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THE EFFECTS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON SURVIVAL AND CD4 CELL PERCENTAGE IN HIV-INFECTED CHILDREN IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

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

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ABSTRACT

ANDREW JAY EDMONDS: The effects of highly active antiretroviral therapy on survival and CD4 cell percentage in HIV-infected children in Kinshasa, Democratic Republic of Congo
(Under the direction of Frieda Behets)

In HIV-infected children, the effects of highly active antiretroviral therapy (HAART) on survival and CD4 responses are understood incompletely. As most pediatric HIV infections occur in lower-income countries, our objective was to provide the first estimates of these effects among children in a resource-deprived setting.

Observational data from HAART-naïve children enrolled into an HIV care and treatment program in Kinshasa, Democratic Republic of Congo between December 2004 and May 2010 were analyzed. Marginal structural models were used to quantify the effects of HAART on survival and CD4 percentage while accounting for time-dependent confounders affected by prior exposure to HAART.

At the start of follow-up, the median age of the 790 children was 5.9 years; 528 (67%) had advanced or severe immunodeficiency and 405 (51%) were in HIV clinical stage 3 or 4. The children were observed for a median of 31 months and contributed 2,090 person-years. Eighty children (10%) died, 619 (78%) initiated HAART, six (1%) transferred care to another facility, and 76 (10%) were lost to follow-up. The mortality rate was 3.2 per 100 person-years (95% CI: 2.4, 4.2) during HAART and 6.0 (95% CI: 4.1, 8.6) during receipt of primary HIV care only. The mortality hazard ratio comparing

HAART to no HAART was 0.25 (95% CI: 0.06, 0.95). Compared to no HAART, the estimated absolute rise in CD4 percentage was 6.8% (95% CI: 4.7%, 8.9%) after six months of HAART, 8.6% (95% CI: 7.0%, 10.2%) after 12 months, and 20.5% (95% CI: 16.1%, 24.9%) after 60 months. HAART-mediated CD4 percentage gains were slowest but greatest among children who had a baseline CD4 percentage <15. The cumulative incidence of recovery to "not significant" WHO age-specific immunodeficiency was lower if HAART was initiated when immunodeficiency was severe rather than mild or advanced.

HAART reduced the hazard of mortality and increased CD4 percentages among HIV-infected children in a resource-deprived setting to a similar degree as previously noted for children in the United States. The more gradual and protracted immunological recovery observed in children with lower baseline CD4 percentages supports earlier initiation of pediatric HAART.

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TABLE OF CONTENTS

LIST OF TABLES ix

LIST OF FIGURES x

LIST OF ABBREVIATIONS xi

Chapter

I. SPECIFIC AIMS 1

Introduction 1

Specific Aim 1 3

Specific Aim 2 3

Specific Aim 3 3

II. BACKGROUND AND SIGNIFICANCE 4

Global Epidemiology of HIV 4

HIV: Clinical, Survival, and Immunological Impacts 5

Clinical Progression and Survival Effects of Antiretroviral Therapy 6

Immunological Effects of Antiretroviral Therapy 17

Immunodeficiency at Antiretroviral Therapy Initiation: Clinical, Survival and Immunological Impacts 22

Summary and Rationale 28

III. RESEARCH METHODS 32

Study Design and Manuscripts 32

Study Context 32

Study Population 34

Patient Follow-up and Data Collection 36

Methodological Context 37

Variables 41

Analysis – Specific Aim 1 43

Analysis – Specific Aim 2 48

Analysis – Specific Aim 3 50

IV. THE EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON THE SURVIVAL OF HIV-INFECTED CHILDREN IN A RESOURCE-DEPRIVED SETTING: A COHORT STUDY 51

Abstract 51

Introduction 52

Methods 54

Results 59

Discussion 61

V. QUANTIFICATION OF CD4 RESPONSES TO HIGHLY ACTIVE ANTIRETROVIRAL THERAPY OVER FIVE YEARS AMONG HIV-INFECTED CHILDREN IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO 72

Abstract 72

Introduction 74

Methods 75

Results 80

Discussion 82

VI. DISCUSSION 93

Summary of Findings....93
Contributions of Findings....94
Strengths and Limitations....97
Future Research Directions....101
REFERENCES....108

LIST OF TABLES

Table

1. Individuals living with HIV, new HIV infections, and AIDS-related deaths, 2009 5

2. Variables included in analyses 41

3. Characteristics of 790 HIV-infected children initiating HIV care in Kinshasa, DRC, between December 2004 and May 2010 67

4. Estimated effect of HAART on mortality among 790 HIV-infected children initiating HIV care in Kinshasa, DRC, between December 2004 and May 2010 69

5. Characteristics of 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC 85

6. Estimated effect of HAART on CD4 percentage, 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC 86

LIST OF FIGURES

Figure

1. Flowchart of HAART and final dispositions (loss to follow-up, death, transfer of care to another facility, or active at time of analysis) for study population.....35

2. Directed acyclic graph representing the causal effect of time-dependent HAART on study outcomes.....40

3. Number of active children by month of follow-up and HAART status 70

4. Cumulative incidence curves depicting the effect of HAART on survival among 790 HIV-infected children 71

5. Duration of HAART (bars) and number of CD4 percentage measurements during each of the six month periods (lines) in 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.....87

6. Estimated effect of HAART on CD4 percentage from marginal structural model (Panel A) and observed, unadjusted CD4 percentage evolutions among children receiving HAART and children not receiving HAART (Panel B),790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.....88

7. Estimated effect of HAART on CD4 percentage, by category of CD4 percentage at baseline, 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.....90

8. Cumulative incidence curves of recovery to “not significant” WHO age-specific immunodeficiency, by category of suppression at the start of HAART, 536 children initiating HAART with mild, advanced, or severe immunodeficiency between December 2004 and May 2010 in Kinshasa, DRC.....92

LIST OF ABBREVIATIONS

ACTG	AIDS Clinical Trial Group
AIDS	Acquired Immune Deficiency Syndrome
AZT	Zidovudine
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
ddC	Zalcitabine
ddI	Didanosine
d4T	Stavudine
DRC	Democratic Republic of Congo
HAART	Highly Active Antiretroviral Therapy
HR	Hazard Ratio
HIV	Human Immunodeficiency Virus
IDV	Indinavir
IPTC	Inverse-Probability-of-Treatment-and-Censoring
IPTCV	Inverse Probability of Treatment, Censoring, and Visit Attendance
IQR	Interquartile Range
3TC	Lamivudine
MSF	*Médecins Sans Frontières*
MSM	Marginal Structural Model
NVP	Nevirapine

OR	Odds Ratio
PEPFAR	President's Emergency Fund for AIDS Relief
RTV	Ritonavir
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNC	University of North Carolina at Chapel Hill
WAZ	Weight-for-Age Z-Score
WHO	World Health Organization

I.

SPECIFIC AIMS

Introduction

Human Immunodeficiency Virus (HIV), the cause of Acquired Immune Deficiency Syndrome (AIDS), is a major source of global morbidity and mortality. As the virus damages cell-mediated immunity by infecting and destroying $CD4^+$ T lymphocytes (i.e., CD4 cells), the CD4 cell level is an essential immunological marker of HIV progression. Given that highly active antiretroviral therapy (HAART) effectively inhibits various phases of HIV propagation, it logically follows that HAART would improve both immunological and clinical outcomes, including survival.

These beneficial impacts of HAART have been demonstrated in a variety of experimental and observational studies. However, despite such evidence, multiple knowledge gaps remain. After HAART was first shown to delay AIDS and improve survival, additional randomized trials comparing HAART to less potent antiretroviral therapy (ART) were precluded due to ethical reasons. This limited future investigations to quantify the effects of HAART on progression to non-experimental data, which are susceptible to biases including confounding by indication. Although prior observational studies are of immeasurable value and have greatly influenced patient care, few have utilized the epidemiological methods necessary to isolate the causal effects of HAART given the complex, time-dependent relationships between exposure, outcomes, and other

factors. The studies that have used those methods have generally examined adult cohorts in the United States. In fact, no such studies have focused on children in resource-deprived, sub-Saharan African settings, where the HIV pandemic is most widespread.

While the simple lack of methodologically adequate observational studies on the clinical progression, survival, and immunological effects of HAART in children justifies further investigation in this arena, increased rollout of antiretroviral drugs in resource-deprived settings has revealed new research priorities as well as highlighted opportunities for novel scientific contribution. Care and treatment programs in the developing world operate in vastly different environments than those of more resource-privileged initiatives, and face specific challenges including a high prevalence of undernutrition, patients who are sicker upon presentation to care, fewer drug options, and decreased laboratory capacity to inform decision making. Although it can be argued that the effects of HAART in children are not definitively known in any context, there is even less evidence on how HAART affects clinical and immunological progression in resource-deprived settings, and questions remain about how outcomes may be impacted by immunological status at time of HAART initiation.

This research was motivated by the opportunity to expand the breadth of understanding on pediatric HAART along with the possibility of informing policy and patient care as, for example, the debate on when to initiate HAART is ongoing. Using observational data from 790 children participating in a comprehensive HIV care and treatment program in Kinshasa, Democratic Republic Congo (DRC), along with appropriate epidemiological methods such as marginal structural models estimated by inverse probability weighted regression, the following aims were addressed:

Specific Aim 1

To estimate the causal effect of HAART on survival.

Hypothesis: Relative to non-initiation of HAART, HAART initiation will decrease the hazard of death.

Specific Aim 2

To estimate the causal effect of HAART on mean change in CD4 percentage at each six month time point up to 60 months following the start of HAART, both overall and by category of baseline CD4 percentage (<15%, 15%–24%, ≥ 25%).

Hypotheses: Relative to non-initiation of HAART, HAART initiation will increase the mean CD4 percentage at each time point. Higher CD4 percentages at baseline will be associated with reduced increases in CD4 percentage at all time points.

Specific Aim 3

To determine whether the degree of immunodeficiency at HAART initiation is associated with recovery to "not significant" WHO age-specific immunodeficiency.

Hypotheses: Children with greater immunodeficiency at time of HAART initiation will be less likely to recover immunologically.

II.

BACKGROUND AND SIGNIFICANCE

Global Epidemiology of HIV

HIV, the virus that causes AIDS, is a significant source of morbidity and mortality worldwide. In 2009, according to the Joint United Nations Programme on HIV/AIDS (UNAIDS), there were approximately 33.3 million people living with the virus, 2.6 million new HIV infections, and 1.8 million deaths due to AIDS.[1] Spread primarily through sexual contact and injection drug use, HIV does not have a uniform global distribution – in 2009, it was estimated that 68% of people living with the virus were in sub-Saharan Africa, where 69% of new infections and 72% of AIDS-related deaths occurred.[1] In less than 30 years, HIV has caused the deaths of 25 million individuals around the world.[2]

Children bear a substantial portion of the global HIV burden. There were about 2.5 million children younger than 15 years of age living with HIV in 2009, with 370,000 new HIV infections and 260,000 deaths due to AIDS.[1] Paralleling the general pandemic, sub-Saharan Africa is the epicenter of pediatric HIV – in 2007, more than 90% of pediatric AIDS-related deaths were in that region,[2] where in 2009, 92% of children with HIV lived. Virtually all children living with HIV were infected via vertical transmission either during breastfeeding, birth, or pregnancy.[1]

Table 1. Individuals living with HIV, new HIV infections, and AIDS-related deaths, 2009.

