical		
Hospitalization	147	111 (76)
WHO clinical stage 4	145	20 (14)
Anthropometry		
Weight for age z-score	142	-2.35 (-3.14, -1.49)
Weight-for-height z-score	133	-1.13 (-1.94, -0.21)
Height for age z-score	141	-2.35 (-3.47, -1.22)
Immunologic		
CD4 count cells/μL	145	286 (101, 552)
CD4 cell percent	137	6.8 (3.6, 11.4)
CD4 percent severe immune category***	137	122 (89)
Total lymphocyte count cells/mm^3	144	3762 (2420, 5788)
Virologic		
Log_{10} HIV-1 RNA copies/mL	127	5.98 (5.44, 6.46)
Haemoglobin g/dl	146	10.2 (9.0, 11.6)
Serum albumin	137	33.0 (28.8, 39.0)

*** Age specific WHO categories for HIV infected children (33)

Table 2: Correlates of mortality among children who initiated HAART

Univariate					
Characteristic	***HR***	***(95% CI)***	***p***	***Number***	***Percentage (out of 149)***
Clinical, sociodemographic					
Age ≥3 yrs)	1.30	(0.47-3.62)	0.6	147	99
Sex (female)	0.79	0.33 - 1.91	0.6	149	100
WHO stage 4	3.08	1.17-8.13	**0.02**	145	97
Hospitalized since birth	7.09	0.95-53.14	0.06	147	97
Weight-for-height z score < -2	3.41	1.28-9.08	**0.01**	142	95
Tuberculosis at baseline	1.49	0.62-3.57	0.4	149	100
Lost parent	1.25	0.50-3.13	0.6	149	100
Laboratory					
Log HIV-1 RNA	0.80	0.58-1.11	0.2	127	85
CD4 count (per 100 cells)	0.86	0.73-1.02	0.09	145	97
CD4 percent < 15%	1.74	0.40-7.56	0.5	137	92
Haemoglobin (< 9 g/dl)	3.00	1.21-7.39	**0.02**	146	98
Total lymphocyte count (per 1000)	0.79	0.63 - 1.00	0.05	144	97
Serum albumin	0.93	0.86-1.00	0.05	137	92
Multivariate					
Weight-for-height z score < -2	2.48	0.87-7.04	0.09	142	95
Haemoglobin (<9 g/dl)	2.95	1.04-8.35	**0.04**	146	98
WHO stage 4	3.03	0.96-9.55	0.06	147	99

Chapter 4:

Long-term virologic response and genotypic resistance mutations in HIV-1infected Kenyan children on combination antiretroviral therapy

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Long-Term Virologic Response and Genotypic Resistance Mutations in HIV-1 Infected Kenyan Children on Combination Antiretroviral Therapy

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ABSTRACT

Background: HIV-infected children may require the use of combination antiretroviral treatment (cART) into adulthood. However, regimens are limited to first line and second line in many African settings. Therefore, understanding the long-term rate of virologic failure and drug resistance during prolonged antiretroviral treatment is important for establishing treatment strategies in African paediatric cohorts.

Methods: Children aged 18 months to 12 years initiated first-line cART and were followed every 1–3 months, for up to 5.5 years. Treatment was switched to second-line cART based on clinical and immunologic criteria according to national guidelines. Virologic failure was determined retrospectively as defined by viral loads greater than 5000 copies per milliliter. Drug resistance was assessed during viral failure by population-based sequencing.

Results: Among 100 children on first-line cART followed for a median of 49 months, 34% children experienced virologic failure. Twenty-three (68%) of the 34 children with viral failure had detectable resistance mutations, of whom 14 (61%) had multiclass resistance. Fourteen (14%) children were switched to second-line regimens and followed for a median of 28 months. Retrospective analysis revealed that virologic failure had occurred at a median of 12 months before switching to second line. During prolonged first line treatment in the presence of viral failure, additional resistance mutations accumulated; however, only 1 (7%) of 14 children had persistent viraemia during second-line treatment.

Discussion: Virologic suppression was maintained on first-line cART in two-thirds of HIV-infected children for up to 5 years. Switch to second line based on clinical/immunologic criteria occurred ;1 year after viral failure, but the delay did not consistently compromise second-line treatment.

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BACKGROUND

Combination antiretroviral therapy (cART) has transformed the natural course of paediatric HIV-1 from a rapidly fatal illness into a chronic disease.[1,2] In Africa where the majority of the world's 2 million HIV-1–infected children reside, improved access to early infant HIV-1 diagnosis, rapid scale-up of antiretroviral drug programs, and current guidelines that recommend initiating treatment in all infants irrespective of CD4 count or clinical disease stage have all contributed to better survival.[3] As children with HIV-1 survive longer on cART, greater emphasis is being placed on the importance of long-term viral suppression. Two recent pooled analyses on the effectiveness of antiretroviral therapy (ART) in resource-constrained settings found that between 40% and 81% of children have complete virologic suppression by 12 months of treatment.[4,5] Although there is substantial literature describing outcomes during the first year of therapy, there is a scarcity of data on long-term outcomes among cART-treated African children.[6,7] Similarly, data on the frequency and pattern of genotypic resistance mutations that arise in response to first-line therapy in this population is largely limited to the first year of treatment.[8,9]

The standard of care in resource-limited settings does not include virologic monitoring and instead relies on clinical and immunologic criteria to indicate failing regimens.3 However, increasing evidence suggests that clinical and immunological failure may not adequately detect failing regimens in HIV-1–infected children[10,11] and that prolonged treatment on failing regimens may accelerate the emergence of multiclass resistance.[12,13] It is anticipated that a large number of the children currently on first-line cART will require second-line therapy in the next few years, and therefore it is important to define the pattern of resistance mutations that arise in African cohorts where HIV-1 non–subtype B is predominant. We describe the pattern of virologic failure and genotypic resistance in a cohort of Kenyan children followed for 3–5 years after treatment initiation.

METHODS

The Pediatric Adherence Study is a prospective cohort established in 2004 to study long-term outcomes of HIV-1–infected Kenyan children initiating cART as previously described.14,15 Children were recruited from the Kenyatta National Hospital (KNH) paediatric wards and HIV Care Clinic and were enrolled after receiving written

informed consent from their legal guardians. Antiretroviral-naïve HIV-1–infected children aged 18 months to 12 years who met clinical (WHO stage 3–4) or immunologic (CD4 < 15%) criteria, which were the WHO recommended criteria for starting cART at the time the study was conducted, were started on nonnucleoside reverse transcriptase inhibitor (NNRTI)–based cART. Thus, initiation of cART and the follow-up in this cohort was similar to what other Kenyan children received at the time, except that entry into the study depended on being hospitalized at KNH, and therefore, this cohort represents children who were sick at the time of enrolment. The specific drugs used in first-line regimens were selected as previously described.[14] The decision to switch to a second-line regimen was based on clinical or immunological criteria according to the current Kenyan National Guidelines.[16] Children were followed prospectively at the KNH research clinic at monthly intervals in the first year and 3 monthly visits subsequently. At every visit, clinical assessment was performed and self-reported adherence was obtained from the caregiver by 3-day and 2- week recall of missed doses. Caregivers were asked to bring the medication, including empty bottles, to each clinic visit. In all cases, the caregiver was either a parent or close family member including grandparent, uncle, or aunt. Overall adherence was the average percent adherence for all clinic visits. CD4 counts were determined using FACSCOUNT, BD Biosciences (Franklin Lakes, NJ) and CD4% determined using a dual platform for absolute lymphocyte count from the Humalyser, haematology analyzer using blood collected at enrollment, at months 3, 6, 15, and every 6 months thereafter.

Viral Load Testing and Virologic Failure

Plasma samples that were collected every 3 months during the first year and 6 monthly thereafter were frozen and shipped to Seattle, Washington, in liquid nitrogen and stored at 280°C until use. HIV-1 RNA levels were measured by the Gen-Probe HIV-1 viral load assay (Gen-Probe, San Diego, CA), which has been validated on the subtypes prevalent in Kenya.[17] We considered a child to have virologic suppression if their viral load dropped and remained below 5000 copies per millilitre after treatment initiation based on the current WHO definition of viral failure in children.[3] Virologic failure was classified into 2 categories as follows: Incomplete viral suppression in which a child's viral load failed to drop below 5000 copies per millilitre after ≥ 3 months of therapy; and viral rebound in which a child's viral load rose above 5000 copies per

millilitre for ≥2 viral load measurements after a period of initial suppression, or if the last sample available was ≥ 5000 copies per milliliter.[3]

Genotypic Resistance Testing

For all children who experienced virologic failure, we performed genotypic resistance testing at baseline (pre-ART) and on either the first or second sample that had a viral load ≥ 5000 copies per milliliter. In children with detectable resistance at the initial point of viral failure, resistance testing was also performed on the last sample available during first-line cART (before initiating second-line cART or at the end of follow-up in children who were not switched). To detect mutations known to confer drug resistance, population-based sequencing was performed on HIV-1 RNA extracted from 140 mL of plasma as previously described.[18] Briefly, a 645-bp region of HIV-1 pol was amplified in duplicate using nested reverse transcriptase–polymerase chain reaction on RNA normalized to 500 viral copies per reaction. Three sequencing reactions were performed on each duplicate polymerase chain reaction product. The sequences were analysed using Sequencher, version 4.5 (Gene Codes Co, Ann Arbor, MI). To differentiate mixed peaks from background noise, a line was drawn such that 95% of secondary peaks were below the line. A site was defined as a "mixed peak" if the secondary peak was above background in at least 3 of 4 sequences. A consensus sequence was submitted to the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu/) for interpretation of drug resistance[19]. In replicate reactions of known mixtures of wild-type and mutant sequences, we reliably detected mutant sequences present at ≥ 20% of total sequence with this method (data not shown).

Statistical Methods

We compared baseline characteristics in children who failed cART to those who did not fail using Pearson χ^2 and Mann–Whitney tests for categorical and continuous variables, respectively. A linear mixed effects model was performed to model the association of immunologic response with virologic response. We performed univariate Cox proportional hazards to model factors associated with virologic failure. The Cox proportional hazard assumptions were confirmed by comparing slopes of the log–log survival plots for each variable and by the global test for proportional hazards based on the Schoenfeld residuals. In children who experienced viral failure, univariate

logistic regression was performed to model the association with resistance mutations. All analyses were performed with Stata version 9.2 (College Station, TX).

RESULTS

One hundred forty-nine children were enrolled and initiated cART between August 2004 and December 2006. Of those enrolled, 14 children did not have a baseline viral load sample available and 35 children either died or were lost to follow-up before their next scheduled appointment. The remaining 100 children had viral load results available at baseline and a median of 9 (range: 1–14) viral load results after cART initiation and were included in the analysis. During follow-up on first-line treatment, 3 of the 100 children died and 16 were lost to-follow-up, and 1 of 14 children died during second-line treatment. The median follow-up of the 100 children on cART was 49 months [interquartile range (IQR): 35–60 months].

Baseline characteristics of these 100 children are shown in Table 1. At enrollment, the median age was 4.5 years, and 33 (33%) of the children were ,3 years of age. Fifty-three percent were female, and 89% were classified as WHO clinical stage 3–4. The median baseline CD4% was 6.8, and median viral load was 6.0 log10 copies per milliliter. None of the children's mothers had received antiretroviral drugs to prevent mother-to-child transmission (PMTCT). In addition, all but one of the children were antiretroviral naive at cART initiation, as they were infected before prophylaxis for PMTCT was widely available. The first-line cART regimen in this cohort consisted of zidovudine (ZDV) plus lamivudine (3TC) with an NNRTI in 75 (75%) children, whereas stavudine (D4T) plus 3TC in combination with an NNRTI was used in 25 (25%) children. The NNRTI backbone consisted of nevirapine and efavirenz in 57 (57%) and 34 (34%) children, respectively.

Virologic Response

In the majority of the 100 children who initiated treatment, virus levels were successfully suppressed during first-line cART (Fig. 1A). Thirty-four (34%) children had virologic failure at a median of 9 months from cART initiation (IQR 6–20 months). Twenty (59%) of the 34 children who experienced virologic failure did so during the first year of cART, whereas an additional 8 (24%) failed during year 2. Twenty-five

(74%) children with virologic failure initially suppressed their virus and later experienced viral rebound, whereas the remaining 9 (26%) never had complete viral suppression.

Immunologic Response Associated With Virologic Outcome

Of the 100 children, CD4 results were available both at baseline and after 6 months of cART for 81 children, after 15 months of cART for 77 children, and for 59 children by 57 months of first-line cART. In all children with available CD4 results during first-line cART, the median CD4% rose from 6.9% at baseline to 17% at 6 months, to 21% by 15 months, and to 34% by 57 months (Fig. 1B). Based on a linear mixed effects model, children who experienced viral failure had a trend toward a lower CD4% at baseline (12.7% versus 15.9%, p = 0.08). Over time during first-line, the rate of increase in CD4% was lower in those with viral failure compared with children who continued to suppress their virus during first-line cART (increase of 2.5% versus 3.3% per year, p = 0.016). This suggests that children with virologic failure experienced a significantly poorer CD4 response.

Predictors of Virologic Failure

Children who experienced virologic failure were younger at enrollment than those with viral suppression (median age: 3.3 versus 4.7 years), but the difference was not statistically significant (Mann–Whitney test; p = 0.07; Table 1). When age was dichotomized to above or below 3 years, children ,3 years at cART initiation had increased likelihood of experiencing virologic failure (HR = 2.25 (95% CI 1.14, 4.42), p = 0.02) using either a univariate cox proportional hazard model (Table 2) or a chi-square analysis (data not shown). None of the other baseline characteristics including sex, WHO clinical stage, weight-for-height Z score, log viral load, CD4%, type of nucleoside reverse transcriptase inhibitor (NRTI) or NNRTI in first-line regimen, or adherence were predictive of virologic failure (Tables 1 and 2). When multivariate analysis was done using Cox proportional hazard models, the results were similar (data not shown).