	Global total (in millions)		% in sub-Saharan Africa	
	All ages	<15 years	All ages	<15 years
Individuals living with HIV	33.3	2.5	68	92
New HIV infections	2.6	0.37	69	no data
AIDS-related deaths	1.8	0.26	72	no data

Data source: Global report: UNAIDS report on the global AIDS epidemic 2010.[1]

HIV: Clinical, Survival, and Immunological Impacts

HIV is deadly due to its deleterious effects on the immune system. The virus infects cells expressing the CD4 glycoprotein,[3,4] including macrophages,[5] dendritic cells,[6] and CD4 cells.[7] During the course of HIV infection, opportunistic diseases increase over time[8,9] as these cells, essential for cell-mediated immunity,[10] are destroyed by induced apoptosis,[11] targeting by cytotoxic $CD8^+$ T lymphocytes,[12] and direct viral killing.[13] The most widely utilized immunological marker of HIV progression is the CD4 count;[14-17] in children, CD4 percentage, regarded as a more stable metric, is typically used.[18] There is perhaps no clearer way to express the importance of CD4 cells and the immunological effects of the virus than by examining the 2008 Centers for Disease Control and Prevention (CDC) case definition for AIDS – an HIV patient aged 13 years or more is considered to have AIDS if the CD4 count ever falls below 200 cells/μL, if the percentage of total lymphocytes that are CD4 cells ever falls below 14%, or if the individual has developed one of 27 immunodeficiency-related conditions.[19] Coincident with the increasing risk of opportunistic diseases as HIV induces immune system failure, an association between low CD4 levels and mortality in adults[20,21] and children[22-24] has been demonstrated.

HAART can effectively inhibit HIV. The compounds comprising HAART, administered in a regimen of three or four drugs,[25] alter the natural history of infection by affecting various phases of HIV propagation via mechanisms including disruption of reverse transcription,[26] blockage of the requisite protease enzyme,[27] prevention of host genome integration by viral DNA,[28] and interference of HIV entry into the target cell.[29] Access to HAART in most high HIV prevalence areas, while improving, is still extremely limited – UNAIDS estimated in 2009 that just 36% of those indicated for HAART in low- and middle-income countries are receiving essential medications.[30] While vertical transmission, the means by which most children acquire HIV, can be prevented with antiretroviral monotherapy,[31,32] complex regimens (e.g., HAART) are even more effective.[33,34] However, just as overall HAART coverage is low, coverage of antiretroviral prophylaxis for HIV-infected pregnant women in low- and middle-income countries was estimated to be only 53% in 2009,[30] meaning that new pediatric infections remain common. HAART has been proven as able to reduce an individual's viral load to undetectable in both antiretroviral-naïve[35,36] and -experienced[35-37] patients, thereby severely limiting if not completely eliminating the devastating cycle of cell infection and destruction caused by HIV. Consequently, it follows that HAART would result in improved clinical and immunological outcomes.

Clinical Progression and Survival Effects of Antiretroviral Therapy

The beneficial effects of ART on the clinical progression and survival of HIV-infected individuals have been established by numerous studies. Given that the first specific aim focuses on survival, an endpoint regularly examined in prior investigations

of the effects of ART on clinical progression, it is apt to provide perspective on what is already known.

The earliest randomized trials of ART evaluated a single drug, zidovudine (AZT), against placebo. A 1987 trial of 282 adult AIDS patients in the United States made clear the lifesaving promise of antiretroviral drugs – after between eight and 24 weeks of follow-up, there was one death in the AZT group compared to 19 deaths in the control group.[38] The combined outcome of progression to AIDS or late stage disease was typically utilized by the influential studies of that time, all of which focused on adults. In a trial of 1,338 individuals in the United States aged 18 years or more with CD4 counts under 500 cells/μL, the rate of progression per 100 person-years, after a mean follow-up of 55 weeks, was 7.4 in the placebo group, 3.6 in the low dose AZT group, and 4.3 in the high dose AZT group.[39] A trial of 993 asymptomatic individuals in Western Europe aged 18 years or more, who had CD4 counts over 400 cells/μL and were followed for a median of 94 weeks, showed that AZT reduced progression – the hazard ratio (HR) for the comparison of AZT to no AZT was 0.56, with a 95% confidence interval (CI) of 0.43 to 0.75.[40] In a trial of 713 mildly symptomatic adults in the United States with CD4 counts between 200 and 800 cells/μL, the rate of progression per 100 person-years after a median follow-up of 11 months was 11.2 in the placebo group compared with 4.5 in the AZT group; correspondingly, 18-month progression-free survival was 91% with AZT and 81% without AZT.[41] Given their clear demonstration of clinical benefits, these trials were instrumental in establishing AZT monotherapy as the first standard of care for pharmaceutical treatment of HIV, but they also made impossible any follow-up placebo-controlled examination of the progression effects of AZT. Once AZT was known to delay

AIDS and death, further such investigation became unethical – in fact, three of the above-cited trials[39-41] were stopped. However, future studies, which would also inform changes in HIV treatment norms, offered additional evidence.

The next set of trials evaluated monotherapy against dual therapy, and like the placebo-controlled AZT trials, found remarkably consistent results. The AIDS Clinical Trial Group (ACTG) 175 study followed 2,467 individuals in the United States aged 12 years or more who had CD4 counts between 200 and 500 cells/μL, 43% of whom were antiretroviral-naïve.[42] An analysis of all participants demonstrated that both the hazard of death (HR: 0.55; 95% CI: 0.36, 0.86) and the hazard of AIDS or death (HR: 0.64; 95% CI: 0.46, 0.87) were lower in the group treated with AZT+didanosine (ddI) than in the group treated with AZT alone; subgroup analyses of previously treated or untreated patients yielded similar findings. In a study of 1,418 previously untreated individuals in Western Europe, Australia, and New Zealand aged 15 years or more who were symptomatic or had CD4 counts under 350 cells/μL, adding ddI to AZT reduced the hazard of death (HR: 0.58; 95% CI: 0.45, 0.75) as well as the hazard of AIDS or death (HR: 0.64; 95% CI: 0.51, 0.79).[43] An analysis of 735 individuals in the United States aged 13 years or more with CD4 counts under 200 cells/μL suggested that the addition of ddI to AZT reduced the hazard of death (HR: 0.62; 95% CI: 0.35, 1.08) and the hazard of AIDS or death (HR: 0.57; 95% CI: 0.36, 0.90).[44] While no trials evaluated AZT against placebo in children, there was a pediatric trial to compare AZT to AZT+ddI, and the same trend as in adults was noted. In an analysis of 542 symptomatic or immunologically impaired children in the United States aged 18 years or less, more than 90% of whom were antiretroviral-naïve, adding ddI to AZT reduced the hazard of progression (defined,

for example, by weight-growth failure or development of at least two opportunistic infections) or death (HR: 0.61; 95% CI: 0.42, 0.88).[45] Another trial of 596 antiretroviral-inexperienced children in the United States aged less than 16 years evaluated ddI monotherapy against AZT+lamivudine (3TC), and found a lower hazard (HR: 0.37; 95% CI: 0.20, 0.67) of progression (death, new AIDS-defining illness, neurological decline, or weight-growth failure) and death (HR: 0.20; 95% CI; 0.06, 1.67) in the two-drug group.[46] Together, these trials demonstrated dual therapy as superior to monotherapy for slowing clinical progression and improving survival, and accordingly helped to usher in a new era of combination ART.

Finally, dual therapy was compared to triple therapy (i.e., HAART). In a trial of 151 antiretroviral-naïve, AIDS-free individuals in Europe, Canada, and Australia aged 18 years or more with CD4 counts between 200 and 600 cells/μL, the proportion of patients who died or experienced a disease progression event during the 52 weeks of follow-up was smaller in the group receiving AZT+ddI+nevirapine (NVP) than in the group receiving AZT+ddI (12% versus 25%; p=0.08 by two-sided Fisher's Exact Test).[47] Another trial assigned 1,313 individuals in the United States aged 13 years or more with CD4 counts below 50 cells/μL, 84% of whom were antiretroviral-experienced (generally with AZT monotherapy), to one of four treatment groups: AZT only, AZT+zalcitabine (ddC), AZT+ddI, or a HAART regimen of AZT+ddI+NVP.[48] The time to death (41% of participants died during the median 65 week follow-up period) as well as AIDS or death was longer in the HAART arm than in either the AZT or AZT+ddC arms ($p \leq 0.01$ for all four comparisons) – HAART also extended survival times relative to AZT+ddI, although the study was not powered to detect a statistical difference. ACTG 320, a 1997 trial

conducted among 1,156 AZT-experienced individuals in the United States aged 16 years or more with CD4 counts less than 200 cells/μL, demonstrated that compared to AZT+3TC, the addition of indinavir (IDV) reduced both the hazard of death (HR: 0.43; 95% CI: 0.19, 0.99) and the hazard of AIDS or death (HR: 0.50; 95% CI: 0.33, 0.76) by approximately 50% during the median 38 weeks of follow-up.[49] This study, one of several to provide empirical support of the superiority of HAART over dual therapy for delaying AIDS and death which resulted in a new triple therapy standard of care, was suspended like other prior studies because it was deemed unethical to provide anything less than HAART to particular participants. These ethical considerations precluded additional trials on the progression effects of HAART; the fact that it was so successful at preventing AIDS and death meant that future trials did not focus on this research question – instead, they explored other themes, like the comparison of established and novel regimens of at least three drugs in terms of their virological and immunological effects,[50,51] or the consequences of planned HAART interruption.[52,53]

There were subsequent investigations of the clinical progression and survival impacts of ART, though they were restricted to non-randomized designs. These studies can be broadly categorized into two groups – those that utilized sufficient epidemiological methods, and those that did not (for discussion of why observational investigation of the causal effects of treatment requires specialized methods, see the *Methodological context* section of Chapter III, Research Methods). Next, the 10 studies to use optimal analytical techniques, which included antiretroviral-experienced individuals unless otherwise noted, will be reviewed.

The earliest methodologically sophisticated study evaluated 2,178 antiretroviral-naïve men from the United States aged 18 years or more who were free of AIDS-defining illnesses, and found that AZT monotherapy reduced the hazard of death relative to no AZT (HR: 0.74; 95% CI: 0.57, 0.96).[54] The next study was similar except for its focus on HAART rather than AZT. For 1,498 AIDS-free individuals in the United States aged 13 years or more who were followed for a median of 5.4 years, HAART reduced the hazard of AIDS or death relative to no HAART (HR: 0.54; 95% CI: 0.38, 0.78).[55] An analysis of the same population using different but still novel methods yielded an AIDS-free survival time ratio of 2.5 for the comparison of HAART to no HAART,[56] a result consistent with the previously reported HR of 0.54. One study found that among women in the United States aged 13 years or more who had initiated HAART, the relative hazard of death attributable to HAART discontinuation was 1.97 (95% CI: 1.17, 3.31).[57] Another found that HAART, relative to no ART, reduced the hazard of AIDS or death (HR: 0.74; 95% CI: 0.49, 1.12) in 917 Spanish adults followed for an average 3.4 years.[58] The next, which analyzed 3,245 Swiss individuals aged 15 years or more, revealed a reduced hazard of death given HAART compared to no ART (HR: 0.14; 95% CI: 0.07, 0.29), and an attenuated but still strong effect compared to two-drug ART (HR: 0.49; 95% CI: 0.31, 0.79).[59] One analysis applied identical methods to 244 adults contemporaneously enrolled in two studies, and reached similar conclusions regarding the beneficial effect of HAART on survival, compared to less potent or no ART – the hazard ratios were 0.34 (95% CI: 0.15, 0.77) and 0.27 (95% CI: 0.11, 0.66).[60] A study published in late 2009 included nearly 63,000 antiretroviral-naïve individuals aged 18 years or more from 12 cohorts in

the United States and Europe, and found that HAART or less potent combination ART, relative to no ART, reduced the hazard of death (HR: 0.48; 95% CI: 0.41, 0.57).[61]

The final two studies are unique for different reasons. The first is unique in its inclusion of individuals who are both from a resource-deprived setting and antiretroviral-naïve. Among 14,267 South Africans aged 16 years or more, who were followed for a median of only four months (maximum 20 months), HAART reduced the hazard of death relative to no ART (HR: 0.14; 95% CI: 0.11, 0.18).[62] The second is unique given its focus on children. Among 1,236 perinatally-infected individuals in the United States aged 21 years or less, HAART reduced the hazard of death compared to less potent ART (HR: 0.36; 95% CI: 0.19, 0.66).[63] It is worth noting that when every one of the above datasets were instead analyzed using suboptimal methods, the revealed beneficial effect was either considerably less strong, or even harmful – this trend, at the very least, is highly suggestive of the inherent limitations of conventional analytic approaches.