Genotypic Resistance at Virologic Failure During First-Line Treatment

Twenty-three (68%) of the 34 children with virologic failure had mutations associated with drug resistance at the initial point of virologic failure. This constitutes 23% of the overall cohort. Of children with initial viral suppression followed by rebound, 72% had detectable resistance mutations, whereas only 56% of those whose virus was never suppressed had resistance ($p = 0.37$). We performed univariate logistic regression to assess potential predictors of resistance and found no significant associations. However, only 34 children with viral failure were tested for resistance, and therefore, power was quite limited. Overall adherence was not associated with development of resistance as 11/23 (48%) children with resistance mutations were less than 100% adherent, whereas 7/11 (64%) children without resistance mutations were ,100% adherent ($p = 0.39$). Table 3 provides a list of all 23 children with specific resistance mutations. Overall, 2 (9%) of the 23 children with resistance had mutations to NRTIs only, 7 (30%) children had resistance to NNRTIs only, and 14 (61%) had multiclass resistance (Table 3). Multiclass resistance was prevalent (n = 13, 52%) in children who experienced viral rebound, but was rare (n = 1, 11%) in children with incomplete viral suppression ($p = 0.03$; Table 3). The most common resistance mutation was M184V present in 15, followed by K103N and G190A/S in 11 and 7 children, respectively. Four children had thymidine analogue resistance mutations (TAMs) including M41L, D67N, K70R, T215Y, and K219EQ. Only 1 child had resistance to the first-line regimen detectable at baseline with a single mutation, V179D (data not shown), that confers low-level resistance to NNRTIs.

Accumulation of Resistance During Extended First-Line Treatment in the Presence of Unrecognized Viral Failure

The decision to switch to second-line therapy, which included 3 new drugs according to Kenyan Ministry of Health guidelines, were based on clinical and/or immunological criteria because viral load testing was not routinely available in 2004 when this cohort began (and is still not widely available in many parts of Kenya). Fourteen (14%) children were switched to second-line regimens (ritonavir-boosted lopinavir) due to clinical and/or immunological failure at a median of 30 months (IQR: 18–36), 12 of whom experienced viral failure before switch (see Figure S1A, Supplemental Digital Content, http://links.lww.com/QAI/A366). Using archived samples, we observed that 12 (86%) of the children who had been switched based on clinical criteria had

experienced virologic failure at a median of 9 months (IQR: 8–17), indicating that virologic failure occurred well before clinical and immunological deterioration. The delay between viral failure and switch to second-line treatment can be seen in the Supplemental Figure (see Figure S1B, Supplemental Digital Content, ttp://links.lww.com/QAI/A366). Eleven (92%) of these 12 children (Table 3) had resistance detectable at the initial point of viral failure, 10 (91%) of them had multiclass resistance. The median delay on first-line Cart in the presence of unrecognized virologic failure was 12.5 months (IQR: 10–19). In addition, of 34 children with viral failure, 22 were not switched to second line but had evidence of viral failure using retrospective samples. However, only 12 (55%) of these 22 (Table 3) had detectable resistance mutations at the initial point of viral failure, 4 (33%) of them had multiclass resistance. After extended first-line treatment in the presence of unrecognized viral failure, we performed resistance testing on the last sample during first-line treatment in 23 children with samples available. Eighteen of these 23 children accumulated additional mutations during the extended time on first-line cART (Table 4). Although the majority of children already had multiclass resistance at the initial point of viral failure (Table 3), 6 of 10 children tested that initially had either no mutations or only single-class resistance, accumulated multiclass resistance after extended first-line cART.

Virologic Suppression and Resistance After Switch to Second-Line Treatment

In children who were switched to ritonavir-boosted lopinavir, the median duration of virologic follow-up on this regimen was 28 months, during which time 5 (38%) children had at least 1 viral level above 5000 copies per milliliter. However, during the entire follow-up period on second-line treatment, only 1 child had sustained viral levels ≥ 5000 copies per milliliter, whereas the remaining 4 had only intermittent viraemia. Only one of these 5 children had evidence of protease resistance during intermittent viraemia and at baseline, with a minor mutation (L10I) which can occur in untreated individuals and is only associated with resistance to protease inhibitors (PIs) when present with other mutations.

DISCUSSION

In this cohort of HIV-1–infected Kenyan children, we observed a virologic failure rate of 34% during a median of 49 months on first-line NNRTI-based cART. This is

comparable to the viral failure rates seen in other paediatric cohorts in similar settings.[7,8,20] The median time to virologic failure on first-line treatment in our study was 9 months, and it is notable that 82% of those that experienced virologic failure did so during the first 2 years on cART. Thus, the rate of failure was low in children who maintained viral suppression at 2 years.

The major strength of our study is the long follow-up, which demonstrates that durable virologic response is an achievable goal in at least two-thirds of HIV-1–infected children treated with first-line cART in similar settings. Nevertheless, the proportion of children who experienced early virologic failure is a cause for concern and indicates the need to further optimize adherence, especially in the initial months of treatment. In this cohort, younger children had a higher likelihood of virologic failure even after controlling for baseline viral load, similar to previous findings.[21] This could result from subtherapeutic drug levels in younger children due to lower adherence or differences in pharmacokinetics. We did not monitor drug levels and therefore cannot confirm the possibility of subtherapeutic treatment. However, younger children are fully dependent on a caregiver for drug administration and only 36% of our cohort reported disclosure to other family members, implying that the pool of potential caregivers able to administer medication in the event of the primary caregiver's absence was limited. Although we inquired about missed doses and spitting out medications, this information was based on self-report and may not be accurate. A study in the same facility found that self-report overestimates true adherence when compared with pharmacy records.[22] In addition, pharmacokinetic data for most antiretroviral drugs is poorly defined for young children and underdosing may occur. These findings suggest that younger children should be prioritized for virologic testing in settings where access to viral monitoring is available on a limited basis. Aside from age, no other baseline characteristics were associated with viral failure; however, our sample size was limited to 100 children in total and only 34 experienced viral failure, thus power was limited. In contrast to findings from a study in Uganda, we did not find that low baseline CD4 or type of NRTI backbone predicted virologic failure.[8] In the Ugandan cohort (n = 222), with shorter follow-up (12 months), lower baseline CD4 counts, male sex, and use of stavudine (D4T)-based treatment was associated with virologic failure. The smaller size of our study and homogeneity of baseline CD4 may explain the lack of detecting a similar association.

Two-thirds of the children with viral failure had resistance detectable at the point of failure, the majority of whom had 2 or more clinically relevant mutations resulting in multiclass resistance. At the point of virologic failure, the 2 most common mutations found were M184V, which confers high-level resistance to 3TC, and K103N, which confers resistance to all first-generation NNRTIs. This is similar to findings from studies in Uganda, Central America, and Cote d'Ivoire and is in part due to the low genetic barrier to resistance for 3TC and NNRTIs.[23–25] The virus that bears the M184V mutation has been found to be relatively unfit, incapable of rapid replication, and has increased susceptibility to ZDV, which may explain why a number of children in our cohort who remained on ZDV in the presence of virologic failure were clinically stable.[26] In fact, in children in our cohort on ZDV-based cART, viral load was significantly lower at rebound compared with baseline in children with detectable M184V compared with children whose mutations did not include M184V (data not shown, $p = 0.01$). The WHO guidelines were recently revised to retain 3TC in second-line paediatric regimens due to the high prevalence and poor replicative capacity of M184V, and our findings confirm the relevance of these guidelines for Kenya.

TAMs and K65R were found at viral failure in 4 children and 1 child, respectively, which was less frequent than the prevalence of NNRTI-associated mutations in our cohort but higher than the prevalence observed in a large cohort in South Africa.[27] These mutations limit the choice of second-line regimens and therefore present a challenge to children failing thymidine-based first line.[27] The Kenyan national guidelines were revised to give preference to abacavir over ZDV in first-line ART to lower the potential for development of TAMs.[16]

There are several reasons why children without detectable resistance may still fail to achieve virologic suppression. The most plausible of these is incomplete adherence which was quite common in this cohort. Other reasons include drug intolerance, drug -drug interactions and inaccurate measures of drug resistance that fail to detect low frequency drug resistance [28]. . In the absence of resistance, it is possible for children to achieve virologic suppression if adherence is improved. Previous studies provide evidence that targeted counselling can lead to viral suppression, averting the need for second-line regimens.[29,30] Therefore, as virologic testing becomes increasingly

available in these settings, optimizing adherence should be the first approach to addressing viral failure when resistance testing is not available. In our study, 22 children who did not meet the clinical criteria to switch to second-line cART had evidence of viral failure upon retrospective testing. However, only 12 (55%) of these children had evidence of antiretroviral resistance at viral failure. Thus, for 10 (45%) children, viral suppression could possibly have been achieved with better adherence. These findings underscore the importance of resistance assays which, when available, add critical information to viral load assays to guide treatment. Switch to second-line treatment was based on clinical or immunological failure, which lagged viral failure by an average of 12 months. This extended period on first-line treatment in the presence of unrecognized viral failure resulted in the accumulation of additional resistance mutations in 18 of 23 children, and multiclass resistance often developed in children who had only single-class resistance or no resistance at the onset of viral failure. There is evidence from other studies that this lag in switching to second-line treatment is associated with increased mortality rates, particularly when the first-line is NNRTI based.[31] A recent study found viral loads of >5000 copies per milliliter was associated with a nearly doubled risk of developing a WHO stage 3–4 event, independent of CD4 count, haemoglobin level, and body mass index.[32] Thus, our study suggests that increased access to virologic testing may be useful for early detection of treatment failure and could improve treatment outcomes. The number of children in our cohort who were switched to PI-ART was relatively small (n = 14). However, a long follow-up (median 28 months after switch) showed that persistent virologic failure on second line was rare. This was true although 10 of the 14 children who switched to PIART had detectable resistance to both NRTIs and NNRTIs before the switch, suggesting that PI monotherapy may be effective in some children as shown in recent studies.[33,34] Despite the lag after virologic failure on first line, most sustained viral suppression on PI therapy well beyond 2 years and the emergence of detectable protease resistance was rare. This is reassuring in settings where third-line regimens, including second-generation boosted PIs or integrase inhibitors, are not feasible due to high cost. Limitations of this study include the fact that the cohort was established primarily for research, which may somewhat limit generalizability. Resistance was assessed by population-based sequencing, which only detects resistant virus that comprises >20% of the viral population, and therefore it is possible that we missed resistance mutations present at lower frequencies in these children. In addition, the cohort was established

in the pre-PEPFAR period, when access to ART was critically limited and therefore may represent very sick children and self-selected survivors. Baseline CD4% at cART initiation in this treatment program has progressively risen from 5%, when this cohort was established, to about 13% currently. Finally, this cohort did not have children with perinatal antiretroviral exposure, and hence the findings may be less relevant to children with prior PMTCT exposure. Strengths of the study include the long follow-up with serially detailed viral and resistance data. In summary, approximately one-third of long-term cART-treated children experienced virologic failure during ;4-year follow-up, the majority of whom had antiretroviral drug resistance. Viral load assays may decrease the lag to treatment switch and thus lessen the accumulation of additional mutations. However, without resistance assays, it is not possible to distinguish failure due to nonadherence from viral rebound due to resistance. Children had excellent suppression on second-line therapy despite the lag in detection of viral failure.

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FIGURES

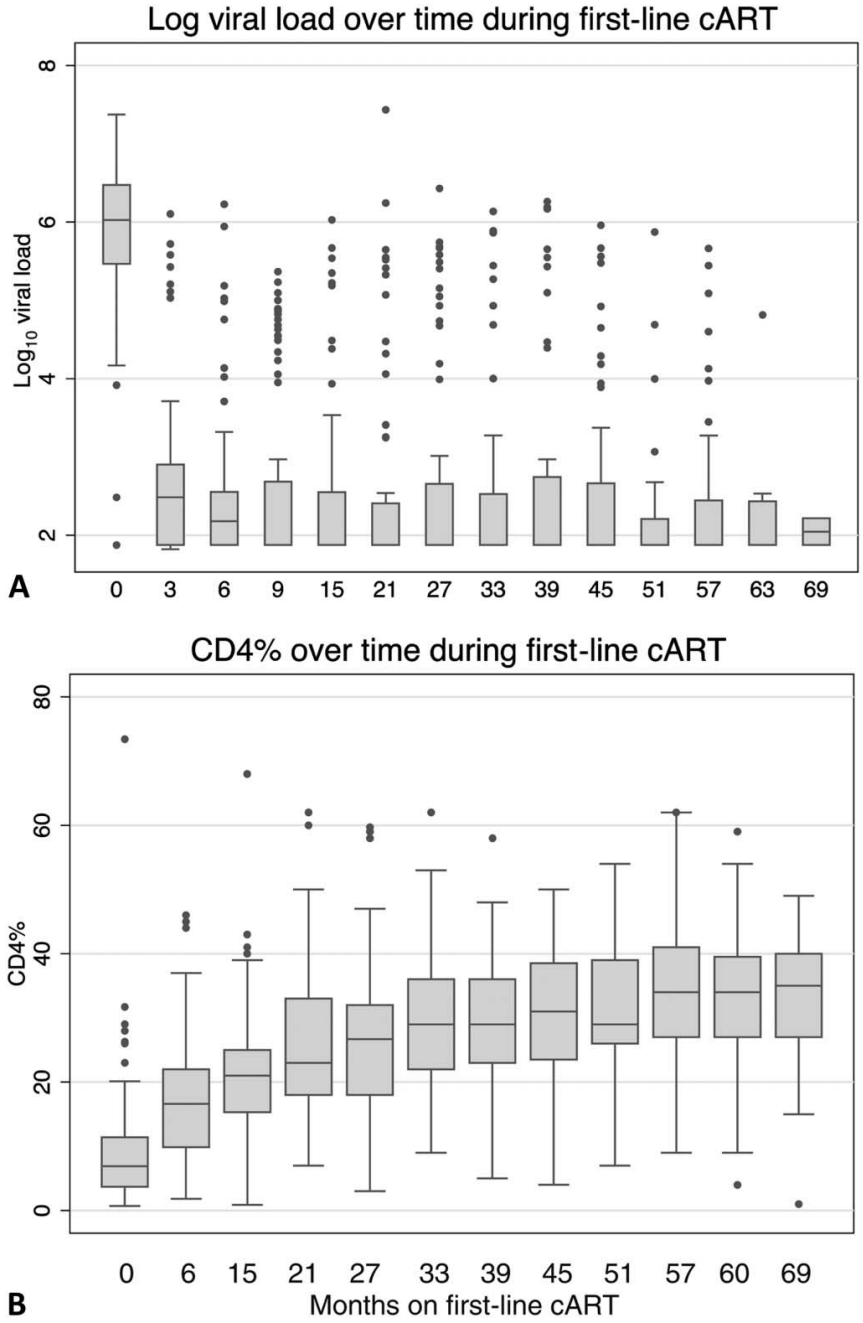

FIGURE 1. Viral load and CD4 kinetics during first-line cART. Changes in (A) log10 viral load; and (B) CD4 percent during first-line cART in the 100 children included in the study. The horizontal bar at the center of each box plot represents the median value, the top and bottom of each box are the 75th and 25th percentiles, respectively. The upper bound and lower bounds of the whiskers are the largest data point ≤ 75th percentile + 1.5 x IQR and the smallest data point ≥ 25th percentile - 1.5 x IQR, respectively. Observed data points beyond these bounds are plotted as filled circles.

TABLES

Table 1 Baseline Characteristics Comparing Children With Virologic Success Versus Virologic Failure

Characteristics	No. Children*	Overall Cohort, n = 100, Median (IQR) or n (%)	Virologic Success, n = 66, Median (IQR) or n (%)	Virologic Failure, n = 34, Median (IQR) or n (%)	P†
Age (yrs)	100	4.5 (2.6–6.2)	4.7 (2.9–6.7)	3.3 (2.2–5.7)	0.07
Female	100	53 (53%)	36 (55%)	17 (50%)	0.67
Lost one or both parents	100	28 (28%)	17 (26%)	11 (32%)	0.49
Hospitalized pre-ART	99	69 (69%)	46 (70%)	23 (68%)	1.00
WHO clinical stage 3–4	97	86 (89%)	59 (89%)	27 (79%)	0.35
Weight-for-age Z score	98	−2.3 (−3.0 to −1.4)	−2.3 (−3.2 to −1.4)	−2.3 (−2.8 to −1.7)	0.75
Weight-for-height Z score	92	−1.1 (−1.9 to −0.2)	−1.2 (−1.9 to −0.2)	−1.0 (−1.9 to −0.3)	0.98
Height-for-age Z score	97	−2.2 (−3.1 to −1.2)	−2.1 (−3.5 to −1.2)	−2.4 (−3.0 to −1.0)	0.86
CD4 count, cells/μL	99	344 (102–666)	300 (82–650)	387 (156–666)	0.27
CD4 cell percent	97	6.8 (3.7–11.4)	6.6 (3.3–10.9)	7.0 (3.8–13.0)	0.53
HIV-1 RNA, $\log_{10}$ copies/mL	100	6.0 (5.5–6.4)	5.9 (5.3–6.5)	6.1 (5.7–6.4)	0.10
Haemoglobin, g/dL	99	10.3 (9.2–11.7)	10.2 (9.0–11.8)	10.4 (9.7–11)	0.58
Started on EFV	100	34 (34%)	26 (39%)	8 (24%)	0.11
Started on ZDV	100	75 (75%)	51 (77%)	24 (71%)	0.47
Adherence <100%	100	60 (60%)	42 (64%)	18 (53%)	0.30

Table 2 Predictors of Virologic Failure Using Univariate Cox Hazard Models*

Baseline Characteristic	n†	HR	(95% CI)	P
Age <3 yrs	100	2.25	1.14 to 4.42	0.02
Sex (female)	100	0.84	0.43 to 1.64	0.60
WHO clinical stage 3–4	97	0.76	0.29 to 1.97	0.57
Weight-for-height Z score <−2	92	0.97	0.40 to 2.38	0.95
Log10 HIV-1 RNA >6	100	1.76	0.88 to 3.51	0.11
CD4 percent <15%	97	1.01	0.44 to 2.33	0.98
Adherence by caregiver report <100%	100	0.64	0.33 to 1.26	0.19

Table 3 Resistance Mutations in Children With Virologic Failure

ID	Subtype	First-Line Regimen	log VL at Failure	Months Post cART Initiation	NRTI Resistance Mutations	NNRTI Resistance Mutations	Multiclass Resistance
PADX1*	A2/D	D4T + 3TC + NVP	4.14	7	K65R	Y181C	+
PADX2*	A	D4T + 3TC + NVP	6.14	33	M184MV	K103N	+
PADX8*	D	D4T + 3TC + NVP	4.90	9	None	K103N	–
PADX9*	A	D4T + 3TC + NVP	4.76	7	M184V	K101E, G190A	+
PADC1*	A	AZT + 3TC + EFV	4.47	20	D67G, M184V	K101H, G190S	+
PADD9*	D	AZT + 3TC + NVP	4.02	6	M184V	Y181C	+
PADF2*	A	AZT + 3TC + NVP	5.07	20	D67N, M184V	K103N	+
PADG5*	A	AZT + 3TC + NVP	5.41	9	D67N, K70R, M184V, K219EQ	K103S	+
PADH3*	A	AZT + 3TC + NVP	5.19	15	M184V	Y181C, G190A	+
PADK4*	A	D4T + 3TC + NVP	5.54	9	L74V, Y115F, M184V	Y181C, H221Y	+
PADM8*	A	AZT + 3TC + NVP	5.00	9	M184IV	K103N	+
PADA1†	A	D4T + 3TC + NVP	4.86	8	None	K103N	–
PADA5†	A	AZT + 3TC + NVP	5.10	11	M184V	K103KN	+
PADB1†	C	AZT + 3TC + NVP	4.82	9	M184V	Y181C, G190AG	+
PADI3†	A	AZT + 3TC + EFV	4.60	55	M184V	K103N	+
PADJ6†	A	AZT + 3TC + EFV	5.33	21	None	G190S	–
PADO0†	C	D4T + 3TC + NVP	3.71	6	None	K103KN	–
PADP6†	A	AZT + 3TC + ABC	4.55	11	M184IMV	None	–
PADB2†‡	A	AZT + 3TC + EFV	5.19	6	None	K103N	–
PADC9†‡	D	AZT + 3TC + NVP	5.58	3	None	K103KN	–
PADG6†‡	A2/D	D4T + 3TC + ABC	5.03	3	M184V	None	–
PADH8†‡	A2/D	AZT + 3TC + NVP	6.03	14	M41L, M184V, T215Y	K101EK, V179E, G190A	+
PADL8†‡	A	AZT + 3TC + NVP	5.21	4	none	K103KN, G190AG	–

Table 4 Accumulation of Resistance Mutations During the Lag Between Viral and Immunologic Failure on First-Line cART

ID*	Months on First-Line After Viral Failure	Resistance After Extended First-Line Treatment During Viral Failure†	Accumulation of New Resistance Mutations
PADX1‡	9	**T69N,** Y181C, **M184V**	+§
PADX2‡	12	K103N, **V108IV,** M184V	+
PADX9‡	8	K101E, M184V, G190A	–
PADC1‡	13	**D67del, T69G, K70R,** K101H, M184V, G190S, **T215F, K219E**	+
PADD9‡	8	**M41L, D67N, K101EK,** Y181C, M184V, **T215F**	+
PADF2‡	6	D67N, **K70R**, K103N, M184V, **K219E**	+
PADG5‡	17	D67N, K70R, K103S, M184V, **T215F**, K219E	+
PADH3‡	40	**D67N, T69N, K70R, A98G**, M184V, G190A**, T215F, K219Q**	+§
PADK4‡	6	L74V, Y115F, Y181C, M184V	–§
PADM8‡	17	K103N, M184V, **T215TF**	+
PADA1¶	27	K103N, **M184V**	+
PADJ6¶	23	**K101EQ**, **M184V,** G190S	+
PADP6¶	27	**K103KN**	+§
PADB2¶	3	**K101EK,** K103N, **M184V**	+
PADC9¶	7	**K101E, M184V, G190A**	+§
PADG6¶	50	**L74V, Y115F,** M184V	+
PADH8¶	35	M41L, **D67N,** V179E, M184V, G190A, **L210W,** T215Y	+
PADL8¶	1	K103KN, G190AG	–
PADL4¶‖	23	**K103N, M184V**	+
PADP4¶‖	26	**A98AG, K101EQ, M184V, G190A**	+
PADA7¶‖	19	**K103N, V108VI, M184V**	+
PADC6¶‖	34	None	–
PADD1¶‖	3	None	–

Mutations in bold were not present at the initial point of viral failure.

Chapter 5:

Survival benefit of early infant antiretroviral therapy is compromised when diagnosis is delayed

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Title

Survival Benefit of Early Infant Antiretroviral Therapy is Compromised When Diagnosis is Delayed

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Abstract:

Late presentation is common among African HIV-1–infected infants. Incidence and correlates of mortality were examined in 99 infants with HIV-1 diagnosis by 5 months of age. Twelve-month survival was 66.8% (95% confidence interval: 55.9–75.6%). World Health Organization stage 3 or 4, underweight, wasting, microcephaly, low hemoglobin, pneumonia and gastroenteritis predicted mortality. Early HIV-1 diagnosis with antiretroviral therapy before symptomatic disease is critical for infant survival.

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Back ground

Many African HIV-1–infected children present late for HIV care, and often at hospitalization.[1–3]This late presentation is likely to undermine the benefits of early antiretroviral therapy (ART) to their survival. The Children with HIV Early Antiretroviral Therapy (CHER) study from South Africa demonstrated that empiric ART provided within the first 6–12 weeks of life resulted in 96% survival at 12 months.[4] However, children enrolled in CHER were asymptomatic with baseline CD4% ≥ 25%. Although encouraging, the inclusion criteria for CHER may limit generalizability to children who present later for medical care. Here, we determined incidence and correlates of mortality among HIV-1– infected infants diagnosed at up to 5 months of age and followed for at least 12 months after ART.

Methods

Study Population

Ethical approval was obtained from the University of Washington and the Kenyatta National Hospital-University of Nairobi Institutional Review Boards. From 2007 to 2009, HIV-1–infected infants were enrolled into a randomized clinical trial (Optimizing Pediatric HIV-1 Therapy 03 [OPH03]) with a 2-year pre-randomization phase (NCT00428116). In Nairobi, HIV-1–infected infants were identified after routine infant HIV-1 DNA polymerase chain reaction testing offered at prevention of mother to child transmission (PMTCT) clinics and hospital wards. Inclusion criteria were: 1) confirmed as HIV-1 DNA positive, 2) no previous ART (except for antiretroviral drugs used for PMTCT), 3) caregiver planned to reside in Nairobi for 3 years, and 4) age < 4.5 months. Before 2009, children with suspected active tuberculosis were excluded.

At enrollment, participants received a physical examination, caregivers provided demographic information and infants and biological mothers provided blood specimens. A follow-up visit was scheduled for ART initiation. ART was initiated generally for infants identified through hospital-based testing after stabilization and discharge, per standard protocol, although in some instances, ART was initiated for these infants while in hospital. ART regimens generally included zidovudine, lamivudine and either nevirapine or lopinavir-boosted ritonavir, for infants with prior nevirapine exposure. Ninety-nine percent of infants received trimethoprim-

sulfamethoxazole (cotrimoxazole) prophylaxis before or at enrollment. Participants attended monthly clinic visits following ART. Likely causes of death were abstracted from hospital medical records or ascertained by verbal autopsy. CD4% and counts were obtained using flow cytometry.

Statistical Analysis

Z-scores for growth parameters were calculated using the World Health Organization (WHO) child growth standards.[5] Underweight, stunting, wasting and microcephaly were defined as weight-for-age, height-for-age, weight-for-height and head circumference Z-score < −2. Anaemia was defined as haemoglobin < 8 g/dL.1 Probability of survival was estimated using Kaplan-Meier survival analysis, and univariate and multivariate Cox proportional hazards models were used to determine correlates of mortality. Survival was evaluated using time from enrollment for analyses including all infants and time from ART initiation for analyses restricted to infants initiating ART. Collinearity between cofactors was evaluated, and multivariate models were presented for non-collinear cofactors. Analyses were performed using Stata IC 10.1 (Stata Corp, College Station, TX).

Results

Cohort Characteristics

Ninety-nine HIV-1–infected infants were enrolled at ages 1–5 months (median, 3.7 months; Table 1). At baseline, most infants were underweight (57.6%), stunted (49.5%) or wasted (30.3%). Ten (12.8%) infants had not been breastfed. Slightly over half (54%) had received PMTCT. Infants were severely immunosuppressed; the median CD4% was 18%, 47 (49.0%) infants were diagnosed with WHO stage 3 or 4 disease and 59 (59.6%) had been hospitalized. None had clinical signs of pulmonary or extrapulmonary tuberculosis.

Infant Follow-up

Infants were followed for a median of 16.1 months (interquartile range: 1.2–27.6) pre-randomization, for a total of 123.1 person-years. Overall, there were 29 (29.3%) deaths, 10 (10.1%) losses to follow-up and 6 (6.1%) withdrawals, of which 12 deaths,

3 losses to follow-up and 4 withdrawals occurred pre-ART. Eighty (80.8%) infants were initiated on ART at a median of 14 days from enrollment (interquartile range: 7–21).

Infant Survival

Overall, the 12-month probability of survival was 66.8% (95% confidence interval [CI]: 55.9–75.6%). Of those who died, 12 (41.4%) had not yet been initiated on ART (median time to death, 11 days [interquartile range: 5–27). The overall rate of death was 23.6 (95% CI: 16.4–33.9) per 100 person-years with decreasing rates from 119.6 per 100 person-years (22 events during 18.4 person-years; 95% CI: 78.7–181.6) during months 0–3, 39.5 per 100 person-years (95% CI: 17.7–87.9) in months 4–6 and 3.2 per 100 person-years (95% CI: 0.46–23.0) during months 7–24. Among the 80 infants who were initiated on ART, the 12-month survival was 77.4% (95% CI: 66.2–85.3%), with an overall rate of death of 14.3 (95% CI: 8.9–23.0) per 100 person-years.