The vast majority of observational studies assessing the clinical progression and survival effects of ART, ranging from AZT monotherapy to HAART, have utilized standard methods. An analysis of 493 patients from an earlier trial[39] who had begun AZT at various times following unblinding revealed that initiation of AZT greatly reduced the hazard of AIDS or death (HR: 0.17), but also that AZT offered no benefit after 2.1 years of treatment.[64] These results were consistent with a study of 4,484 European adults. A multivariable Cox proportional hazards model adjusting for four baseline characteristics as well as time-varying *Pneumocystis carinii* (*Pneumocystis jiroveci*) prophylaxis yielded a lower hazard of mortality given AZT monotherapy relative to no AZT during the first (HR: 0.45; 95% CI: 0.41, 0.50) and second (HR: 0.78; 95% CI: 0.70, 0.88) years, but a

higher hazard after two years (HR: 1.24; 95% CI: 1.05, 1.47).[65] In four other observational studies of adults,[66-69] AZT monotherapy, relative to no AZT, reduced the hazard of clinical progression and/or survival to a similar degree as noted in comparable trials. In two analyses of sizeable adult populations that adjusted for prognostic factors such as CD4 count using time-dependent Cox proportional hazards models, two-drug ART was noted to reduce mortality compared with AZT only – the hazard ratio was 0.55 (95% CI: 0.41, 0.74) for AZT plus either ddI or ddC versus AZT;[70] 0.79 (95% CI: 0.67, 0.93) for AZT+ddI versus AZT,[71] and 0.74 (95% CI: 0.59, 0.92) for AZT+ddC versus AZT.[71] A methodologically similar study of 398 adults in the United States aged 20 years or more found that HAART reduced mortality (HR: 0.44; 95% CI: 0.28, 0.68), although it is unstated whether the referent group was naïve to HAART or all antiretrovirals.[72] Among adult patients in Brazil, after adjusting for CD4 count, HAART was noted to extend survival relative to one- or two-drug ART.[73]

One compelling investigation was completed by Phillips et al.[74] In this study, data from three large cohorts were used to mimic prior trials that had influenced HIV ART standards, including three evaluating AZT monotherapy against two-drug ART[42-44] and two comparing two-drug ART to HAART.[49] In general, analysis of the cohort data with traditional observational methods yielded estimates close to those from the trials, but one cohort dataset yielded a harmful effect of HAART on AIDS or death (HR: 1.20; 95% CI: 1.01, 1.44) compared to a two-drug regimen, a result that starkly opposes the protective trial finding. This discrepancy, like the above noted trend of effect attenuation resulting from the application of suboptimal methods, reveals the potential for bias if observational data are not appropriately analyzed.

While trial data on the ART impacts of clinical progression and survival in pediatric populations are extremely limited, there are conventional observational studies of children in addition to the optimal one previously described.[63] In a study of 1,028 individuals in the United States aged less than 21 years who were followed for a maximum of four years, HAART reduced the hazard of death relative to less potent combination ART (HR: 0.33; 95% CI: 0.19, 0.58) according to a multivariable Cox proportional hazards model that accounted for baseline and time-varying factors[75] A similar study, which followed 1,142 Italian children for a median of 5.9 years, found that monotherapy (HR: 0.77; 95% CI: 0.55, 1.08), two-drug ART (HR: 0.70; 95% CI: 0.42, 1.17), and HAART (HR: 0.29; 95% CI: 0.13, 0.67) reduced the hazard of death compared with no ART.[76] In a study of 107 Spanish children followed for a median of 65 months which adjusted for only CD4 percentage and age at HAART initiation in addition to gender, it was noted that HAART reduced the hazard of death (HR: 0.03; 95% CI: 0.01, 0.14) relative no HAART, and that a lesser proportion of the group of children receiving HAART progressed to AIDS (26% versus 76%, $p<0.01$).[77]

One recent pediatric HIV research area that is distinct from other studies of HAART, yet has still tangentially supported its beneficial effects on clinical progression and survival, is the question of whether HAART should be initiated in early or late infancy. A randomized trial demonstrated that among asymptomatic South African infants with CD4 percentages above 25%, mortality was reduced by 76% in those who started HAART before 12 weeks of age compared with those who initiated it later.[78] This finding was echoed in an observational study of 210 European infants, which indicated

that deferring HAART increased the hazard of AIDS or death (HR: 3.0; 95% CI: 1.2, 7.9).[79]

Although ART has been consistently demonstrated to delay the clinical progression and improve the survival of HIV-infected individuals, knowledge on this relationship is not comprehensive. The aforementioned studies reveal various gaps, including a conspicuous scarcity of data on children – most research, whether prior to or during the HAART era, has been completed in adult populations in resource-privileged settings. Additionally, the stepwise historical progression from monotherapy to HAART has meant that placebo-controlled trials were limited to evaluation of a single-drug intervention, and that HAART has only been experimentally compared to less potent combination ART. In essence, there are no trials – which offer strong evidence, as "randomization is a powerful technique for ensuring valid causal inferences"[80] – that directly assess the clinical progression or survival impacts of HAART vis-à-vis no ART in any population, even though this is a substantively important contrast. Although the randomized design is no longer ethically plausible, it is still possible to quantify the causal effects of HAART in an epidemiologically sound fashion using observational data.[81] However, the prior observational studies quantifying how HAART affects survival that are most methodologically rigorous, and thus the source of the highest quality non-experimental evidence, have been generally limited to the same types of populations as earlier trials. Only one such study – one of just two to offer the contrast of HAART with no ART – was completed outside of North America or Europe, and it focused on adults.[62] To date, there is just a single observational study of the effects of HAART on pediatric survival that has used the methods necessary to yield an effect measure defendable as no

worse than minimally biased, and that study included children in the United States who were antiretroviral-experienced if not receiving HAART.[63] Other studies of children – all of which were completed in resource-privileged areas – used inadequate methods and hence provide information that, while potentially accurate, is inherently debatable in light of the possibility for bias previously noted.

Another shortcoming is that studies designed to quantify the clinical progression and survival effects of ART rarely report effects across strata of immunodeficiency, despite associations between CD4 levels, opportunistic infections, and mortality. As highlighted, trial populations are often restricted to specific, narrow scopes of CD4 values, such as under 200 cells/μL[44] or 200 to 500 cells/μL,[42] which prevents extrapolation beyond those ranges. While most studies that did not restrict based on CD4 criteria simply present no more than an overall estimate, two methodologically ideal studies of adults additionally reported differential effects by level of immunodeficiency. One study revealed that HAART, compared to less potent ART, reduced the hazard of AIDS or death to a greater degree given a lower CD4 count – the HR was 0.36 (95% CI: 0.20, 0.64) if the CD4 count was less than 200 cells/μL at baseline, 0.46 (95% CI: 0.27, 0.81) if the CD4 count was between 200 and 350 cells/μL, and 0.82 (95% CI: 0.54, 1.27) if the CD4 count was more than 350 cells/μL.[55] This trend was also evident in the South African study,[62] which showed that HAART less profoundly improved survival relative to no ART when the analysis was restricted to those with baseline CD4 counts above 200 cells/μL (HR: 0.33; 95% CI: 0.17, 0.64), as well as in the study of 63,000 adults,[61] which revealed a stepwise reduction in the effect of HAART with increasing initial CD4 counts.

No studies of the clinical progression or survival effects of HAART in children present results by baseline level of immunodeficiency.

The studies presented in this section collectively inform the hypothesis that HAART initiation, relative to non-initiation of HAART, will decrease the hazard of death.

Immunological Effects of Antiretroviral Therapy

The immunological effects of ART, like its effects on clinical progression and survival, have been extensively researched. This study's second specific aim, which focuses on the immunological effects of HAART, necessitates appraisal of existing knowledge on this association. Because the questions of how ART affects clinical and survival outcomes and immunological markers of HIV infection are conceptually and substantively related, they are oftentimes investigated concurrently, and the histories of research on these subject areas logically run in parallel. That is, trial evidence on the immunological effects of ART also reflected (and shaped) the movement from monotherapy to two-drug therapy to HAART, with later observational studies – some methodologically stronger than others – addressing the effects of ART on CD4 levels.

Most randomized trials of the clinical progression and survival effects of ART also examined immunological endpoints, including four previously described trials that evaluated AZT monotherapy against placebo. In two studies, AZT significantly increased CD4 counts relative to no AZT,[38,39] while in another, it reduced the hazard of a CD4 count decline to below 350 cells/μL by 40%.[40] In the third trial, AZT increased CD4 counts after six months among those with pre-randomization counts between 200 and 500

cells/μL (p=0.001), but not among those with a lesser degree of immunodeficiency (p=0.79).[41]

Immunological outcomes were also evaluated in five previously described trials evaluating two-drug ART against AZT monotherapy. The first stratified by prior ART – among those previously treated, there was an increase in mean CD4 count from baseline over the first 32 weeks in the AZT+ddI and AZT+ddC groups, but a decrease in the group receiving AZT only; over longer follow-up, there was a decrease from baseline in all groups, but a larger decrease in the group receiving AZT only.[42] Among those previously untreated, individuals in the two-drug groups had a mean increase in CD4 count from baseline at most follow-up points, while those in the AZT group had a mean decrease at most follow-up points. An analogous pattern was noted in a separate but similarly designed trial.[43] Another trial did not stratify by previous ART, but showed mean CD4 count increases at two months in the AZT+ddI (19.2 cells/μL) and AZT+ddC (12.9 cells/μL) groups but a decrease (-4.0 cells/μL) in the AZT only group.[44] A trial in children demonstrated that at four weeks the mean CD4 percentage increased from baseline in the AZT+ddI group but not in the AZT only group (p<0.001); at 96 weeks, the AZT+ddI group had a 76% higher mean CD4 percentage than the AZT only group (p<0.001).[45] Another pediatric trial noted that the mean CD4 count increase (72.9 cells/μL) at weeks 36 to 48 increased more (p=0.01) given AZT+3TC than ddI only (3.6 cells/μL).[46] Other trials contrasting two-drug regimens with monotherapy examined immunological outcomes, including two that compared AZT only to AZT+3TC. In a study of 129 antiretroviral-naïve Europeans aged 18 years or more, the mean CD4 count increase after 24 weeks in the AZT+3TC group (80 cells/μL) was greater (p<0.001) than

that in the AZT only group (20 cells/μL).[82] In a study of 366 North American adults, there was a greater 24 week increase in mean CD4 percentage (p<0.001) and count in the AZT+3TC group (p=.002 and 0.015 for low- and high-dose 3TC, respectively) than in the AZT only group, and these differences were durable over 52 weeks of follow-up.[83]