Correlates of Infant Mortality

Among all infants, significant baseline correlates of mortality included younger age (hazard ratio [HR] = 0.66), baseline CD4% < 10% (HR = 5.94), baseline WHO stage 3 or 4 (HR = 4.05), being underweight (HR = 3.28), wasting (HR = 5.60), microcephaly (HR = 2.71), haemoglobin < 8 g/dL (HR = 3.44), pneumonia (HR = 2.31) and gastroenteritis (HR = 4.73; Table 1). In a multivariate model, baseline WHO stage 3 or 4 (adjusted HR = 4.07) and haemoglobin < 8 g/dL (adjusted HR = 4.63) remained significant cofactors of mortality.

In analyses restricted to infants who were initiated on ART, significant correlates of mortality were generally similar to those for the overall cohort, although microcephaly and gastroenteritis were no longer associated with mortality in this subset. Plasma HIV-1 RNA level (HR = 2.52) was associated with mortality for infants initiating on ART but not the overall cohort.

Discussion

This study demonstrated substantial mortality in infants with acute HIV-1 infection who received HIV care by 5 months of age. The 12-month survival among the cohort was 66.8%. Younger age, WHO stage 3 or 4, being underweight, wasting, microcephaly,

low haemoglobin, presence of pneumonia and presence of gastroenteritis predicted mortality.

Among infants who did not start ART and died, median time to death was 11 days, suggesting a very short window of opportunity to provide ART in HIV-1–infected infants. The median time from enrollment to ART was 14 days. Standard adherence counseling sessions took 1–2 weeks, and hospitalized infants were stabilized and discharged before ART, suggesting that streamlined adherence counseling and emergent ART for hospitalized infants warrant consideration.

Consistent with previous studies of older children with chronic HIV-1,[1,3,6–9] HIV-1 disease severity, poor growth, low hemoglobin, pneumonia and gastroenteritis were all associated with mortality, and may reflect the contribution of these factors toward delay of ART or may reflect irreversible HIV-1 disease. Gastroenteritis and microcephaly correlated with mortality for the overall cohort, but not for infants who were initiated on ART, implying that these cofactors are linked to particularly rapid disease course.

In combination, pneumonia and gastroenteritis contributed to approximately two-thirds of mortalities (see Table, Supplemental Digital Content 1, http://links.lww.com/INF/B187, listing causes of death). In addition, there was overlap between these diagnoses at enrollment and at death: 2 of 8 (25%) subjects who died of gastroenteritis had gastroenteritis at enrollment, and 5 of 9 (54%) subjects who died of pneumonia had pneumonia at enrollment. Our results highlight a need for rapid management strategies for pneumonia and gastroenteritis in HIV-1–infected children.

Slightly over half (54%) of OPH03 infants had access to PMTCT, but in general, still lacked early infant diagnosis (by 6 weeks of age). We found substantially higher mortality than did the South African CHER trial (10 deaths per 100 person-years), which enrolled infants with HIV-1 diagnosis at age < 3 months (median: 1.7 months) and randomized to immediate versus deferred ART.[4] The 12-month survival was significantly higher in CHER than in our study (92% versus 71%; $p < 0.0001$). CHER infants had higher baseline CD4% (35% versus 18%) and weight-for-age (−0.7 versus −2.6). These differences are likely due to rapid clinical course in HIV-1–infected

infants[10] and further reflect the survival benefit of early infant HIV-1 diagnosis and treatment.

For context, we compared overall survival in OPH03 versus HIV-1–infected untreated infants born between 1999 and 2002 (participants in a perinatal cohort study)[10] and surviving to age 4 months. Mortality was lower overall in OPH03 than in untreated infants (24 versus 48 per 100 person-years; p = 0.07; see Fig., Supplemental Digital Content 2, http://links.lww.com/INF/B188, depicting survival by cohort). Still, improved survival in OPH03 infants versus historical untreated infants was not apparent until more than 6 months after HIV-1 diagnosis. This lag suggests that infants with symptomatic HIV-1-disease may not be salvageable and/or the full impact of ART in symptomatic infants may not be immediate.

The main strength of this study is inclusion of infants with advanced HIV-1 disease and late HIV-1 diagnosis, both typical in Africa.[1–3] Our comparisons between cohorts are limited by demographic and regional differences. Differences in PMTCT coverage (higher for both CHER and CTL than for OPH03), and type (majority with single-dose nevirapine alone for CHER and OPH03 and majority with zidovudine alone for CTL10) may have influenced subsequent disease progression. Many OPH03 infants were identified at hospital, limiting comparability with CTL and CHER, which were identified through PMTCT. Although retention was similar in CTL (89%) and in OPH03 (90%), retention was higher in CHER (96%), and it is possible that the mortality difference between CHER and OPH03 was underestimated if infants who were lost to follow-up actually died.

The analysis in this paper entirely utilized the 24-month pre-randomization phase of the OPH study. We did not utilize the post-randomization phase in this thesis. The reported loss to follow-up of 10% that was experienced is substantial and may impact findings. Selection bias is possible because children lost to follow-up may have an over-representation of more severe forms of pathology and/or poorer medication adherence hence result in an under-estimation of the mortality. Differential loss of follow-however up does not apply at this stage because loss happened in the pre-randomization phase. This explanation is added to the discussion section on page

Our study underscores the importance of early identification and prompt treatment of HIV-1–infected infants by 6 weeks of age. For infants diagnosed later, it will remain important to discern whether accelerated ART improves survival. Early access to HIV-1 diagnosis and treatment, before onset of symptoms, will remain the key intervention for survival among HIV-1–infected infants.

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Tables

Table 1: Summary of Baseline Characteristics and Univariate Analyses of Correlates of Mortality

Correlate	n (%) or Median (IQR)	HR (95% CI)‡	*p*	n (%) or Median (IQR)	HR (95% CI)‡	*p*
Age (months)	3.7 (2.9–4.0)	0.66 (0.44–0.99)	0.043	3.7 (2.9–4.0)	0.59 (0.35–1.0)	0.048
Male	50 (50.5%)	1.62 (0.77–3.40)	0.2	37 (46.3%)	1.05 (0.41–2.73)	0.9
Weight at birth	3.0 (2.7–3.4)	1.10 (0.59–2.05)	0.8	3.0 (2.7–3.4)	1.15 (0.51–2.60)	0.7
Received to PMTCT	50 (53.8%)	0.96 (0.46–2.03)	0.9	39 (52.0%)	0.65 (0.24–1.75)	0.4
HIV-1 disease status						
Plasma HIV-1 RNA $\log_{10}$ copies/mL	6.55 (5.99–7.08)	1.24 (0.75–2.06)	0.4	6.59 (5.98–7.21)	2.52 (1.06–5.98)	0.037
WHO stage 3 or 4	47 (49.0%)	4.05 (1.73–9.49)§	0.001	34 (43.6%)	3.39 (1.19–9.62)	0.022
CD4%	18 (14–24)	0.95 (0.90–1.00)	0.063	18 (14–24)	0.96 (0.90–1.03)	0.2
CD4% < 10	10 (10.4%)	4.94 (2.07–11.78)	<0.001	7 (8.8%)	4.92 (1.60–15.17)	0.006
CD4 count (cells/μL)	1302 (755–1953)	0.96 (0.91–1.02)	0.2	1302 (766–1908)	0.94 (0.87–1.01)	0.095
Growth		3.28 (1.33–			2.25 (0.79–	
Underweight (WAZ < −2)	57 (57.6%)	8.07)	0.01	44 (55.0%)	6.38)	0.13
Stunted (HAZ < −2)	49 (49.5%)	1.73 (0.82–3.67)	0.15	40 (50.0%)	1.58 (0.60–4.14)	0.4
Wasted (WHZ < −2)	30 (30.3%)	5.60 (2.63–11.91)	<0.001	19 (23.8%)	4.59 (1.76–11.91)	0.002
Microcephalic (HCZ < −2)	18 (18.2%)	2.71 (1.23–5.98)	0.013	11 (13.8%)	1.40 (0.40–4.89)	0.6
Morbidity		3.44 (1.45–			3.36 (1.09–	
Hemoglobin < 8 g/dL	13 (13.5%)	8.19)	0.005	9 (11.3%)	10.34)	0.035
Hospitalized since birth	59 (59.6%)	2.22 (0.98–5.01)	0.055	44 (55.0%)	1.61 (0.60–4.36)	0.3

Fever	8 (8.4%)	1.14 (0.34–3.77)	0.8	6 (7.8%)	0.63 (0.08–4.78)	0.7
Upper respiratory tract infection	9 (9.1%)	0.60 (0.14–2.52)	0.5	8 (10.0%)	0.52 (0.07–3.90)	0.5
Pneumonia	31 (31.3%)	2.31 (1.11–4.78)	0.025	22 (27.5%)	2.40 (0.93–6.23)	0.07
Gastroenteritis	8 (8.2%)	4.73 (1.91–11.74)	0.001	3 (3.8%)	1.33 (0.17–10.04)	0.8

Results were similar in a multivariate model including WHO Stage 3 or 4 (HR: 4.07, 95% CI: 1.71–9.71; *P* = 0.002) and hemoglobin < 8g/dL (HR: 4.63, 95% CI: 1.45–8.19; *p* = 0.001).
WAZ indicates weight-for-age; HAZ, height-for-age; WHZ, weight-for-height; HCZ, head circumference; IQR, interquartile range; HR, hazard ratio.

Chapter 6:

Cytomegalovirus viraemia and clinical outcomes in Kenyan children diagnosed with human immunodeficiency virus in hospital

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Title

Cytomegalovirus Viraemia and Clinical Outcomes in Kenyan Children Diagnosed With Human Immunodeficiency Virus (HIV) in Hospital.

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Abstract

Background: Cytomegalovirus (CMV) viraemia is common in human immunodeficiency virus (HIV) infection and is associated with worse long-term outcomes. To date, no studies have assessed CMV viraemia in children diagnosed with HIV in hospital.

Methods: We studied CMV viraemia and clinical outcomes in 163 Kenyan children aged 2 months to 12 years, diagnosed with HIV in hospital. CMV DNA levels in plasma were measured using quantitative polymerase chain reaction (PCR). Regression models were used to assess associations between CMV viraemia ≥1000 IU/mL and the risk of continued hospitalization or death at 15 days, duration of hospitalization, and 6-month mortality.

Results: At enrollment, 62/114 (54%) children had CMV viraemia, and 20 (32%) were ≥1000 IU/mL. Eleven CMV reactivations were observed after admission. The prevalence and level of CMV viraemia were highest in children −2 ($p = 0.02$). Adjusting for age and $\log_{10}$ HIV RNA, the relative risk of death or continued hospitalization at 15 days was 1.74 (95% confidence interval [CI] = 1.04, 2.90), and the hazard ratio of 6-month mortality was 1.97 (95% CI = .57, 5.07) for children with CMV DNA ≥1000 IU/mL compared to lower-level or undetectable CMV DNA. Children with CMV DNA ≥1000 IU/mL were hospitalized a median ~5 days longer than children with lower-level or undetectable CMV DNA ($p = .002$).

Conclusion: In this nested observational study, CMV viraemia was common in hospitalized children with HIV, and levels ≥1000 IU/mL were associated with increased risk of mortality and longer hospitalization.

Background

The association between cytomegalovirus (CMV) coinfection and poor human immunodeficiency virus (HIV) outcomes is well established in both children and adults [1, 2]. In addition to being an opportunistic infection, CMV likely contributes to non-AIDS morbidity and mortality risk even during effective HIV suppression on antiretroviral therapy (ART) [3]. CMV infection is associated with greater expansion of activated and exhausted T cells, and augmented host inflammation and microbial translocation [4–7], which are hallmarks of HIV pathogenesis. Clinical trials of CMV suppression in the pre-ART era were designed to use ganciclovir, high-dose acyclovir, or valacyclovir over many months [8–10]; but because many of these were terminated early due to adverse events, there are limited data on the benefit of CMV suppression. Short-course oral CMV antivirals were found to reduce frequencies of activated CD8 T cells in blood of ART-treated adults [11] and in the semen of ART-naive men [12], suggesting that CMV therapy even in the absence of apparent disease could reduce host immune activation. Despite a wealth of research focused on CMV and long-term HIV outcomes, there are virtually no data on the impact of CMV viraemia in children who are diagnosed with HIV while hospitalized. This question is highly relevant as the yield of HIV testing in African hospitals is now highest compared to other testing venues [13]. In sub-Saharan Africa, hospitalized children with HIV have extremely high mortality (16–39% [14– 16]), despite rapid initiation of ART [14, 15, 17]. In the recent Pediatric Urgent Start of Highly Active Antiretroviral Therapy (HAART) (PUSH) Study, starting ART within 48 hours of hospital HIV diagnosis in hospitalized children was found to be safe but similar to post-stabilization ART initiation for survival [14]. We used archived specimens and data from PUSH to evaluate the association between CMV viraemia and outcomes, to determine whether CMV suppression during ART initiation should be studied as a novel interventional approach to improve outcomes in this population.

Methods

Recruitment and Follow-up

This study was nested in the PUSH randomized clinical trial (RCT) of urgent versus post-stabilization ART initiation among children diagnosed with HIV infection during hospitalization (NCT02063880). Human subjects' approvals were obtained from

University of Nairobi/ Kenyatta National Hospital (UoN/ KNH) Ethics Research Committee (ERC) and the University of Washington Institutional Review Board (IRB). The study was conducted in 2 hospitals in Nairobi (KNH and Mbagathi County Hospital) and 2 in western Kenya (Jaramogi Oginga Odinga Teaching and Referral Hospital and Kisumu County Hospital). Details on recruitment, interventions, and main study outcomes have been reported previously [14]. Eligibility criteria for children enrolled in the PUSH trial included: hospitalized, age 0–12 years, HIV-positive, antiretroviral therapy (ART) naive, and no clinical evidence of central nervous system infection. Hospital staff tested children older than 18 months for HIV using 2 different rapid tests in compliance with Kenya National Guidelines; those with confirmed HIV infection were referred to the study team for screening. Children younger than 18 months who met Kenya National Guidelines as “HIV-exposed” received an HIV polymerase chain reaction (PCR) test; PCR result turnaround time was 48 hours after sample collection. Enrolled children were randomized to receive either ART within 48 hours (urgent) or 7–14 days (post-stabilization), with regimens selected according to contemporaneous Kenya and World Health Organization (WHO) guidelines.

Children were followed for 6 months after ART initiation. At 8 scheduled visits (enrollment, week 1, 2, and monthly from 1 to –6 months post-ART), detailed sociodemographic and clinical data were collected. Laboratory samples were collected at 5 time-points (enrollment, week 2, month 1, month 3, month 6). Plasma samples were stored at all sampling visits when possible.

Clinical Data

Reason for hospital admission was recorded at enrollment, with each case diagnosed by standard hospital protocols. At enrollment and each follow-up visit, growth indicators (weight, height, mid-upper arm circumference [MUAC]) were collected and used to calculate Z-scores using the WHO reference population. Severe acute malnutrition (SAM) was defined as WHZ <−3 and visible wasting or oedema. Baseline laboratory values included neutrophil count, CD4, and $\log_{10}$ HIV viral load. Clinical stage and severe immunosuppression were defined using CD4% thresholds by age (<12 months <30%, 12–35 months <25%, 36–59 months <20%, over 5 years <350 cells/uL) according to WHO criteria [18, 19].

CMV DNA Measurements and Definitions

CMV DNA levels were measured from stored plasma specimens using real-time quantitative PCR as previously described [26]. Briefly, viral nucleic acids were extracted from 200 uL of thawed plasma using a QIAamp Ultrasens Virus kit (Qiagen, Inc., Valencia, California, USA) according to manufacturer's instructions. TaqMan PCR was performed targeting the UL55/ UL123-exon 4 regions of CMV, and cycle thresholds compared to a standard curve to determine viral copies/mL; primers and probes are provided in reference [26]. Values were transformed to express measurements in international units (IU)/mL by dividing by 1.4. The PCR limit of detection was 1 copy per reaction (35.7 IU/mL). CMV reactivation was defined as CMV DNA detection > 1 copy/rection following one or more initially negative CMV DNA tests.

Statistical Analysis

Stata v.14 (StataCorp, College Station, Texas, USA) was used for all analyses, and all comparisons were 2 sided with alpha = 0.05. The Wilcoxon rank sum test was used to compare the distribution of CMV levels between groups. Potential correlates of CMV viraemia were selected from previous literature in adult critical care settings (corticosteroids, clinical acuity, clinical laboratory values) [20] and expected confounders in HIV-infected children (HIV RNA, CD4 percent, age, malnutrition) as well as site of recruitment (Nairobi/Kisumu). Correlates of CMV viraemia were identified using Poisson regression to estimate prevalence ratios and 95% confidence intervals (CI), adjusting for baseline $\log_{10}$ HIV RNA level and age.

Primary outcomes defined a priori included death within 6 months, the combined outcome of death or continued hospitalization on day 15, and duration of hospitalization among survivors. Fifteen days were chosen for assessment of the combined endpoint because this is the median duration of hospitalization in the high-dependency acute wards and intensive care unit at Kenyatta National Hospital (Saini et al, manuscript in preparation). The primary exposures investigated were CMV viraemia $\geq$1000 IU/mL and baseline CMV viral load (log IU/mL). There is no universally agreed-upon plasma CMV level of clinical significance, however levels $\geq$1000 IU/mL have higher statistical reliability than lower levels [21]. This level was predictive of mortality in immunocompetent adults admitted to intensive care in the United States

[22] and has been associated with decreased lung function and stunting in Zimbabwean youth with HIV infection [23].

Generalized linear models were used to compare the relative risk of the combined endpoint, and Kaplan-Meier survival analysis was used to compare 6-month mortality risk between groups. Variables that were independently associated with both CMV viraemia at enrollment and mortality in our earlier report [24] at $p < .05$ (child age and baseline $\log_{10}$ HIV RNA level) were assessed as confounders between CMV viraemia and survival outcomes. We assessed potential effect modification by age on 6-month survival, stratifying by $\geq$2 years and < 2 years and present these analyses as Supplementary data. We used the Benjamini-Hochberg method at a false discovery rate of 0.05 to control for multiple testing for the correlates analysis.

Among children surviving to 6 months, the Wilcoxon rank sum test was used to compare duration of hospitalization between children who were CMV viraemic and aviraemic at enrollment.

Because this study was nested into an RCT, we additionally assessed potential effect modification by randomization arm (urgent vs post-stabilization initiation of ART) for each analysis but found no evidence that results differed by randomization arm (data not shown).

Results

Population Characteristics

A total of 163 (88%) of 181 children enrolled in PUSH had plasma available for CMV PCR. Among these, median age was 2 years (interquartile range [IQR] = 1.0, 5.4), 47% were female, and 93% were under the care of their biological mother (Table 1). Most (78%) were severely immunosuppressed at admission with very high HIV RNA levels. Many children were classified as stunted (59%) or wasted (45%), and a quarter had severe acute malnutrition. The most common admission diagnosis was pneumonia (65%). Median time between admission and enrollment was 4 days (IQR = 2, 6).

Twenty-five (15%) of 163 children included in this analysis died, and 61 (37%) died or were still hospitalized on day 15 since admission. Among 138 survivors at 6 months, 36 (26%) were still hospitalized 15 days after admission

Prevalence of CMV Viraemia

Of the possible 5 sample collection time points, the majority of children had between 3 and 5 CMV DNA measurements (data not shown). Overall, 72% (118/163) of children had CMV DNA detected at least once during follow-up. Sixty-two (54%) of 114 children were CMV viraemic pre-ART at enrollment, and of these, 32% (20/62) had CMV levels ≥1000 IU/mL.

Figure 1A shows the prevalence of CMV detection in children grouped by age; the prevalence of viraemia was highest for children <1 year old (88%) and lowest in the children aged 5–12 years (21%). Of 72 children with CMV viraemia at any time during the study, 11 (9%) were CMV reactivations, and these children were mostly older (7 of these children were aged 2–5 or 5–12 years, Figure 1B).

CMV viral load trajectories from admission to 6 months

The overall median CMV level of children who were viraemic at baseline was 2.41 $\log_{10}$ IU/mL (IQR = 2.06, 3.21), and their peak was 2.82 $\log_{10}$ IU/mL (IQR = 2.18, 3.41); CMV viral load trajectories generally decreased following ART initiation but remained detectable in 21/75 (28%) at 6 months (Figure 2A). Follow-up time was much shorter in children who died, and there was high variability in their CMV viral load trends over time (Figure 2B). CMV levels followed similar trajectories for younger (<2 years) and older (≥2 years), with both groups declining over time (Figure 2C). Children aged <2 years old had higher median CMV levels compared to children ≥2 years old at enrollment (median 3.0 vs 2.2, p = 0.0001).

The median first CMV level measurement of children reactivating CMV was 1.71 log10 IU/mL (IQR = 1.34, 2.07), and their peak was 1.84 (IQR = 1.71, 2.28); these were both significantly lower than the baseline and peak levels of children who were viraemic at admission ($p < 0.001$ for each comparison). There was limited follow-up time post-reactivation to observe longitudinal changes, but generally their CMV levels remained low through follow-up (<1000 IU/mL, Figure 2D).

Correlates of CMV Viraemia at Enrollment

Clinical and laboratory characteristics were compared between children with CMV viraemia ≥1000 IU/mL at enrollment (hospital admission) versus children who were CMV undetectable or had CMV DNA levels <1000 IU/mL (Table 2). Each variable was assessed individually and then adjusted for age and/or HIV RNA level. Age <2 years (adjusted prevalence ratio [aPR] = 3.71 [95% CI = 1.18, 11.8], p = .026), HAZ < -2 (aPR = 4.74 [95% CI = 1.23, 18.3], p = .024) and HIV RNA level (aPR = 1.91 [95% CI = 1.16, 3.16], p = .012) were independently associated with CMV viraemia ≥1000 IU/mL. Covariates that were significantly different in univariate analysis but did not retain significance after adjustment included receipt of steroids, WHO clinical stage III and C-reactive protein level. After adjustment for multiple testing, none of the correlates remained significantly associated with CMV viraemia ≥1000.

Correlates of any CMV DNA viraemia above versus below the limit of detection (35.7 IU/mL) are provided in Supplementary Table 1. Variables independently associated with CMV detection included younger age (aPR = 0.85 [95% CI = .77, .93], p = 0.001) and higher baseline HIV viral load (aPR = 1.25 [95% CI = 1.03, 1.53], p = 0.026). Covariates that were significantly different in univariate analysis but did not retain significance after adjustment included clinical diagnosis of malaria and total neutrophil count. After adjustment for multiple testing, none of the correlates remained significantly associated with CMV DNA viraemia.

Admission Diagnosis and CMV Viraemia

The prevalence of CMV viraemia and CMV levels are shown for children by admission diagnosis in Table 3, ranked by median CMV level at enrollment. No children were diagnosed with active CMV disease during the study period and no children with treated with anti-CMV medications. CMV was most prevalent (77%) and detected at the highest level in children with diarrhoea or gastroenteritis (median $\log_{10}$ 2.6 IU/mL). CMV viraemia was detected in a fifth of children with pneumonia, at a median of 2.4 $\log_{10}$ IU/mL. Median oxygen saturation levels (SpO_2) among children admitted for pneumonia with hypoxia were 86% (IQR = 80, 88) in CMV aviraemic children, 82% (IQR = 75, 86) in children with CMV viraemia, and 78% (IQR = 72, 84) in children with

CMV ≥ 1000IU/mL (comparing SpO_2 CMV ≥ 1000 IU/mL vs CMV < 1000 mL or aviraemic p = 0.17).

CMV levels were higher with more advanced WHO clinical stage at enrollment (median 1.9 log10 IU/mL [IQR 1.6, 2.5] for those in stage I/II, 2.1 [IQR 1.5, 2.9] for stage III, and 2.9 [IQR 1.5, 3.9] for stage IV). Clinical characteristics of the 11 children who reactivated CMV during the study are described in detail in Supplementary Table 2.

CMV Viraemia and Outcomes Over 6 Months Follow-up

Figure 3A shows Kaplan-Meier survival curves over the 6 months of follow-up by CMV level in the 114 children with enrollment CMV testing. Mortality rates in CMV aviraemic children and children with low level CMV viraemia (< 1000 IU/mL) had similar time to death (p = 0.5). Children with CMV viraemia ≥1000 IU/mL had a significantly shorter time to death compared to children with lower CMV levels (p = 0.02) and aviraemic children (p = 0.002). Children who were aviraemic or had low-level viraemia (<1000 IU/mL) were grouped together for further analyses (Figure 3B). Kaplan-Meier survival curves for children stratified by age <2 years and ≥2 years are shown in Supplementary Figure 1.

Crude and adjusted point estimates for CMV viraemia and clinical outcomes are shown in Table 4. CMV levels ≥1000 IU/mL were associated with a 74% increased risk of attaining the combined endpoint of death or continued hospitalization at 15 days, independent of log10 HIV RNA level and age (aRR = 1.74 [95% CI = 1.04, 2.90]). Enrollment CMV DNA level was associated with increased risk of the combined endpoint but did not retain significance after adjusting for age and baseline log10 HIV viral load (aRR = 1.17 [95% CI = 0.87, 1.58]).

Among children surviving to 6 months post-admission, the median duration of hospitalization was approximately 5 days longer (14.5 days [IQR = 13, 16]) in the children with CMV level ≥1000 IU/mL compared to children who were aviraemic or had a CMV level <1000 IU/mL (9 days [IQR = 5, 12], p = 0.002). Enrollment CMV DNA level was not associated with longer duration of hospitalization (p = 0.3). We did not

adjust for age and HIV RNA as confounders in this analysis because they were not associated with duration of hospitalization.

CMV level ≥1000 IU/mL was associated with increased hazard of death over 6 months in crude analyses (hazard ratio [HR] = 3.78, [95% CI = 0.54, 9.25]) but did not retain significance when adjusting for HIV RNA level and age. Enrollment CMV DNA level was not significantly associated with higher 6-month mortality.

Discussion

In this study of Kenyan children diagnosed with HIV at hospital admission, we found a very high rate of CMV viraemia, which was inversely associated with child age. Most children were viraemic at admission, but a substantial number were also observed to reactivate CMV infection during study follow-up. We found that children with CMV DNA levels ≥1000 IU/mL in plasma had a higher risk of continued hospitalization or mortality at 15 days post-admission, and longer duration of hospitalization compared to children who were CMV aviraemic or had low-level CMV viraemia. These data suggest CMV levels above 1000 IU/mL identify children at high risk for poor outcomes while starting ART during hospitalization.

CMV viraemia was present in more than half of children at baseline and overall was detected in nearly three quarters of children during follow-up. To date, the only similar data to ours are from a study in Zambia where 55% of hospitalized children with HIV who were <2 years old were CMV viraemic at admission [25]. Consistent with our study, Tembo et al found that CMV viraemia was associated with being underweight and younger age; however, they did not report outcomes by CMV viraemia. Younger age and higher HIV RNA level were independently associated with an increased risk of having baseline CMV viraemia, but surprisingly CD4 percent and WHO clinical stage were not; we hypothesize that this is because host inflammation and global immune dysregulation also contribute to CMV viraemia. Given age-related changes in CD4 numbers over infancy, there may also be residual confounding by age. Children under 2 years old comprised the majority of mortalities in the PUSH Cohort, suggesting

that quantitative CMV PCR testing in this age group may be important for clinical prognosis and future evaluation of the role of anti-CMV therapeutics.

We previously reported detection of CMV viraemia by 6 months of age in ~80% of ART-naive Kenyan children with *in utero* or very early (<1 month) HIV acquisition, with many children remaining CMV viraemic for a year or more [26]. Because the PUSH study included infants and children across a wide age range, the viraemia we found is most likely a mix of primary CMV infections and reactivations. Children with CMV viraemia at admission had higher levels than those with CMV observed after enrollment, and none of the children with observed CMV reactivation died. Given our sampling intervals and the small number of reactivations observed, we cannot conclude whether reactivations occurred as a manifestation of treatments received in hospital. The observed reactivations occurred across diverse clinical diagnoses and did not appear to be related to transfusion or receipt of steroids. None of the children with CMV reactivation had evidence of immune reconstitution inflammatory syndrome (IRIS). Because of the small number and short follow-up time post-CMV detection, our study cannot determine the potential clinical relevance of these CMV reactivation events.