HAART was shown to improve CD4 counts relative to two-drug ART in three previously cited trials. In the first, the mean increase in CD4 count after one year of follow-up was 52 cells/μL greater in the AZT+ddI+NVP group than in the AZT+ddI group.[47] In the second, HAART improved the CD4 count to a greater degree after 16 weeks than alternating monotherapy or either two-drug regimen (all p<0.001).[48] And in the third, in strata of patients with severe (CD4 count less than 51 cells/μL) or moderate (CD4 count 51-200 cells/μL) immunodeficiency at ART initiation, two-drug ART led to a greater increase in CD4 counts at four, eight, 24, and 40 weeks than did AZT+3TC+IDV.[49]

While there is limited trial evidence on the clinical progression and survival effects of HAART, there is plentiful such data on the immunological effects of HAART. Among 320 adults from the United States, Europe, and Australia with CD4 counts below 50 cells/μL who had previously received AZT monotherapy, those randomized to HAART had a greater mean CD4 count increase (p<0.001) at 24 weeks (95 cells/μL) than those receiving AZT+3TC (6 cells/μL).[84] In 97 patients who were similar but less immunodeficient (CD4 count between 50 and 400 cells/μL), the same conclusion was reached.[85] One research group examined two different HAART regimens in antiretroviral-naïve adults from Europe, Australia, and Canada. In a study of 103 individuals with CD4 counts between 150 and 500 cells/μL, there was a greater mean

increase in CD4 count at 52 weeks in the AZT+3TC+IDV group than in the AZT+3TC group (p=0.01).[86] In a trial of 105 individuals, those receiving AZT+3TC+nelfinavir had a greater mean CD4 count increase (p=0.027) at 28 weeks (101.5 cells/μL) than those receiving AZT+3TC (47 cells/μL).[87] In a trial of 297 children aged less than 18 years in the United States who were antiretroviral-experienced but naïve to both AZT and 3TC, those randomized to AZT+3TC+ritonavir (RTV) had a higher median CD4 percentage at 48 weeks (p<0.01) than those randomized to RTV+stavudine (d4T).[88] In a study of 162 European and Brazilian children less than 17 years of age, adding 3TC to AZT+ddI, AZT+ddC, AZT only, or ddI only resulted in a mean CD4 count that was 48 cells/μL higher at 24 weeks (p=0.03).[89]

Observational studies of the immunological response to ART, like those that quantify the effects of ART on clinical progression and survival, can also be categorized into two groups of methodological sophistication. However, while there are observational studies using standard methods to evaluate the effects of ART vis-à-vis less potent or no ART on clinical progression and survival, typical observational studies of immunological outcomes do not directly assess therapy against those referents. Rather, the norm is simply to observe changes in CD4 levels within a population that is uniformly receiving ART. These studies, whether conducted among adults or children, have provided extensive evidence for the ART-mediated improvement of immunological competency. For example, a study of 861 antiretroviral-naïve Spanish adults starting HAART showed an increase (p<0.001) in median CD4 count from 214 cells/μL to 499 cells/μL over a median of 173 weeks;[90] among 57 children in the United States aged less than 16 years who were antiretroviral-experienced but HAART-naïve, initiation of HAART increased

the median CD4 count by 3% over 48 weeks.[91] The paradigm of examining treated patients exclusively means that beyond the above cited randomized trials, the only other studies to quantify the immunological response to ART (versus less potent or no ART) were a limited number of observational investigations that employed adequate methods.

To date, just five such studies have been completed. The first analyzed a population of 1,486 antiretroviral-naive adults in the United States to estimate that AZT monotherapy increased the CD4 count by 5.4 cells/μL (95% CI: -1.8, 12.7) every six months, compared to no AZT.[92] The next included 1,763 men, 48% of whom were antiretroviral-experienced but HAART-naïve, from the same cohort.[93] It was estimated that HAART relative to no HAART increased the mean CD4 count by 71 cells/μL in the first year and 29 cells/μL per year thereafter, with a greater first year gain in those with the lowest baseline CD4 counts. A similar study of 867 women showed not only that HAART improved the CD4 count at two years post-initiation, but also that the effect was stronger among those with more severe initial immunodeficiency.[94] A study of South African adults,[62] already described as it also examined survival, found that each month of HAART increased the CD4 count by 15.1 cells/μL (95% CI: 14.7, 15.5) over the first 19 months, a result that was similar across strata of baseline immunodeficiency.[62] The only analysis in children,[95] a companion study to that which assessed the effect of HAART on survival in exactly the same population,[63] found that HAART increased the mean CD4 percentage by 2.34% (95% CI: 1.35%, 3.33%) over the first year relative to no HAART, that increases were sustained over five years, and that responses were most robust among those with the most profound immunodeficiency at baseline.[95] As before, these studies all noted attenuated effects when traditional methods were applied to the same data,

stressing the importance of appropriate analysis for any quantification of the immunological response to ART.

Considering that the clinical progression, survival, and immunological effects of ART are biologically associated, and that investigations of these outcomes use related techniques and often identical study populations, it is unsurprising that the collective bodies of literature on these topics share many of the same shortcomings and limitations. The previously described gaps which characterize existing studies of the clinical progression and survival effects of ART – for example, a lack of methodologically strong observational studies comparing HAART to no ART in children, whether overall or within levels of immunodeficiency, and no studies of children in resource-deprived settings – are also evident in the scientific literature on the immunological effects of ART. These mutual deficiencies provide a common direction for future research into these substantially overlapping subject areas.

The studies presented in this section collectively inform the hypothesis that HAART initiation, relative to HAART non-initiation, will increase the mean CD4 percentage at all time points.

Immunodeficiency at Antiretroviral Therapy Initiation: Clinical, Survival and Immunological Impacts

While there is much known about the impact of immunodeficiency at time of ART initiation on clinical and immunological outcomes, the debate on when HAART should be started is not yet resolved, meaning that more information on these associations is needed. As the second specific aim of this study focuses on how the

immunological effects of HAART are influenced by category of CD4 percentage at baseline, with the third specific aim addressing whether the degree of immunodeficiency at HAART initiation is associated with recovery to "not significant" WHO age-specific immunodeficiency (see Table 2 for definition), it is pertinent to discuss the scope and implications of existing knowledge. Although this research does not examine the relationship between immunodeficiency and clinical progression or survival, this topic is included in the literature review for the sake of completeness and parallelism, and also because it remains only partially understood.

In adults, greater immunodeficiency at the start of HAART has been linked to an increased likelihood of clinical progression and mortality. Progressively lower CD4 counts were associated with reduced survival in four previously described studies, each of which contends that their results support not delaying HAART until the onset of severe immunodeficiency.[72,90,96,97] An analysis explicitly completed to provide information on when HAART should be started in resource-deprived settings demonstrated that Thai adults with a baseline CD4 count less than 200 cells/μL had an elevated hazard of AIDS or death during the median 62 months of follow-up compared with those with a baseline CD4 count greater than 350 cells/μL (HR: 3.67; 95% CI: 1.31, 10.27).[98] Fewer CD4 cells at HAART initiation has also been associated with reduced survival in a study of adults in South Africa;[99] analyses of adults in the United States[100] and more than 20,000 adults from 12 cohorts in Europe and North America[101] revealed that more severe immunodeficiency at the start of HAART was associated with a higher hazard of AIDS or death.

Findings in children – mostly from sub-Saharan Africa, as there are few children starting HAART in the developing world – are generally concordant with those in adults, although there is sparser evidence, and some is conflicting. The previously described Zambian study[102] revealed that the hazard of death was approximately doubled if children started HAART with a CD4 percentage under 20% rather than above 20%, while the study of children in Haiti[103] showed that the hazard of death was higher in those with an initial CD4 percentage under 5% than in those with higher percentages (HR: 1.78; 95% CI: 1.38, 2.83). A study in *Côte d'Ivoire* also noted the danger of profound immunodeficiency, as the probability of survival 24 months after HAART initiation was 72.8% given an initial CD4 percentage under 5% versus 97.8% otherwise.[104] A pooled analysis of 2,405 children from 16 sub-Saharan African cohorts showed that initial World Health Organization (WHO) age-specific severe immunodeficiency,[105] compared to lesser or no immunodeficiency, was independently associated with death over the median 20.3 months of follow-up (HR: 2.57; 95% CI: 1.29, 5.12).[106] However, results from several studies indicate that immunodeficiency at the start of treatment does not affect survival. The probability of survival at one and two years did not differ across strata of baseline immunodeficiency in a study of 586 children less than five years of age from 14 sub-Saharan African countries,[107] and an initial CD4 percentage under 5% was not associated with death among 151 South African children less than 16 years of age who were followed for a median of eight months.[108]

It makes intuitive sense that the degree of immunodeficiency at the start of ART would influence eventual immunological outcomes. Insight on this association also contributes to the discussion of when HAART should be started, and it has been directly

examined, though there are relatively few studies with extended follow-up, particularly from resource-deprived settings or amongst children. One previously cited study of adults showed CD4 count gains, which leveled off after approximately three years, in each of four strata of baseline immunodeficiency.[90] However, if the initial CD4 count was between 200 and 350 cells/μL, the likelihood of eventual immunological recovery (i.e., over 500 cells/μL) was reduced, with the chance of recovery even less if baseline immunodeficiency was more profound. In Australian adults, while the same relationship between initial CD4 count and immunological recovery at two years was noted, CD4 count gains waned about two years after HAART initiation,[109] the same point at which immune reconstitution slowed in 237 adults from the United Kingdom.[110] Studies in the United States[111] and the Netherlands,[112] both of which conclude that HAART should be initiated earlier, yielded consistent results – less robust immunological recovery if the baseline CD4 count was less than 350 cells/μL, with no additional CD4 count gains after three to four years. One recent analysis, novel given its examination of long term CD4 count trajectories among patients from resource-deprived areas, included 19,967 adults from 27 cohorts in sub-Saharan Africa, Latin America, and Asia and found that immunological recovery plateaued at roughly two years regardless of initial immunodeficiency. The authors of this study state that "baseline CD4 cell count was the most important determinant of subsequent CD4 cell count trajectories" and strongly contend that their data support starting HAART at higher CD4 counts.[113]

Of the limited number of investigations of pediatric populations, only a minority examined long term immunological responses, with none of those studies completed in resource-deprived settings. A study of 1,012 children in the United States who were

antiretroviral-experienced, less than 18 years of age, and followed for up to three years revealed that those who started HAART with a CD4 percentage under 25%, while exhibiting gains that occurred over two years, failed to recover to the 25% level.[114] Another study in the United States had a median follow-up of 39 months and also showed that those with a baseline CD4 percentage under 25% increased but did not recover to 25%, but it included a total of only 85 children and did not describe when CD4 gains ceased.[115] In 177 children from Ireland and the United Kingdom who were antiretroviral-naïve when starting HAART, each additional 5% in initial CD4 percentage raised the odds of attaining a CD4 percentage of 30% at six months (odds ratio [OR]: 2.60; 95% CI: 1.76, 3.84); trends were apparently similar at 12 months although data were not presented.[116] A study of 71 Dutch children presented a conflicting result – successful immune reconstitution independent of initial CD4 percentage – although few children were followed for the entire 96 week period and the distribution of baseline immunodeficiency was not presented.[117]

Two previously cited studies examined immunological responses in children. The first, unique in its sub-Saharan African setting but limited in its duration of follow-up, noted that the probability of attaining a CD4 percentage of 25% one year after HAART initiation decreased with increasing baseline immunodeficiency.[107] The second, unique in its long term assessment of outcomes, revealed that CD4 levels among children with immunodeficiency at baseline improved for two years after the start of HAART before stabilizing during the subsequent three years.[95] It also showed while those with an initial CD4 percentage between 15% and 25% were able to recover to 25%, such recovery did not occur among those with an initial CD4 percentage under 15% even after five years, a

finding that "supports the initiation of HAART in children before severe immunosuppression occurs to maintain normal CD4 cell percentages."