CMV level ≥1000 IU/mL was associated with an increased risk of continued hospitalization or death by 15 days, independent of $\log_{10}$ HIV viral load and age. In survivors, high-level CMV viraemia was associated with 5 days longer hospitalization. Although we cannot ascertain if CMV viraemia is causally related to these poor outcomes due to the observational nature of our study, our findings are consistent with previous literature demonstrating an association between CMV viraemia and poor long-term outcomes (growth, morbidity, mortality) in children and adults living with HIV [3, 27–29] and short-term hospitalization outcomes (including increased mortality, increased oxygen dependency, and longer hospital stay) in HIV-negative, immunocompetent adults [20, 30].

The most common hospital admission diagnoses in the PUSH trial were pneumonia or diarrhoea; these are also the 2 most frequent causes of hospitalization in Kenya and children from areas with high rates of malnutrition globally [31]. CMV viraemia ≥ 1000 IU/mL was especially common in children with diarrhoea and pneumonia and

could reflect the primary source of CMV viral replication in the lung or gastrointestinal tract. Our data suggest SpO_2 levels may be lower in the CMV viraemic children admitted for severe pneumonia and warrants further study. CMV has wide tropism and can cause pneumonia and colitis. Immune modulation/increased susceptibility to other infections, and CMV-induced aggravation of lung inflammation is hypothesized to precipitate clinical decline in critically ill immunocompetent adults [20], and a similar mechanism could be proposed in the gastrointestinal tract. The association we found between CMV viraemia and stunting, a marker of chronic malnutrition, also supports a potential role for CMV in the gastrointestinal tract of these children. Malnutrition contributes to immune dysregulation and gut dysbiosis, and both animal and human studies have found that CMV replication disrupts the gut microbiome [32, 33]. Systematic evaluation of CMV replication in fluids and tissues from the gastrointestinal tract and lung would be informative but present unique implementation challenges in children.

Our study has several strengths and limitations. Strengths include the large and unique hospital cohort, with systematic and detailed sampling and clinical data collection. Despite this, the children had diverse clinical experiences and investigations during hospitalizations that limit our ability to analyse the clinical course of children, and we do not have detailed data on cause of death. We were not able to perform histology on lung or gastrointestinal tract samples, which would be mechanistically informative, and we did not perform autopsies on children who died. We also had low statistical power for mortality. Because some specimens had been used for the parent study, we did not have complete sampling at baseline on all participants, further limiting statistical power. Finally, because our study was observational, we cannot conclude whether a causal relationship exists between CMV and the outcomes we studied.

In summary, CMV viraemia was found in the majority of ART-naive children diagnosed with HIV at hospital admission, and levels $\geq$1000 IU/mL were associated with worse clinical outcomes, including longer duration of hospitalization and a higher risk of continued hospitalization or death at 15 days. Together, these data support further

research into the relevance of CMV viraemia in this population and CMV suppression as a potential novel intervention.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclaimer

The publication's contents are solely the responsibility of the authors and do not represent the official views of the funders.

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Potential conflicts of interest.

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Figures

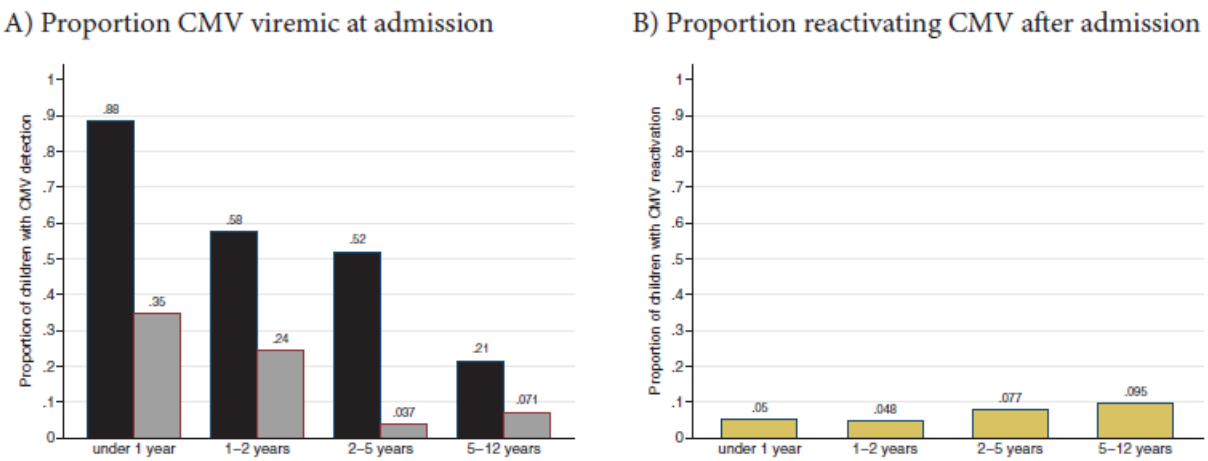

Figure 1. Proportion of children with CMV viraemia at hospital admission or reactivation during follow-up, by age at enrollment.

A, Proportion of children with CMV viraemia at enrollment. N = 114 children with admission samples screened in children aged ≤1 year (n = 26), 1–2 years (n = 33), 2–5 years (n = 27), and 5–12 years (n = 28). Black bars = CMV viraemia ≥35.7 IU/mL (above the limit of detection) detected at enrollment, gray bars = CMV viraemia ≥1000 IU/mL detected at enrollment.

B, Proportion of children with CMV reactivation. N = 118 children first screened at enrollment or their next visit; 11 were initially negative then experienced CMV viraemia at a later visit. Reactivations occurred in 2 children aged ≤1 year, 2 children aged 1–2 years, 3 children aged 2–5 years, and 4 children aged 5–12 years. *A*, Proportion CMV viraemic at admission.
B, Proportion reactivating CMV after admission. Abbreviation: CMV, cytomegalovirus.

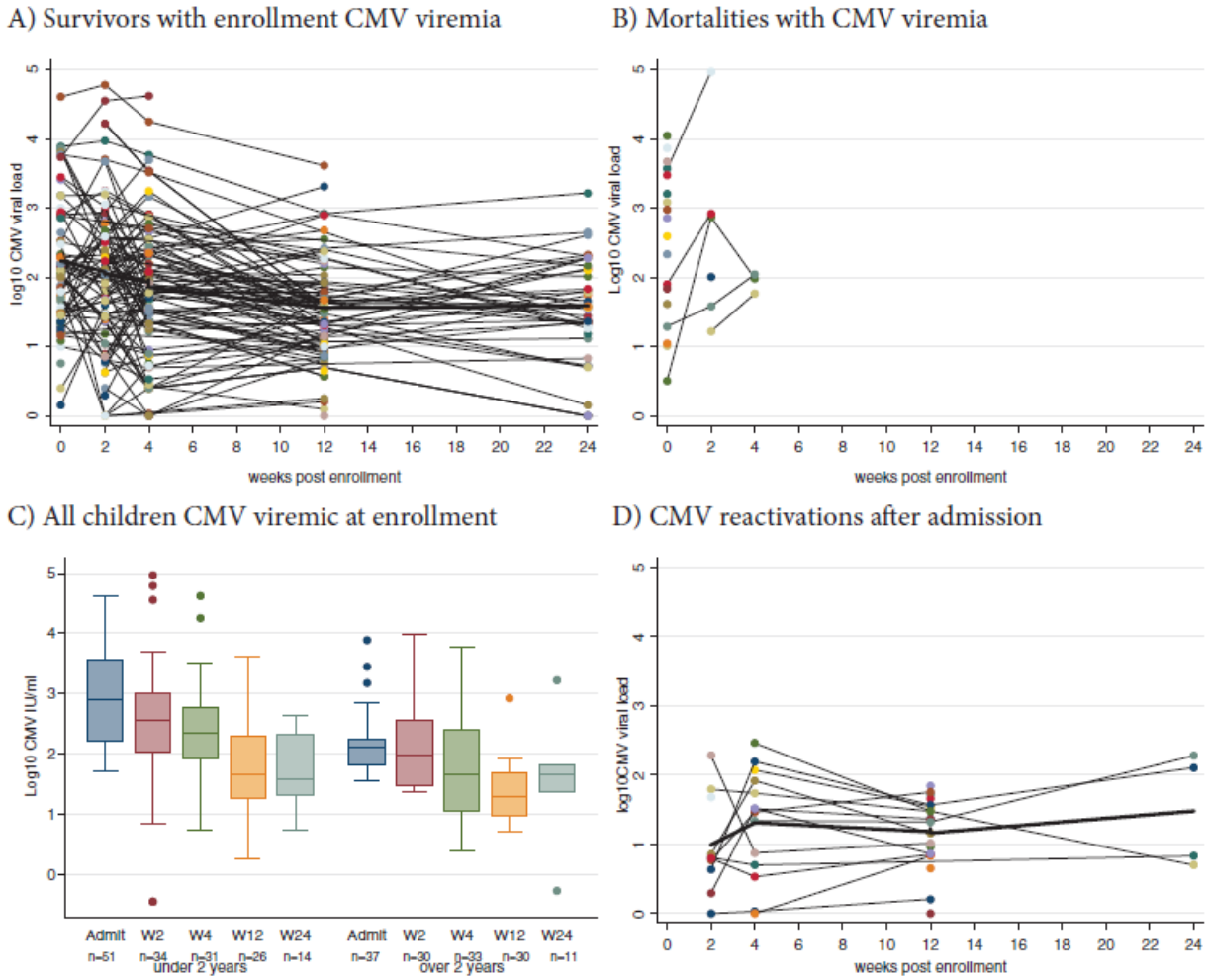

Figure 2. Longitudinal CMV levels in children with CMV viraemia at admission. Longitudinal trajectories of children who were CMV viraemic, who (*A*) survived or (*B*) died during follow-up. *C*, CMV IU/mL of plasma is shown longitudinally at each study timepoint (W = week) for children who had CMV viraemia at hospital admission (Admit), categorized by age under 2 years and 2 years and older. *D*, Longitudinal trajectories of 11 children who reactivated CMV while in hospital. *A*, Survivors with enrollment CMV viraemia. *B*, Mortalities with CMV viraemia. *C*, All children CMV viraemic at enrollment. *D*, CMV reactivations after admission. Abbreviation: CMV, cytomegalovirus.

Tables

Table 1. Baseline Characteristics of Kenyan Children Diagnosed With Human Immunodeficiency Virus (HIV) in Hospital Who Were Tested for Cytomegalovirus (CMV) DNA

	N	Median (IQR) / n (%)
Demographics		
Age	163	2.0 (1.0, 5.4)
Age < 1 years old	163	40 (25%)
Age ≥1to <2 years old	163	42 (26%)
Age ≥2 to <5 years	163	39 (24%)
Age ≥5 to <13 years	163	42 (26%)
Female	163	76 (47%)
Primary caregiver mother	163	152 (93%)
Enrolled at Kisumu site	163	90 (55%)
Growth		
WAZ < −2SD	158	98 (62%)
WHZ < −2SD	121	55 (45%)
HAZ < −2SD	159	94 (59%)
Severe acute malnutrition	119	31 (26%)
Enrollment diagnosis and disease stage		
Pneumonia	163	106 (65%)
Pneumonia with hypoxia	94	22 (23%)
Suspected pulmonary TB	161	26 (16%)
Malaria	162	36 (22%)
Gastroenteritis	163	29 (18%)
Persistent diarrhea	163	17 (10%)
Baseline WHO stage III/IV	162	109 (67%)
Laboratory and treatment		
Total neutrophil count	157	3.4 (2.3, 5.1)
CD4%	162	15 (9, 23)
CD4% ≥15%	162	83 (51%)
Severe immunosuppression (WHO age and CD4% criteria)	162	127 (78%)
Log_{10} HIV RNA copies/mL	154	5.7 (5.0, 6.3)
Lab confirmed TB	163	10 (6%)
Received steroids	155	8 (5.0%)
Key outcomes		
Died before 6 months	163	25 (15%)
Died or continued hospitalization at 15 days	163	61 (37%)
Continued hospitalization among survivors (day 15)	138	36 (26%)
Median duration of hospitalization (days)[a]	151	10 (7, 16)

WAZ, HWZ, HAZ = weight for age, height for weight, and height for age z-score, respectively. Severe acute malnutrition was defined as WHZ < −3 SD, visible wasting, ± oedema. Abbreviations: IQR, interquartile range; MUAC, mid-upper arm circumference; SD, standard deviation; TB, tuberculosis; WHO, World Health Organization. [a]Among children surviving to 6 months.

Table 2. Correlates of Cytomegalovirus (CMV) Viraemia ≥1000 IU/mL at Admission in Hospitalized, ART-Naive Kenyan Children With Human Immunodeficiency Virus (HIV)

	N	n (%) or median (IQR) in Category	CMV≥1000 IU/mL n (%) or median (IQR)	Crude PR (95% CI)	*P* value	Adjusted PR (95% CI)	Adjusted *P* value
Demographics							
Age < 2 years	114	59 (52%)	17 (29%)	5.28 (1.63, 17.1)	.006	3.71 (1.17, 11.8)	.03[a]
Age ≥2 years		55 (48%)	3 (5%)				
Recruitment site Kisumu	114	78 (68%)	11 (14%)	0.56 (.26, 1.24)	.2	0.76 (.37, 1.56)	.5
Recruitment site Nairobi		36 (32%)	9 (25%)				
Nutritional status							
Underweight	110	70 (64%)	15 (21%)	1.71 (.67, 4.38)	.3	1.26 (.50, 3.20)	.6
Not underweight		40 (36%)	5 (13%)				
Wasted	86	40 (47%)	10 (25%)	1.44 (.63, 3.30)	.4	1.17 (.52, 2.62)	.7
Not wasted		46 (53%)	8 (17%)				
Stunted	111	65 (59%)	16 (25%)	5.66 (1.36, 23.6)	.02	4.74 (1.23, 18.3)	.02
Not stunted		46 (41%)	2 (4%)				
SAM[c]	85	21 (25%)	6 (38%)	1.83 (.75, 4.45)	.2	1.79 (.79, 4.06)	.2
Not SAM		64 (75%)	10 (16%)				
Immune status							
WHO stage IV	113	36 (32%)	5 (45%)	5.45 (1.53, 19.4)	.009	2.44 (.69, 8.68)	.2[b]
WHO stage III		66 (58%)	12 (18%)	2.18 (.65, 7.27)	.2	2.00 (.62, 6.43)	.2[b]
WHO stage I/II		36 (32%)	3 (8%)	Ref			
Severe immunosuppression[d]	113	85 (75%)	18 (90%)	2.96 (.73, 12.1)	.1	1.08 (.27, 4.38)	.9[b]
Not severe immunosuppression[d]		28 (25%)	2 (7%)				
On steroids at enrollment	108	5 (5%)	3 (60%)	4.12 (1.75, 9.73)	.001	2.05 (.86, 4.92)	.1
Not on steroids		103 (95%)	15 (15%)				
Urgent RCT ART arm	114	57 (50%)	12 (21%)	1.50 (.66, 3.40)	.3	1.53 (.70, 3.33)	.3
Early RCT ART arm		57 (50%)	8 (14%)				
Laboratory							
Total neutrophil count	112	3.34 (2.12, 4.92)	3.02 (1.73, 6.81)	1.01 (.95, 1.06)	.9	1.04 (.98, 1.11)	.2
C-reactive protein	71	14 (3, 59)	3.8 (3, 36)	0.98 (.97, .99)	.01	0.99 (.97, 1.00)	.06
Hemoglobin level (g/dL)	114	8.8 (7.5, 9.6)	8.7 (7.9, 9.8)	1.15 (.94, 1.40)	.2	1.14 (.95, 1.38)	.2
CD4%	113	16.1 (10.0, 22.9)	11.6 (8.95, 19.6)	0.97 (.94, 1.01)	.1	0.97 (.93, 1.02)	.2
Log_{10} HIV RNA copies/mL	110	5.71 (5.02, 6.25)	6.29 (5.94, 6.70)	2.51 (1.44, 4.38)	.001	1.91 (1.16, 3.16)	.01[b]

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; PR, prevalence ratio; RCT, randomized clinical trial; SAM, severe acute malnutrition; WHO, World Health Organization. [a]Adjusted for viral load only. [b]Adjusted for age only. [c]Severe acute malnutrition, defined as WHZ < −3 S, visible wasting, ± edema. [d]Severe immunosuppression defined by age and CD4 count according to WHO criteria. After the Benjamini-Hochberg false discovery rate correction, none of the correlates remained significantly predictive of CMV viraemia.

Table 3. Prevalence and Levels of Cytomegalovirus (CMV) Viraemia at Hospital Admission, by Diagnosis

		Prevalence of CMV	Median log_{10} CMV IU/mL in Children	Prevalence of CMV > 1000
	n/N (%)	Viraemia	IU/mL	With CMV Viraemia
Diarrhoea and/or gastroenteritis	37/114 (23%)	21 (70%)	8 (27%)	2.6 (2.1, 3.6)
Persistent diarrhoea	15/114 (13%)	10 (67%)	5 (33%)	3.0 (2.2, 3.7)
Gastroenteritis	22/114 (19%)	17 (77%)	6 (18%)	2.6 (2.1, 3.4)
Pneumonia	74/114 (65%)	42 (57%)	14 (19%)	2.4 (2.1, 3.2)
Pneumonia with hypoxia	19/70 (27%)	11 (58%)	4 (21%)	2.3 (2.0, 3.6)
Malaria	27/114 (24%)	9 (33%)	2 (7%)	2.1 (1.8, 2.6)
Confirmed tuberculosis	5/114 (5%)	1 (20%)	0	2.1 (2.1, 2.1)

Table 4. Survival Outcomes by Enrollment Cytomegalovirus (CMV) Viraemia in 114 Kenyan Children Diagnosed With Human Immunodeficiency Virus (HIV) Infections in Hospital

	Total events	Events (%) or Median (IQR) in CMV Aviraemic or Low-level Viraemia n = 62	Events (%) or Median (IQR) in CMV Viraemic ≥ 1000 IU/mL n = 20	CMV Aviraemic or Low-Level Viraemia vs Viraemic ≥ 1000 IU/mL, crude	CMV Aviraemic or Low-Level Viraemia vs Viraemic ≥ 1000 IU/mL, Adjusted for HIV RNA and Age	Log_{10} CMV IU/mL, Crude	Log_{10} CMV IU/mL, Adjusted for HIV RNA and Age
Death or continued hospitalization at day 15	40/114	28 (45%)	13 (65%)	RR = 2.26 (1.44, 3.56) *P* = <.001	aRR = 1.74 (1.04, 2.90) *P* = .04	RR = 1.34 [95% CI = 1.05, 1.69], *P* = .02	aRR = 1.17 [95% CI = .87, 1.58], *P* = .3
6-month mortality	20/114	15 (24%)	8 (40%)	HR = 3.78 (1.54, 9.25) *P* = .004	aHR = 1.97 (.77, 5.07) *P* = .2	HR = 1.42 [95%CI = .98, 2.07], *P* = .06	aHR = 1.05 [95% CI = .66, 1.68], *P* = .8

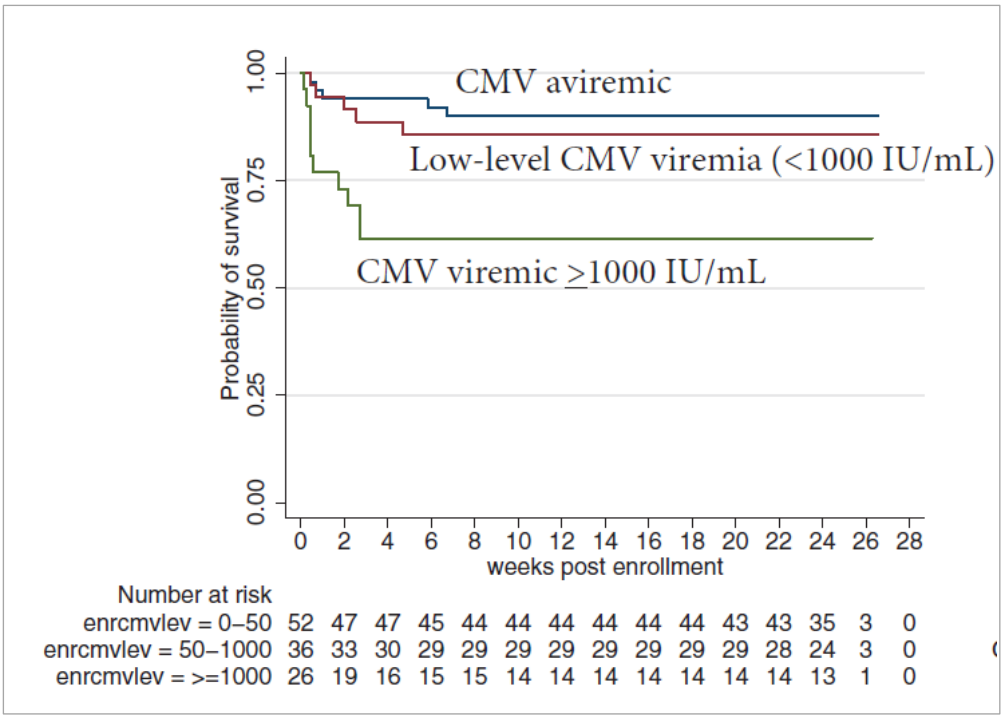

Figure 3. Survival probabilities for hospitalized children by admission CMV viraemia

Chapter 7:
General Discussion

7.0 General Discussion

This thesis brings together research on paediatric HIV treatment and covers a ~15-year time-frame starting from the period when ART first became available for children with advanced disease to the current universal treatment era (test and treat) when all children living with HIV are eligible for ART. In spite of the passage of time, uncontrolled HIV in children remains a highly aggressive disease with high mortality. In Kenya an estimated 83,000 children below 14 years live with HIV and there is a worrying trend of stagnation of PMTCT efforts which threatens the gains made in preventing new HIV infections in children (1,2). While progress has certainly been made in reaching more children and placing them on treatment, the challenges of late presentation, appropriate formulations for young infants, impact of caregiver and family dynamics on paediatric adherence and co-infections remain as pertinent today as they were when we embarked on these studies.

The studies highlighted in this thesis have contributed significantly to a better understanding of the outcomes of paediatric HIV treatment broadly including the rates and predictors of clinical, virologic and immunologic response to ART in the short and long-term in Kenyan children. We have quantified the levels and spectrum of drug resistance to commonly used ART regimens and defined the role of cytomegalovirus, an important and common but poorly recognized co-infection on treatment outcomes thus laying the foundation for future translational work in improving clinical outcomes. Being among the first published data on paediatric HIV treatment some of our studies have directly informed national HIV treatment policy and contributed to guideline formulation in Kenya and globally.

The studies presented address several related key points that form part of the experience of HIV from infancy through childhood. The first aspect touches on infants with HIV whose survival is severely delayed by late diagnosis. We showed that a delay of approximately 2 months in HIV diagnosis is extremely costly and may result in decreased survival from 96% to 68%. Late diagnosis which is defined by presentation with advanced clinical features regardless of the age highly compromises the value of early ART. This observation also means that children who survive untreated to older ages represent survivors who did not succumb to HIV in infancy. This is important in interpreting the results from cohorts of older children – there is a risk of underestimating true mortality due to survivor bias. The fact that in our study

of older children (median age 4.5 years) had lower mortality than infants should be interpreted in this light. The importance of defining predictors of mortality cannot be over-emphasized because some of these factors are modifiable. We identified two main sets of predictors of outcome; the first relating to advanced HIV (WHO stage, low hemoglobin, severe wasting) the second a specific co-infection (CMV). By addressing stage of presentation through intensified efforts in early identification mortality can be averted. While studies that define response are important in framing the burden of HIV-related mortality, identifying predictors is more pertinent for designing interventions to optimize outcomes. Our studies show that the search for safe and effective CMV suppressive therapy for children living with HIV starting ART is justifiable.

The initial studies on Early response to ART in children with advanced HIV (Chapter 2) leveraged the Paediatric Adherence Study cohort which was launched in an environment where ART was not available to Kenyan children and challenged the prevailing premise that the WHO 2003 ART guidelines had settled for a sub-optimal ART backbone (NNRTI-based compared to the protease inhibitors which were recommended in Europe and the US at the time) (3). In addition, it was well recognized that the children initiated on ART in Africa not only had more advanced clinical disease and lower CD4 counts and percentage than their counterparts in Europe and US but faced additional pathology such as tuberculosis and malnutrition. In summary, treatment outcomes for this unlikely population with sub-optimal regimens was unknown and the chances of success uncertain. Our results demonstrated that good clinical, immunologic and virologic outcomes were achievable with an NNRTI-based regimen and at least two-thirds of the cohort achieve viral suppression < 400 copies within 9 months of ART. Substantial immunologic recovery depicted by an increase in CD4 percentage from 5% to 15% and 18% at 9 and 15 months respectively and prompt growth recovery as measured by restoration of weight-for- age and height-for age was observed. The rate of hospitalization dropped from 58% in the 6-month pre-ART period to 17% following ART thus saving costs of hospital admission. This study also provided an accurate estimate of drug toxicity with under 10% of the children requiring change of treatment as a result of an adverse drugs reaction. Our findings contributed to strengthening WHO recommendation and NNRTI based ART was rapidly scaled up across the region and remained in wide-spread use for at least 10 years before

evidence supported the superiority of protease inhibitors (4) and subsequently integrase strand transfer inhibitors (5). It is worth noting that this cohort could not include infants < 18 months because confirmation of HIV diagnosis in this sub-population which requires PCR testing was not available in public sector health setting. Further as per prevailing treatment guidelines only children with advanced HIV or low CD4 counts were eligible for treatment.

We used the same cohort expanded it and with extended follow-up to better define mortality (Chapter 3) in children initiated on ART. The overall mortality was 13% however most deaths occurred in the first 4 months and the rate of mortality dropped sharply after this period. In order to correctly contextualize this mortality burden a comparison with pre-ART mortality is warranted. Before availability of ART CLHIV in Kenya and Rwanda were found to have mortality exceeding 50 and 60% respectively (6,7). Regarding correlates of mortality, it was evident that children starting treatment with advanced HIV as indicated by WHO stage 4, severe wasting (WHZ < -2) and low haemoglobin <9 g/dL were at highest risk of death. Similar findings have been reported in cohorts of CLHIV from Zambia and Ethiopia (8,9). Collectively this and other evidence played important advocacy role in guideline changes between 2010 and 2012 to provide ART to all children regardless of clinical stage or CD4 counts. This is based on the understanding for some children with advanced HIV treatment are brought in too late and hence the best approach to address this is to initiate ART before too far advanced disease (10,11). The causes of death in this cohort included a combination of infectious conditions (pneumonia, sepsis, tuberculosis) and non-communicable (cardiac failure resulting from chronic lung disease, Non-Hodgkin's lymphoma) which are generally common in advanced stages of HIV in children. Efforts to prevent tuberculosis which have been put in place and averted TB-related deaths include Isoniazid-preventive therapy for all newly diagnosed CLHIV (12,13).

Since ART is life-long it is important to evaluate long-term findings and we used extended follow-up to characterize outcomes after a median 5 years of follow-up on ART in the same cohort (Chapter 4). Of particular importance is the ability to achieve long-term viral suppression which is the single most important indicator of effective HIV treatment. For this analysis serial sampling for plasma viral load (quarterly was used to both establish virologic suppression as well as presence of drug resistance mutations for those failing treatment. We found the rate of virologic failure to be 34%

over the follow-up period and most children who failed did so in the first year of ART with a smaller proportion during the second year. Significantly younger children (< 3 years) were more likely to experience virologic failure and out of those who failed majority (68%) had presence of drug resistance mutations. NNRTI associated mutations were the most common followed by NRTI mutations which is expected given the low genetic barrier to resistance. We had an opportunity to follow a small number of children (n=14) who switched to second line protease -inhibitor based therapy for 28 months and while some of these children experienced episodes of high viral loads in the course of follow-up, none had major PI- resistance mutations indicating the high genetic barrier to resistance for this class of ART. An important observation out of this study was that while clinical teams would recommend treatment switch on basis of clinical failure when this was superimposed on the timing of virologic failure the clinical failure occurred on average 15 months after the virologic failure. Thus, relying only on clinical assessment in this scenario would typically result in keeping children on a failing regimen for at least an extra year with potential accumulation of further resistance.