It has been stated that understanding the associations between baseline immunodeficiency and eventual clinical and immunological outcomes would help to inform when HAART should be initiated, a claim that is substantiated by the authors of essentially every one of the preceding studies asserting that their respective findings should contribute to the ongoing debate. However, even those most ardently advocating for earlier initiation do not contend that results from these studies can alone impart change, not only because of the inherent potential biases of observational data such as confounding, but also because they do not precisely answer the question of when HAART should be started. That question is best addressed by a randomized trial comparing outcomes of individuals initiating HAART in a particular immunological range (e.g., CD4 count between 350 and 500 cells/μL) to those of individuals deferring HAART until advanced immunodeficiency. While such trials have not yet been completed,[118] several recent studies have used observational data in concert with sophisticated methods that account for lead time bias and informative censoring in order to mimic trials assessing immediate versus deferred HAART.[119,120] This work – along with a post-hoc analysis which allowed for a randomized contrast of HAART initiation above 350 CD4 cells/μL to below 250 CD4 cells/μL[121] – has provided compelling evidence, in addition to that presented above, that HAART should be started in adults before the CD4 count falls below 350 cells/μL, as is currently recommended by WHO.[122] So, although there is increasing clarity on this issue in adults, the question remains far from resolved in children.[123]

The studies presented in this section collectively inform the hypotheses that children with greater immunodeficiency at HAART initiation will be less likely to recover immunologically, and that higher CD4 percentages at baseline will be associated with a reduced increase in CD4 percentage at all time points. The latter hypothesis is also informed by previously cited studies of the immunological effects of HAART in adults which examined the impacts of baseline CD4 levels.[55,61,62] These hypotheses are also influenced by fact that CD4 levels at adjacent time points are logically connected – that is, it makes innate sense that those with lower baseline CD4 percentages will have lower CD4 percentages post-HAART, and that there is a narrower window for HAART-mediated immunological improvement when CD4 percentages at baseline are higher.

Summary and Rationale

There is a general knowledge gap on the effects of ART on survival and immunological and clinical progression outcomes in HIV-infected children, with even less known among children in resource-deprived settings. Although research questions related to pediatric ART outcomes that are no longer feasibly answerable via randomized trial can now be addressed using observational data with specialized analytical methods, only two studies to date have utilized such an approach. The simple fact that the effects of HAART on survival and CD4 percentage, relative to no ART, have not been robustly quantified among children in a resource-deprived setting is alone sufficient to justify further research. Add the reality that questions remain about whether immunological responses to HAART are affected by baseline CD4 percentage, and there is ample opportunity and motivation for additional investigation.

It has been suggested that resource-deprived settings are typified by a distinct contextual milieu, but this theme has been explored in little detail. An article by De Baets et al.[124] provides detailed description of this context and underscores realities commonly faced by pediatric HIV programs in Africa. These include challenges at the clinic level, such as limited diagnostic capacity, fewer drug options, dilapidated facilities, and suboptimal training, as well as challenges related to the patient and their environment, including delayed care seeking, concurrent comorbidities and undernutrition, and frequent loss to follow-up due to various poverty-related factors. Callens et al. highlight issues specific to the DRC, including food insecurity and inadequate health infrastructures.[125] These factors collectively raise the possibility that the effects of HAART might not be heterogeneous in resource-deprived and -privileged settings; in fact, this has been observed in a meta-analysis of adults.[126] The Antiretroviral Therapy in Lower Income Countries Collaboration points out that mortality after the initiation of HAART in low- and middle-income countries is higher than in high-income countries; UNAIDS speculates that is likely due to more advanced HIV upon presentation to care and a high incidence of co-occurring conditions.[2] This example illustrates the danger of assuming equivalent effects of HAART in all children, and reveals the need for this research area to be explored in a resource-deprived setting.

The impetus for this study is also built on the contention that additional specific knowledge in children is needed. Simply stated, HIV infection is not the same in children and adults. Following infection, because of deleterious effects of HIV on the immature thymus[127] and heavy replication within the expanding lymphoid cell mass,[128] children typically attain a higher peak viral load and correspondingly take longer to reach a

virological steady state than adults.[129] However, although HIV can damage the thymus, children experience enhanced immunological recovery during HAART because the thymus is more active during childhood.[130] This fact likely contributes to the results of a study which examined immunological responses of nearly 50,000 antiretroviral-naïve Europeans initiating HAART – after adjustment for potentially prognostic factors, individuals six to 29 years of age had a higher probability of response than those aged 30 to 39 years, while individuals more than 60 years of age had a lower probability of response.[131] These biological differences compel a need for research in children, just as profound differences in programmatic contexts compel a need for research in resource-deprived settings. It cannot be assumed that observations in adults are applicable in younger populations, a sentiment perhaps best expressed in a study by the Pediatric ACTG group: "disease progression manifests itself differently in children… this often makes it inappropriate to extrapolate findings in adult studies to children."[24]

This work was completed to mitigate the incomplete understanding of the effects of HAART on survival and CD4 percentage in HIV-infected children, and to additionally inform the debate on when HAART should be initiated in terms of immunological status. It features sufficiently long follow-up and provides estimates of the effects of HAART to a lack thereof, a contrast that is relevant and prognostic because the majority of patients initiating HAART globally are antiretroviral-naïve, and unique because very few past studies in adults, and none in children, have presented that comparison. One study expressed that when data from randomized trials are not available, "observational data are often the best available evidence for assessment of

therapeutic effects"[55] – in that spirit, with the tools necessary for proper analysis of the longitudinal observational data at hand, this research was pursued.

III.

RESEARCH METHODS

Study Design and Manuscripts

The study's aims – including estimation of the effects of HAART on survival and CD4 percentage in HIV-infected children – were pursued via analyses of longitudinal, observational, clinical cohort data from an ongoing comprehensive HIV care and treatment program in Kinshasa, DRC. This study produced two manuscripts. The first, entitled "The Effect of Highly Active Antiretroviral Therapy on the Survival of HIV-Infected Children in a Resource-Deprived Setting: A Cohort Study" addresses Specific Aim 1. The second, entitled "Quantification of CD4 Responses to Highly Active Antiretroviral Therapy Among HIV-Infected Children in Kinshasa, Democratic Republic of Congo" addresses Specific Aims 2 and 3.

Study Context

As this study is ancillary to an existing program, context can be gleaned by examining the specifics of that initiative. Since 2003, with funding from the President's Emergency Fund for AIDS Relief (PEPFAR) and the CDC Global AIDS Program, the University of North Carolina at Chapel Hill (UNC) has worked to improve HIV prevention and care in Kinshasa in collaboration with various international, national, and local partners including the Kinshasa School of Public Health, the National AIDS Control

Program, the Elizabeth Glaser Pediatric AIDS Foundation, the Belgian Development Cooperation, the Salvation Army, the William J. Clinton Foundation, the United Nations Children's Fund, and the Global Fund to Fight AIDS, Tuberculosis, and Malaria. A cornerstone of that effort, and the source of data for this analysis, is an evidence-based, family-centered program which provides primary care, prophylaxis and treatment for opportunistic infections, and HAART (if indicated) to HIV-infected children and their first-line family members, pregnant and post-partum women and their HIV-exposed infants, and tuberculosis co-infected HIV patients at Kalembe Lembe Pediatric Hospital and Bomoi Healthcare Center.

The program was implemented with various programmatic and research objectives. The programmatic objectives include evaluating the feasibility of comprehensive HIV care and treatment in Kinshasa, developing a sustainable and reproducible model for resource-deprived settings, providing community-based psychosocial support, sexual and reproductive health services, and nutritional counseling and support for participants and their families, and increasing local expertise of primary HIV care, specifically opportunistic infection and HAART management. The research objectives include estimating the incidence of antiretroviral toxicities and immune reconstitution syndromes, determining post-HAART changes in anthropometric parameters, observing adherence to HAART, and assessing the safety and utility of simplified laboratory monitoring. One research goal, to examine the clinical and immunological progression of individuals receiving HAART, is directly addressed by this study.

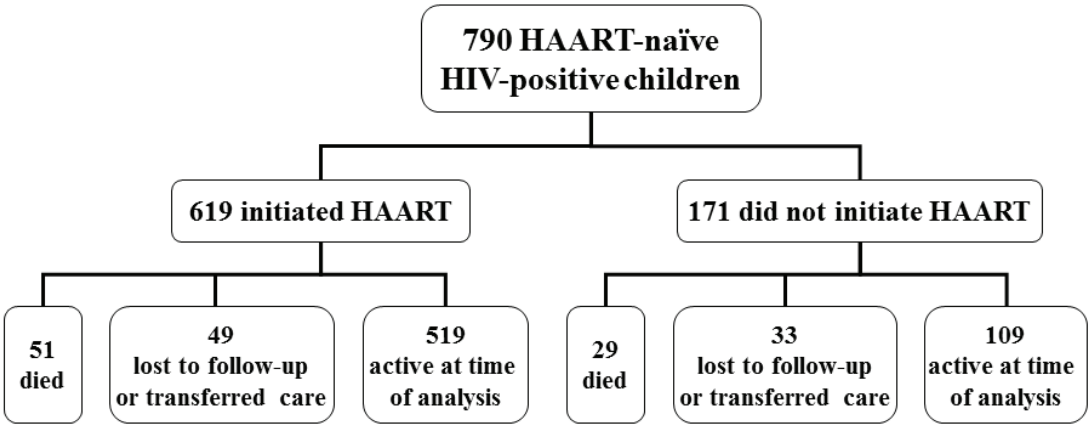
Study Population

As the program is ongoing, to maximize the person-time and the number of children included in the analysis, datasets were finalized as late as possible. Patients enrolled through May 2010 were considered, with follow-up continuing through no later than August 2010. Between November 30, 2004, when the program began, and May 31, 2010, a total of 3,445 individuals enrolled for care, 1,927 (55.9%) of whom were children (henceforth, "children" refers to those who were less than 18 years of age at the time of enrollment). Of the 1,360 individuals who initiated HAART by May 31, 2010, 770 (56.6%, a figure nearly identical to the pediatric proportion of enrollees) were children. As of May 31, 2010, there were 2,303 active participants; 1,085 (47.1%) were children and 1,128 (49.0%) were receiving HAART. There were 600 children receiving HAART, meaning that 53.2% of those receiving HAART were children and 55.3% of children were receiving HAART. A total of 3,064.0 person-years had been accrued by children by May 31, 2010, with 1,793.6 (58.5%) of those person-years accrued during receipt of HAART.

Because the focus of Specific Aims 1 and 2 was to estimate the effects of HAART relative to no HAART, it was necessary to exclude from the study population any children who were receiving HAART at time of program enrollment. To ensure that effect estimates represented those from an antiretroviral-naïve population, any children who were not receiving HAART at time of enrollment, but had received any ART previously, were also excluded. These criteria reduced the population negligibly. Also excluded were all children for whom documentation of confirmed HIV infection, by serology at age greater than 18 months or by viral load or DNA PCR, was lacking. This

criterion did result in the exclusion of a significant proportion of the 1,927 total enrolled children, not because of poor documentation, but because the program provides care to many HIV-exposed infants who are ultimately determined to be HIV-uninfected, at which time they are immediately deactivated. As in prior studies,[55,63,93,95,132] complete baseline confounder data was required, and baseline could be shifted to the first visit with complete data. The only descriptor with non-negligible incomplete data at baseline was CD4 percentage; baseline was shifted in just 5.2% of children. Allowing follow-up to start at first CD4 rather than just at enrollment resulted in the inclusion of individuals that would have been otherwise excluded due to a single missing laboratory result. The final study population, once all exclusion criteria were applied, was comprised of 790 children who contributed a total of 2,089.8 person-years. HAART was started by 619 children (78.4%); children were receiving HAART during 1620.9 (77.6%) of the person-years. Eighty children (10.1%) died, 76 (9.6%) were lost to follow-up, 6 (0.8%) transferred care to another facility, and 628 (79.5%) were active at time of analysis.

Figure 1. Flowchart of HAART and final dispositions (loss to follow-up, death, transfer of care to another facility, or active at time of analysis) for study population.



In the second manuscript, the Specific Aim 3 analysis was restricted to subset of the total population who initiated HAART and did so when immunodeficiency was mild, advanced, or severe. Similar to a prior study that used data from this cohort in which just 12.2% of children who started HAART had "not significant" immunodeficiency,[133] only 13.4% of the 536 HAART initiators had "not significant" immunodeficiency.

Patient Follow-up and Data Collection

At each HIV care visit, participants were evaluated and demographic, clinical, and psychosocial data were collected as specified in the program protocol. According to this protocol, the initial decision whether HAART should be initiated was made one week following enrollment based on physician assessment and WHO[105,134] and DRC[135] antiretroviral guidelines; if HAART was deferred, it could have been started at any subsequent visit if clinically indicated. The standard prescribed regimen was AZT or d4T, 3TC, and NVP or efavirenz. If immunological treatment failure, toxicities, or potential drug interactions arose, patients were switched to alternative regimens. In analyses, patients switched to alternative regimens were still considered to be receiving HAART. Once initiated, HAART was never discontinued. Those receiving HAART were scheduled to visit monthly, while those not receiving HAART were scheduled to visit quarterly. Individuals needing acute medical care could also make unscheduled visits. Patients were lost to follow-up if they withdrew from care or could not be located by three tracking attempts following a missed visit. Specimens for CD4 count and percentage, assayed at the DRC National AIDS Reference Laboratory, were obtained at program enrollment and biannually thereafter.

Parental informed consent was obtained for all children; children at least 12 years age also provided their assent. In the consent forms, it was explicitly stated that collected data could be used for research. Data were collected using standardized paper forms and entered into a password-protected Microsoft Access database, which was securely transmitted on a monthly basis from Kinshasa to UNC. Routine and ad hoc procedures to identify and correct logically or biologically implausible values ensured a high degree of data quality prior to time of analysis.

Methodological Context

In the *Clinical Progression and Survival Effects of Antiretroviral Therapy* section, it is asserted that observational investigation of the causal effects of treatment is strengthened by specialized epidemiological methods, but no details are offered. The purpose of this section is to illustrate the reasons why that statement was made, as well as to provide the specifics of those methods, which figure prominently in the execution of the study.

Observational data can present a number of analytical challenges. First, because exposure in a non-experimental study of treatment is not randomized like in a clinical trial, confounding by indication both at baseline and throughout the course of follow-up may result in substantial biases. Additionally, if the data are longitudinal, another scenario may further complicate estimation of an unbiased causal effect of an exposure on an outcome. This occurs when a particular factor is a common cause (confounder) of the exposure (at time t) and the outcome, while also a causal intermediate between the exposure (at times prior to t) and the outcome.

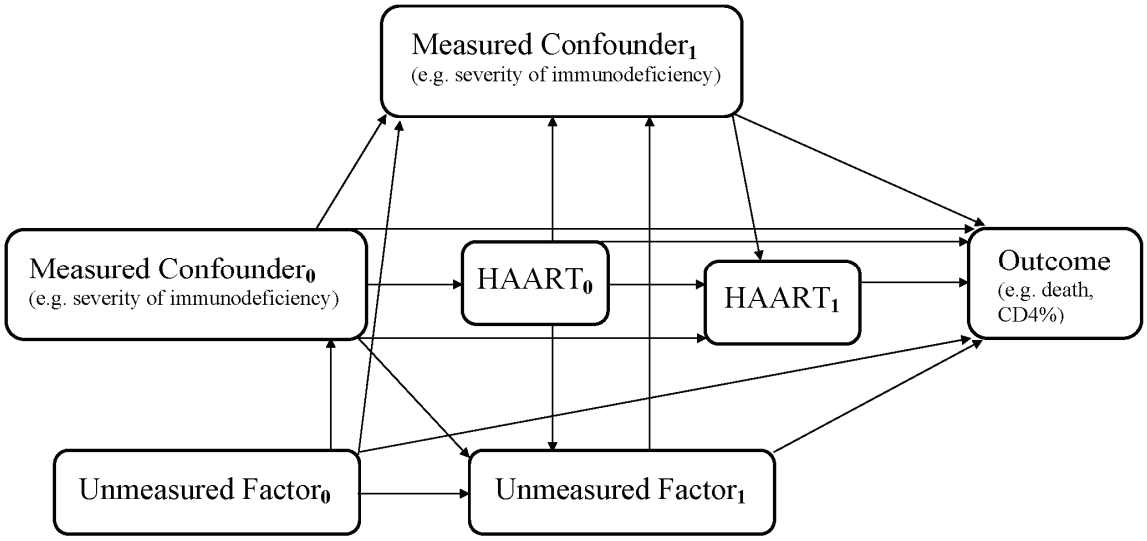
It has been demonstrated in the presence of time-dependent exposures and factors that are simultaneously confounders and causal intermediates (hereafter referred to as time-dependent confounders affected by exposure), standard epidemiological methods for the estimation of causal effects are rendered inadequate.[81,136] Several novel techniques to overcome this situation have been developed, including G-estimation of structural nested models[137,138] and marginal structural models (MSMs).[81] The most commonly applied approach is MSMs, as evidenced by their utilization in nearly all of the previously cited "methodologically sophisticated" studies.[54-63,92-95] The parameters of an MSM – whether estimated via modification of linear, logistic, Cox proportional hazards, or other models – can be consistently obtained using estimators based on inverse-probability-of-treatment-and-censoring (IPTC) weighting.

Weighting is the underlying reason why the measure of effect produced by an MSM is causal rather than associational. While an IPTC-weighted model to estimate the parameters of an MSM is similar to other models in that it uses observed data to predict an outcome as a function of exposure and covariates, it is unlike other models in that it weights observations by the inverse of a joint probability of treatment and censoring that is unique for each individual at each point of follow-up. Application of IPTC weighting effectively removes any association of time-dependent confounders with subsequent exposure, thus creating a "pseudopopulation" unaffected by this phenomenon. Given that weighting does not modify the original causal association between exposure and outcome, the association between exposure and outcome in the "pseudopopulation," assuming adjustment for baseline confounders, is therefore equivalent to the causal association between exposure and outcome in the original population.

Under the assumptions of consistency, positivity, and no model misspecification, unmeasured confounding, or informative censoring – most of which are empirically unverifiable – the measure of effect obtained from an MSM is causal.[81,139] The exact interpretation depends on the type of model. For example, the effect measure from a Cox proportional hazards MSM is the log hazard ratio comparing the hazard of the outcome had all individuals been continuously treated since baseline to the hazard had they never been treated during follow-up, while that from a linear MSM represents the mean change in the outcome had all individuals been continuously treated since baseline compared to that had they never been treated during follow-up. Though derived from the observed outcomes of individuals experiencing exposed and unexposed person-time, contrasts from MSMs (while causal) are ultimately hypothetical given that IPTC weighting transforms an associational model into a model for counterfactual outcomes in the source population.

This study's first specific aim was to estimate the causal effect of HAART on survival, and its second specific aim was to estimate the causal effect of HAART on mean change in CD4 percentage. The following directed acyclic graph (DAG)[140] – a causal diagram that depicts assumed relationships between an exposure, an outcome, and other related factors – is illustrative for both aims. It shows that there is a time-dependent exposure (HAART), and reveals the structural contribution of a time-dependent confounder affected by exposure, several of which are believed to figure into the associations between HAART and study outcomes. Thus, in order to best elucidate the causal effect of HAART on 1) survival, and 2) CD4 percentage change, this analysis used weighted regression models to estimate the parameters of MSMs.

Figure 2. Directed acyclic graph representing the causal effect of time-dependent HAART on study outcomes.



Numeric subscripts 0 and 1 denote the enrollment visit and subsequent visits, respectively. Measured time-dependent confounders affected by exposure and unmeasured causal risk factors (i.e., non-confounders) for the study outcomes are shown. For clarity, factors that are baseline confounders (i.e., not time-dependent confounders affected by exposure) are not shown.

Variables

Table 2. Variables included in analyses.

Variable	Function	Specification	Notes
HAART	Exposure (aims 1,2)	Binary (aim 1) Categorical (aim 2)	As shown in Figure 1, HAART is time-dependent. When binary, as an exposure in aim 1 or as a predictor in models for weight numerators and denominators (see *Analysis – Specific Aim 1*), this variable toggled from "0" to "1" if a child initiated HAART. As an exposure for aim 2, this variable represented cumulative HAART exposure and was coded into indicators for six month categories (no HAART, >0 to 6 months HAART, 6 to 12 months HAART, and so on up to a maximum of 66 months).
Death	Outcome (aim 1)	Binary	This variable toggled from "0" to "1" if a child died.
CD4 percentage	Outcome (aim 2) Covariate (aims 1,2) Stratification (aim 2)	Continuous Cubic spline	As an outcome, this variable was specified continuously as it was in related studies.[62,92-95] As a time-dependent confounder affected by exposure, it was modeled as a cubic spline.[62,92,93,95] It was grouped into three categories (<15%, 15%–24%, ≥ 25%) in stratified analyses for aim 2.
WHO HIV clinical stage	Covariate (aims 1,2)	Categorical (4 level)	Measured only at enrollment, this variable was a baseline confounder and defined as in WHO guidelines.[105,134] Categories were asymptomatic, mild, advanced, and severe.
WHO age-specific severity of immunodeficiency	Covariate (aims 1,2) Exposure (aim 3) Outcome (aim 3)	Categorical (4 level)	Derived from age and immunological criteria defined as in WHO guidelines.[105] Categories were not significant, mild, advanced, and severe. The definition of "not significant" is outlined in detail in the table footnotes.[a] This variable was a baseline confounder for aims 1 and 2, and an exposure and outcome for aim 3.
Gender	Covariate (aims 1,2)	Binary	While it is arguable that this variable may not fit the conceptual definition of a confounder, it nonetheless was included based on precedent in prior related studies.[59,61,62,93,95]

Variable	Function	Specification	Notes
Age	Covariate (aims 1,2)	Cubic spline	This variable, a baseline confounder, was specified as in a related study.[62]
HIV-related symptom or condition	Covariate (aims 1,2)	Binary	A time-dependent confounder affected by exposure, this variable was coded as "1" if a child was diagnosed with at least one of a list of HIV-symptoms or conditions (see *Chapters IV and V*) and "0" otherwise.
Cotrimoxazole	Covariate (aims 1,2)	Binary	A time-dependent confounder affected by exposure, this variable was coded as "1" if a child was receiving cotrimoxazole and "0" otherwise.