The study on long-term virologic response and drug resistance has important practical and policy implications in the monitoring and sequencing ART. First it demonstrated that a failing NNRTI based regimen is associated with a high probability of NNRTI (and less often NRTI) resistance mutations and therefore a treatment switch can reasonably be made without performing expensive and often inaccessible drug resistance testing. Conversely for children on protease inhibitors virologic failure often exists in the setting of non-adherence with resistance less common, hence before a switch drug resistance testing is warranted so that unnecessary switches are avoided. Secondly the study pointed out the perils of overdependence on clinical judgement without viral load monitoring for making treatment changes. These findings have been corroborated by other studies (14, 15) and current Kenyan antiretroviral treatment guidelines recommend 6-monthly viral load testing for children and adolescents on ART (16).

The next series of studies focused exclusively on infants (Chapter 5) in recognition of the vast differences between HIV treatment response in infants versus children (17). It is well recognized that some of the highest rates of HIV disease progression and mortality is seen in young infants and some scholars consider any infant with untreated HIV who survives the first 1-2 years of life to be a slow progressor (18). The Infant

Disease progression study conducted in HIV infected infants followed up at Kenyatta National Hospital in the pre-ART era reported a 6-month mortality rate of 46% (6).

Following the CHER trial in South Africa in which ART was initiated early (1.7 months) with markedly reduced mortality international guidelines were switched to recommend ART for all infants upon diagnosis (19) and programs quickly adapted these guidelines. It was generally anticipated that survival of infants with HIV would equally improve to levels that approximate those seen in children who started immediate ART in the clinical trial (76% survival). However, a close evaluation of the presentation and timing of children in the CHER study in comparison with the typical infant presenting to our facility revealed significant differences that would most likely impact outcomes. Specifically, the children in the CHER trial were younger, asymptomatic and had normal CD4+ T-lymphocyte counts and percentage (20). The Optimizing pediatric HIV-1 Therapy (OPH) cohort enrolled HIV-1 infected infants < 4.5 months and initiated ART at a median age of 3.7 months (Chapter 5). Most infants had been hospitalized at least once and at baseline 49% had advanced clinical disease (WHO stage 3-4). The median CD4 percentage was 18% compared to the 35% in CHER indicating more advanced immune deficiency before ART. After a median follow-up of 16 months we observed a high mortality of 14% in infants who initiated ART and the 12-month probability of survival was 77%. A head-to-head comparison between this cohort and the CHER cohort revealed differences in age, CD4 count, nutritional status and hospitalization and although the difference in age was 2 months this was probably enough to confer the high mortality difference. In our cohort CD4 < 10%, WHO stage 3-4, growth failure, Hb < 8 g/dL and gastroenteritis predicted mortality. Overall this study provided some of the clearest evidence that even with guideline change unless infants are identified early (where early means younger age, asymptomatic and high CD4%) the benefit of ART is highly compromised. Following this study the Kenyan MOH-NASCOP embarked on more aggressive identification of HIV-infected infants including birth testing and promoting PITC along with PMTCT efforts.

A related important observation from the OPH cohort was the fact the nearly 40% of overall mortality occurred in infants waiting for stabilization before ART initiation (median time to ART was found to be 14 days). This delay led us to design a trial to address delay to ART within hospital. In the study (designated ‘Paediatric Urgent Start of HAART’ - PUSH) infants and children were randomized to urgent ART (within 48

hours of hospital admission) versus post-stabilization ART (within 14 days) and followed up for 6 months to evaluate mortality (21). Although we did not demonstrate a survival benefit from urgent ART initiation the practice was safe and this had led to a shift in which ART initiation is promptly initiated in infants and children with HIV in most Kenyan facilities.

The final research study leveraged the PUSH study and is based on our efforts to elucidate additional often overlooked drivers of mortality that may form future targets for intervention beyond ART HIV-infected children (Chapter 6). Focusing on hospitalized infants and children who have the highest mortality we sought to evaluate the potential role of cytomegalovirus infection on clinical outcomes among hospitalized CLHIV initiating ART. We have previously shown that CMV is a common co-infection among HIV-infected children with above 75% of Kenyan HIV-infected infants harbouring CMV at one point or other (22). Additionally, we have shown that CMV viraemia persists longer in the setting of paediatric HIV (23). In the PUSH which enrolled and initiated ART in HIV-1 infected children aged 0-12 years study we measured CMV viral load levels at baseline and serially. We considered high CMV viraemia to be levels above 1000 IU/mL and compared outcomes (mortality and prolonged hospitalization) between children who had high CMV viraemia with those with either no viraemia or low level viraemia. In this study 54% of the children CMV viraemia at baseline, 32 % had high CMV viraemia and had 72% had CMV viraemia at one point in follow-up. Age below 2 years predicted high CMV viraemia and the risk of mortality or hospitalization beyond 15 days was 74% higher in children with high CMV viraemia compared to those with either low level or no CMV viraemia and this effect was independent of HIV viral load. On average high CMV viraemia conferred 5 days longer hospitalization and increased the odds of death. This study illustrates some pertinent findings regarding the relationship of CMV to HIV in hospitalized children and provides an opportunity for intervention. First the prevalence of persisting CMV viraemia is high and secondly it had a hazardous effect independent of HIV viral load. Although we cannot directly causality due to the study design and lack of histopathologic diagnoses (we did not perform autopsy), our findings are consistent with others that indicate that CMV leads to or worsens outcomes in patients with HIV (24).The mechanisms of this effect have been the subject of multiple studies, however, at the cellular level CMV increases the levels of immune activation this making more

target cells available for HIV and also is associated with the development of a larger reservoir of HIV latent T cells (25-26). The practical implications of thus study is that if CMV is suppressed either before or during antiretroviral treatment this may improve outcomes. Small adult studies have demonstrated this in HIV -infected patients in intensive care, however paediatric data on this is lacking (27).

Alternative approach towards HIV control and cure are considered under eradication, functional or hybrid strategies. While eradication may be an unrealistic goal, functional cure which refers to achievement of immune control without necessarily removing all HIV reservoirs) and without using antiretroviral drugs is possible. Key approaches to achieve functional cure include genome editing, engineered T cells to control cells infected with HIV, broadly neutralizing and monoclonal antibodies and latency reversing agents. Two examples of antibodies are broadly neutralizing antibodies for HIV envelope and monoclonal antibodies for CCR5. CCR5 is a key co-receptor utilized by HIV for cell entry and the absence of this receptor confers natural protection from HIV. Evaluation of monoclonal antibodies to eliminate the CCR5 receptor is promising and is expected to also reduce HIV reservoir size (28)

Strengths and Limitations

The studies used for this thesis employed prospective cohort design and involved a prolonged duration of follow-up which allowed us obtain longitudinal data. The cohorts were established primarily for research and data collection was detailed, systematic and well planned. Efforts were made to reach patients to missed clinics and overall retention was high. Biological outcomes were carefully documented and multiple laboratory data was obtained including some tests that are not routinely available such as cytomegalovirus and genotypic drug resistance tests. Antiretroviral drugs were constantly available through the well-funded PEPFAR program at the Kenyatta National Hospital (KNH), a large tertiary facility and for the PUSH studies the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in Kisumu, a city in Western Kenya. The medical care of children including ART regimens strictly adhered to the Ministry of Health HIV treatment guidelines which increases generalizability of our findings. The children enrolled in the studies were largely typical of those that would access care in a public facility and represented lower income population which is the main target for the KNH.

The studies nonetheless had important limitations that must be considered in interpretation of findings. All studies were all based tertiary public facilities which are often better resourced than non-tertiary sites. This is however mitigated to some extent by the fact that HIV care is fairly standardized across different levels of sites. Availability to specialized care would be more in KNH and JOOTRH than in lower tier facilities. The sample sizes for the cohorts was relatively modest in relation to the high numbers of children living with HIV in Kenya. Due to the observational study design we are not able to definitely ascribe causality since confounding is well recognized in observational studies. Finally, for children who died autopsy which is the gold standard for determining pathologic diagnosis was not available which may result in misclassification.

Impact of thesis on scientific journey

While I conducted these studies and as part of building an independent research career my PhD has provided an opportunity to place my findings in context of the fast evolving filed of HIV treatment including the development of new drugs, fixed dose combinations and now the long-acting injectable antiretroviral drugs. I have been able to more clearly see the link between the various studies, how they build on each other to improve treatment for children with HIV. I now have better appreciation of the incremental nature of scientific inquiry starting from simple to complex. For instance, we initially focused purely on clinical parameters as predictors of outcome but over time the importance of more hidden factors such as co-infections which can only be detected through more sophisticated tests emerged. Through this process I have learnt first-hand the importance of continual investment for improvement in diagnostic and therapeutic measures. My enthusiasm to continue improving the lives of children with HIV has been re-energized. Finally this process has reminded me strongly of the need to advocate for improved uptake of measures to prevent HIV in children.

Conclusions

In these series of studies on HIV treatment in children we demonstrated that:

1. NNRTI based ART introduced for children aged 18 months to 12 years with advanced HIV resulted in good early clinical, immunologic and virologic outcomes with at least 67% achieving viral suppression within 9 months.

2. The mortality rate in children initiating NNRTI-based ART in the first 2 years was 13% and advanced WHO stage (4), severe wasting (WHZ < -2) and low haemoglobin (< 9 gm/dl) predicted mortality.
3. Following a median of 5.5 years on NNRTI-based ART, 34% of children experienced virologic failure and at least two thirds of those failing ART had evidence of drug resistance. In a smaller group that switched to protease inhibitor no major resistance developed to protease after 28 months of follow-up.
4. HIV-1 infected infants initiating ART at a median age of 3.7 months with symptomatic HIV disease and immunosuppression (49% in WHO stage 3-4, median CD4 18%) had much higher mortality at 14% after 12 months indicating the high cost of delaying diagnosis.
5. High levels of cytomegalovirus//CMV viraemia (> 1000 IU/mL) in HIV-1 infected children aged 0-12 years initiating ART was associated with higher risk of a combined endpoint of prolonged hospital stay or 6-month than those with either low level viraemia or no viraemia.

Recommendations

1. Accelerate efforts to identify and promptly initiate antiretroviral therapy for infants with HIV-1. This includes a policy change to conduct the first HIV-1 PCR test for infants exposed to HIV before 6 weeks, preferably within two weeks of life.
2. Enhance uptake of regular virologic testing for infants and children on antiretroviral to identify those failing treatment and thus prevent emergence of HIV drug resistance.
3. Evaluate the role of empiric CMV viral load suppression among children with HIV from the point of initiating antiretroviral therapy to improve outcomes. Where possible this may be coupled with measuring the level of CMV viremia in these children.

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APPENDICES 1. ETHICS APPROVAL PAEDIATRIC ADHERENCE STUDY

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Ref. No.KNH/ERC/R/42

15th March 2013

Dr. Dalton Wamalwa
Principal Investigator
Dept.of Paediatrics & Child Health
School of Medicine
University of Nairobi

Dear Dr. Wamalwa

Re: Approval of annual renewal – study titled "Effect of medication diaries on Adherence to Highly Active Antiretroviral therapy among HIV-1 infected Kenyan children" (P185/10/2005)

Refer your communication of February 22, 2013.

This is to grant you annual extension of approval for ethical research Protocol P185/10/2005 for data analysis only.

The renewal periods are 7th February 2013 – 6th February 2014.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN -ERC website **www.uonbi.ac.ke/activities/KNHUoN**

Kindly forward the informed consent documents for endorsement with updated stamp.

Yours sincerely

PROF. A.N.GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Dean, School of Medicine,UoN
The Chairman, Dept.of Paediatrics & Child Health, UoN

2: ETHICS APPROVAL OPTIMIZING PEDIATRIC HIV-1 THERAPY

KENYATTA NATIONAL HOSPITAL
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Ref: KNH-ERC/ 01/ 3538

Date: 23rd May 2006

Dr. Grace John-Stewart
Dept. of Paediatric & Child Health
Faculty of Medicine
University of Nairobi

Dear Dr. Stewart

RESEARCH PROPOSAL: "OPTIMIZING PEDIATRIC *HAART* 0-3 MONTHS" (P4/01/2006)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** revised version of your above cited research proposal for the period 23rd May 2006 – 22nd May 2007.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

c.c. Prof. K.M.Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
The Dean, Faculty of Medicine, UON
The HOD, Medical Records, KNH
Co-investigator: Dr. D. Wamalwa, Dept. of Paediatrics & Child Health, UON

3: ETHICS APPROVAL PAEDIATRIC URGENT START OF HAART (PUSH)

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Ref: KNH-ERC/ MOD/9

8th January 2013

Dr. Dalton C. Wamalwa
Dept.of Paediatrics & Child Health
School of Medicine
University of Nairobi

Dear Dr. Wamalwa

Re: Approval of modifications study titled "Poststabilization vs urgent start of HAART in HIV-1 infected children with severe co-infection" (P378/09/2011)

Your request dated November 5, 2012 refers.

The KNH/UoN-ERC has reviewed and approved the following –

1. PUSH Protocol 01 August , 2012 version 3
2. Assent Form(Ages 7-12) for Screening 01 Aug 2012 Version 2
3. Assent(Ages 7-12) for Enrollment 01 August 2012 Version 2
4. Informed Consent for HIV-1 Testing 01 August 2012 Version 3
5. Informed Consent for Enrollment 01 Aug 2012 Version 4

The documents are duly stamped and endorsed for use.

Yours sincerely

PROF.A.N. GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Dean, School of Medicine, UoN
The Chairman, Dept.of Paediatrics, UoN