[a] Immunodeficiency is categorized as "not significant" if the CD4 percentage is >35 at age <12 months, >30 at age ≥ 12 to <36 months, >25 at age ≥ 36 to <60 months, or if the CD4 count is >500 cells/mm^3 at age ≥ 60 months.

Analysis – Specific Aim 1

The study's first specific aim, to estimate the causal effect of HAART on survival, was pursued via Cox proportional hazards MSMs. Because most software packages cannot accommodate subject-specific time-varying weights in the fitting of Cox models, parameters of Cox proportional hazards MSMs are typically approximated via weighted pooled logistic regression.[54,55,58-63,133] The parameters of a Cox model, whether the model is weighted or not, are well approximated by pooled logistic regression when the hazard of the outcome per person-time interval is small,[141] as is the case in this study.

Let $D(t)=1$ if an individual experienced the outcome between visit (t-1) and visit t and $D(t)=0$ otherwise. Let the $A(t)$ represent an individual's HAART status at visit t; let $a(t)=1$ if an individual initiated HAART at or before visit t and $a(t)=0$ otherwise. Let $\bar{A}(t)$ represent an individual's HAART history up to visit t; let $\bar{a}(t)$ be HAART history up to visit t. Let $L(0)$ represent the vector of baseline confounders; let $l(0)$ be the vector of baseline confounders observed. Let $L(t)$ represent the vector of time-dependent confounders affected by exposure; let $l(t)$ be this vector observed at visit t. Let $C(t)=1$ if an individual is censored between visit (t-1) and visit t, and $C(t)=0$ otherwise, where an individual is censored if they are lost to follow-up or have not experienced the outcome at the end of study. k denotes days since the start of follow-up.

Using weighted pooled logistic regression – where individuals' contributions to the risk set at each visit t are weighted by the subject- and time-specific stabilized weights $sw_i(t)$ – the following model was fit, where $\beta_0(t)$ is a time-dependent intercept:

$$(1) \qquad \text{logit}\, pr[D(t) = 1 \mid D(t\text{-}1) = 0, \bar{A}(t\text{-}1), L(0)] = \beta_0(t) + \beta_1 A(t\text{-}1) + \beta_2 L(0)$$

The time-dependent intercept, wherever noted, was modeled using restricted cubic splines with four knots at the 5th, 35th, 65th, and 95th percentiles of time, per precedent.[55,93]

The stabilized weight $sw_i(t)$, most easily conceptualized as an inverse-probability-of-treatment-and-censoring weight, is not simply the product of an inverse-probability-of-treatment weight and an inverse-probability-of-censoring weight. More specifically, sw_i(t) is the product of the following: 1) the conditional probability that an individual received their own exposure at visit *t* given their exposure history and baseline confounders, divided by the conditional probability that the individual received their own exposure at visit *t* given their exposure history and baseline and time-dependent confounders affected by exposure, and 2) the conditional probability that the individual was uncensored at visit *t* given their exposure history and baseline confounders, divided by the conditional probability that the individual was uncensored at visit *t* given their exposure history and baseline and time-dependent confounders affected by exposure. Thus, as outlined by Hernán et al.,[54]

$$sw_i(\mathrm{t}) = \prod_{k=0}^{\mathrm{int}(t)} \frac{pr(A(k) = a_i(k) \mid \overline{\mathrm{A}}(k-1) = \overline{a}_i(k-1), L(0) = l(0)_i)}{pr(A(k) = a_i(k) \mid \overline{\mathrm{A}}(k-1) = \overline{a}_i(k-1), L(0) = l(0)_i, \overline{L}(k) = \overline{l}_i(k)} \times \prod_{k=0}^{\mathrm{int}(t)} \frac{pr(C(k) = 0 \mid \overline{\mathrm{C}}(k-1) = 0, \overline{\mathrm{A}}(k-1) = \overline{a}_i(k-1), L(0) = l(0)_i)}{pr(C(k) = 0 \mid \overline{\mathrm{C}}(k-1) = 0, \overline{\mathrm{A}}(k-1) = \overline{a}_i(k-1), L(0) = l(0)_i, \overline{L}(t) = \overline{l}_i(k))}$$

This subject- and time-specific weight is stabilized, meaning that the numerator of each of the terms in the above equation is as depicted, rather than equaling 1 (which would produce a non-stabilized weight). Stabilized weights have been shown to yield less variable and no less biased parameters than non-stabilized weights.[81] Applying time-dependent weights to model (1) induces within-subject correlation, necessitating the use

of robust variance estimators (i.e. generalized estimating equations with an independent working covariance matrix).[54]

It is necessary to fit four logistic models to obtain $sw_i(t)$, needed to weight model (1). The first model, fit only using those observations representing visits at which individuals have not yet initiated HAART or have just been assigned HAART, features HAART as the outcome and the baseline confounders and time-dependent intercept as predictors. This model yields a predicted probability of remaining off HAART for each of the non-HAART or first HAART visits. For each individual, this probability for visit t is multiplied by that for all previous times, leading to the conditional exposure probability in the numerator of the stabilized weight.

The second model is identical to the first, except for that the time-dependent confounders affected by exposure are added as predictors. The predicted probabilities from this model, while analogous to those of the first model, instead contribute to the conditional exposure component of the stabilized weight denominator. The third model, which uses all observations, features the censoring indicator as the outcome and the HAART indicator, baseline confounders, and the time-dependent intercept as predictors. Hence, the predicted probabilities of remaining uncensored obtained from this model contribute to the conditional censoring component of the stabilized weight numerator. The fourth model is identical to the third, except for that the time-dependent confounders affected by exposure are added as predictors – the predicted probabilities contribute to the conditional censoring component of the stabilized weight denominator. In these four models, the time-dependent intercept reduces the number of free parameters, leading to reduced variability necessary for consistent IPTC estimation.[142]

A stabilized weight is obtained for each observation by dividing the product of the numerator components by the product of the denominator components. Then, with each observation in the pooled dataset weighted by its respective stabilized weight, model (1) is fit using robust variance to account for induced clustering. As previously described, the resultant exposure parameter is equivalent to the log hazard ratio comparing the hazard of the outcome had all children continuously received HAART since baseline to the hazard had they never initiated HAART.

It has been demonstrated that a dynamic visit schedule, evident in this HIV program because patients visited more regularly if they were receiving HAART and could also visit when acute care was needed, can induce biases in longitudinal studies of time-varying treatment.[143] Thus, in addition to the customary IPTC-weighted model, an alternative weighted model was used. The weight in this model was the IPTC weight, as described above, multiplied by a visit attendance weight, to yield an inverse probability of treatment, censoring, and visit attendance (IPTCV) weight. The numerator and denominator models for having a visit were identical for those for the censoring weight models, besides their inclusion of one additional predictor: time since last visit.

A DAG informed by previous related analyses[55,62,63,93,95,133] and a priori knowledge was used to identify the included baseline confounders (age, gender, and WHO HIV clinical stage and severity of immunodeficiency) and time-dependent confounders affected by exposure (CD4 percentage, HIV-related symptom or condition, and cotrimoxazole), which are detailed in Table 2. This strategy was empirically supported, assuming that the causal diagram was correctly specified.[144] The pooled dataset included one observation per person-day. If information was missing for a time-

dependent covariate, it was be filled by the most recently observed prior value (whether at a scheduled or unscheduled visit), as is standard.[62,63,93,95,132] For example, if a child was observed on days one and four, data for the "missing" days two and three were filled from day one.

At baseline, the total study population (as well as the subsets of children who eventually initiated or did not initiate HAART) was described in terms of variables in Table 2 – e.g., the number and percentage of children in each immunodeficiency category, and the median and interquartile range (IQR) for CD4 percentage, were calculated. These groups were also characterized in terms of other descriptors, including person-years contributed, median duration and IQR of follow-up, median number and IQR of program visits, and outcomes including death and deactivation due to transfer of care to another facility or loss to follow-up. Proportions were compared via the mean score or chi-square test, as appropriate, and medians were compared via the Mann-Whitney test. Mortality rates, rate ratios, and 95% CIs were calculated via Poisson regression.

Associations between HAART initiation and both baseline confounders and time-dependent confounders affected by exposure, produced by the logistic model to obtain the denominators for the inverse-probability-of-treatment weights, were examined. As in methodologically comparable studies,[54-63,92-95] to demonstrate whether gains in estimate validity were afforded by the utilized analytical approach, MSM results were directly compared to methods that do not appropriately account for time-dependent confounders affected by exposure (e.g., unweighted time-dependent Cox proportional hazards regression). Models that included an interaction term between categorical time and

HAART were used to assess the constancy of HRs during follow-up. To visualize the effect of HAART on survival, Kaplan-Meier curves were constructed, with unstratified, unconditional IPTCV-weighted curves generated using unstabilized weights.[145] Estimates of cumulative incidence were depicted by plotting the compliments of Kaplan-Meier curves. The timing of HAART initiation, as well as the decreasing size of the study population over time due to transfer of care to another facility, loss to follow-up, and death, was also illustrated.

Analysis – Specific Aim 2

Although the focus of Specific Aim 2 was to estimate the causal effect of HAART on CD4 percentage rather than death, there was substantial methodological overlap with Specific Aim 1. Commonalities included the same baseline confounders and time-dependent confounders affected by exposure, specification of time as days, carrying data forward, and fitting of weighted models with robust variance. Thus, for the sake of concision, this section highlights the precise areas in which the analysis differed from that already described.

As in the survival analysis, MSMs were a central component. However, there was an essential difference – instead of estimating the parameters of Cox proportional hazards MSMs via weighted pooled logistic regression, the parameters of MSMs were estimated via repeated measures linear regression, as in previous studies.[62,92,93,95,132] While stabilized IPTC weights were again derived via four pooled logistic models, with six models used to generate IPTCV weights, weights were applied not to model (1), but instead to model (2):

$$(2) \qquad E[Y(t) \mid \bar{A}(t), L(0)] = \beta_0(t) + \beta_1 Z(t) + \beta_2 L(0)$$

E[Y(t)] represents CD4 percentage at time t and Z(t) represents cumulative HAART exposure as of time t. Examination of previous studies[93,132] informed how Z(t) was categorized (see *Variables* section). Within-subject correlation resulting from repeated observations of individuals were accounted for by using generalized estimating equations with an independent working correlation matrix, per precedent.[92,93,95,132] This model yielded the mean difference in CD4 percentage had all individuals continuously received HAART since baseline compared to that had they never received HAART during follow-up.

As CD4 percentage was assessed biannually, although program visits were scheduled either monthly or quarterly, model (2) was fitted using only observations with a measured CD4 percentage result. If HAART was started at a visit where CD4 percentage was not measured, the precise time of initiation nonetheless informed the cumulative HAART exposure variable (i.e., cumulative exposure was coded as ">0 to 6 months" if HAART began 90 days prior to CD4 assessment).

In addition to the overall effect of HAART on CD4 percentage at six month time points, the effect within categories of baseline CD4 percentage was estimated by fitting stratum-specific models as in the studies by Hernán et al.[61] and Fairall et al.[62] Mean changes in CD4 percentage were presented graphically, as were the durations of HAART and the number of CD4 percentage measurements during each of the six month periods. The observed evolutions in CD4 percentage for children receiving HAART and children not receiving HAART were also depicted using box plots.

Analysis – Specific Aim 3

To determine whether the degree of immunodeficiency at HAART initiation was associated with recovery to "not significant" WHO age-specific immunodeficiency, the immunodeficiency categories as defined by WHO guidelines[105] and outlined in Table 2 were first collapsed into two groups: mild plus advanced, and severe. An HR for the time-to-event outcome of recovery to "not significant" immunodeficiency was estimated via Cox proportional hazards regression, with the proportional hazards assumption verified by visually inspecting log-negative-log survival estimates. Kaplan-Meier curves were generated to examine the survival functions of the two severity of immunodeficiency groups, with the log-rank test used to assess differences between groups. Time started at HAART initiation and ended at the first of loss to or end of follow-up, transfer of care to another facility, death, or attainment of "not significant" immunodeficiency. A published SAS macro was used to construct cumulative incidence curves.[146]

IV.

THE EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON THE SURVIVAL OF HIV-INFECTED CHILDREN IN A RESOURCE-DEPRIVED SETTING: A COHORT STUDY

Abstract

Background

The effect of highly active antiretroviral therapy (HAART) on the survival of HIV-infected children has not been well quantified. Because most pediatric HIV occurs in low- and middle-income countries, our objective was to provide a first estimate of this effect among children living in a resource-deprived setting.

Methods and Findings

Observational data from HAART-naïve children enrolled into an HIV care and treatment program in Kinshasa, Democratic Republic of Congo between December 2004 and May 2010 were analyzed. We used marginal structural models to estimate the effect of HAART on survival while accounting for time-dependent confounders affected by exposure. At the start of follow-up, the median age of the 790 children was 5.9 years, 528 (66.8%) had advanced or severe immunodeficiency, and 405 (51.3%) were in HIV clinical stage 3 or 4. The children were observed for a median of 31.2 months and contributed a total of 2089.8 person-years. Eighty children (10.1%) died, 619 (78.4%)

initiated HAART, six (0.8%) transferred care, and 76 (9.6%) were lost to follow-up. The mortality rate was 3.2 deaths per 100 person-years (95% CI: 2.4, 4.2) during HAART and 6.0 deaths per 100 person-years (95% CI: 4.1, 8.6) during receipt of primary HIV care only. The mortality hazard ratio comparing HAART to no therapy from a marginal structural model was 0.25 (95% CI: 0.06, 0.95).

Conclusions

HAART reduced the hazard of mortality in HIV-infected children in Kinshasa by 75%, an estimate that is similar in magnitude but with lower precision than the effect of HAART on survival that has been reported among children in the United States.

Introduction

Highly active antiretroviral therapy (HAART) clearly improves the survival of adults living with HIV[25,147] even when initiated at higher CD4 cell counts,[119,120] but less is known about the degree to which HAART affects the survival of HIV-infected children. The course of HIV disease in children, due in part to deleterious impacts of the virus on the immature thymus[127] leading to high HIV RNA viremia[129] and rapid death,[148] is distinct from that in adults.[24] Response to antiretroviral treatment also differs across age groups.[131] Given that the natural history of HIV and response to therapy vary by age and that over two million children worldwide are living with HIV,[2] the extrapolation of results from studies of adults to pediatric populations is not appropriate. It is imperative that the effect of HAART on survival be quantified specifically in children.

Most observational studies of the effects of treatment on survival in children have not used the epidemiological methods necessary to account for potential biases inherent to their design, including confounding by indication. One study not employing those methods found that HAART, relative to no therapy, decreased the mortality rate by 71% among 1,142 Italian children.[76] A recent study of 1,236 children in the United States,[63] which used optimal analytical methods, observed a result similar to that from the Italian study.

Greater than 90% of children receiving HAART live in low resource areas.[2,149] Because multiple factors that may affect adversely treatment outcomes including delayed presentation to care and a higher incidence of co-occurring conditions are more common in such environments,[150] there is a specific need for information on the effects of HAART on patients living in these areas. Studies in adults have shown higher mortality after HAART initiation in resource-poor settings than in resource-privileged settings after adjusting for age, CD4 cell count, and disease stage.[126,151] Although investigations in pediatric cohorts from Zambia[102] to Haiti[103] to *Côte d'Ivoire*[104] have shown that treatment improves immunological, hematological, and growth outcomes and results in mortality rates lower than those observed in the pre-antiretroviral era,[148] the effect on mortality of HAART itself has never been accurately quantified among children in a resource-deprived setting. This is true of two recent multi-site studies in sub-Saharan Africa, [106,152] as well as studies in Thailand,[153] Zambia,[154] *Côte d'Ivoire*,[155] and Lesotho[156] that have provided valuable information on mortality among children receiving HAART, including rates during the early and late therapeutic periods.

In this study, we investigated the effect of HAART on mortality in an observational clinical cohort of HIV-infected children in the Democratic Republic of Congo (DRC). As HAART is typically initiated in sicker patients, for example those with lower CD4 cell percentages, it is necessary to adjust for this confounding by indication to estimate its effect on survival. But adjusting for a time-dependent factor such as CD4 cell percentage that is itself affected by prior HAART exposure can yield a measure not interpretable as the total effect of HAART, because the effects of therapy are mediated in part through CD4. We therefore used a method that adjusts for time-dependent confounding by indication while accounting for confounders affected by prior exposure, marginal structural models (MSMs).

Methods

Ethics Statement

Parental informed consent for the HIV care program, in addition to assent from minors at least 12 years of age, was obtained for all participants. All research was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of the Kinshasa School of Public Health (approval numbers ESP/CE/010 and ESP/CE/014) and the University of North Carolina at Chapel Hill Institutional Review Board (study numbers 04-1007, 05-2311, and 10-0661).

Study Population, Measurements, and Follow-up

The source of information for this study was a comprehensive HIV care and treatment program serving children and family members at Kalembe Lembe Pediatric Hospital and Bomoi Healthcare Center in Kinshasa, DRC.[133] HIV infection was confirmed by serological testing, with HIV viral load or DNA PCR used in infants under 18 months of age. Patients were managed in accordance with World Health Organization (WHO)[105,134,157] and national[135] guidelines regarding diagnosis and treatment of opportunistic infections, laboratory monitoring, and provision of cotrimoxazole and HAART when clinically or immunologically indicated. HAART could be initiated as soon as one week following enrollment or at any visit thereafter, with the standard first-line regimen comprised of zidovudine or stavudine, lamivudine, and nevirapine or efavirenz. Because virological diagnostics were occasionally unavailable, enrolled infants under two years of age did not always begin treatment immediately as recommended by WHO.[157] Visits were scheduled monthly for patients receiving HAART and quarterly for those not receiving HAART, with additional unscheduled visits made by individuals needing acute care. Clinical data, documented by physicians during patient visits using standardized forms, were collected. CD4 cell count and percentage were evaluated every six months at the DRC National AIDS Reference Laboratory, but viral load was not routinely assessed due to infrequent availability of the assay.

The population for this analysis was HIV-infected children who were naïve to antiretroviral therapy and under 18 years of age at the start of follow-up. The beginning of follow-up, also referred to as baseline, was the initiation of HIV care (between December 2004 and May 2010) unless a child's CD4 percentage was not obtained at that visit, in which case baseline was defined as the date of the first available CD4 percentage

result. Consistent with precedent,[63,93] allowing follow-up to start at first CD4 meant that children did not have to be excluded due to missing immunological data. Follow-up ended at death or at the last clinic visit prior to transfer of care, loss to follow-up, or August 2010. If children withdrew from care or could not be located by three tracking attempts after a missed visit, they were classified as lost to follow-up. Children contributed non-HAART person-time until they initiated HAART. Program personnel gathered information on children who died, including the date and suspected cause of death.

Statistical Analysis

Mortality rates were expressed as deaths per 100 person-years, with rates and rate ratios calculated via Poisson regression. To compare proportions, we used the chi-square or mean score test, while medians were compared by the Mann-Whitney test. SAS version 9.2 (SAS Institute, Cary, NC) was used for all analyses.

To estimate the total effect of HAART on mortality, one must adjust for confounders measured at baseline as well as time-varying confounders affected by prior exposure, factors that are causal intermediates between treatment and death while simultaneously common causes of subsequent treatment and death.[81] We did so by fitting four logistic regression models to predict subject and time-specific probabilities of treatment and censoring as a function of covariate histories, and using these predicted probabilities to construct stabilized inverse-probability-of-treatment-and-censoring (IPTC) weights. The model for the denominator of the treatment weight included as independent variables baseline confounders, time-varying confounders affected by

exposure, and time, while the model for the treatment weight numerator included only time and baseline confounders. The models for the censoring weight numerator and denominator were identical to those for the treatment weights, except that the censoring models also included time-varying HAART as a predictor. Then, these IPTC weights were used in a weighted pooled logistic model, which included baseline confounders, to estimate the parameters of a Cox Proportional Hazards MSM.[54,158] Assuming no unmeasured confounding, informative censoring, or model misspecification, weighting disassociates time-varying confounders with subsequent treatment and censoring, effectively eliminating the causal intermediates complicating estimation of the effect of HAART on mortality. With the intent-to-treat assumption that children starting HAART received it uninterruptedly throughout follow-up, approximately true in our program because adherence was checked at every visit and treatment was never discontinued for active patients, the MSM yields a hazard ratio (HR) comparing the hazard of death had all children initiated HAART to that had no children initiated HAART during follow-up.

For comparison, we also fit unadjusted as well as adjusted but unweighted pooled logistic models. Additionally, to address possible bias resulting from the dynamic visit schedule,[143] we utilized an alternative weighted model which employed the above described IPTC weight multiplied by a visit attendance weight, that is, an IPTCV weight. The models for the probability of having a visit were equivalent to the censoring weight models, except for time since last visit was also included as a predictor. The constancy of HRs over follow-up was assessed with models that included an interaction term between HAART and categorical time. Kaplan-Meier curves to visualize the impact of HAART on survival were constructed; unstabilized weights were used to obtain unconditional,

unstratified IPTCV-weighted curves.[145] The complements of Kaplan-Meier curves were plotted as estimates of cumulative incidence. 95% confidence intervals (CIs) for MSM HRs were based on robust variance to account for within-subject correlation induced by weighting. Our pooled dataset included one row per person-day, with missing covariate data carried forward from last observation.

Confounders were selected based on a posited causal directed acyclic graph[140] and previous studies. Baseline confounders were WHO HIV clinical stage and severity of immunodeficiency, age, and gender; time-varying confounders affected by prior exposure were cotrimoxazole prophylaxis, HIV-related symptoms or conditions, and CD4 cell percentage. HAART, gender, cotrimoxazole, and symptoms or conditions were coded dichotomously. HIV-related symptoms or conditions included one or more of the following: Kaposi's sarcoma, oral or esophageal candidiasis, severe weight loss, tuberculosis, fever or diarrhea of one month or more, lymphocytic interstitial or *Pneumocystis jirovecii* pneumonia, chronic herpes simplex, oral hairy leukoplakia, cryptococcal meningitis, toxoplasma or HIV encephalopathy, or HIV-associated nephropathy. Because it was generally assessed only at enrollment, clinical stage was that at HIV care initiation for all children, including those for whom follow-up began at first CD4 result. Severity of immunodeficiency was calculated according to WHO guidelines[105] using CD4 and age at baseline. Both clinical stage and severity of immunodeficiency were coded into four levels and treated as indicator variables in multivariable analyses. CD4 percentage, age, and time were modeled as restricted cubic splines with four knots, at the 5th, 35th, 65th and 95th percentiles.

Results

Characteristics of the 790 children at baseline are shown in Table 1. The median age was 5.9 years (interquartile range [IQR]: 2.7, 9.8), and roughly one-half were female (52.5%). The majority of patients had severe immunodeficiency (57.2%) as reflected in the low median CD4 percentage of 15 (IQR: 9, 22). Most children had advanced HIV as indicated by clinical stage 3 or 4 (51.3%), and 19.9% had evidence of at least one HIV-related symptom or condition.

The 790 children, 619 of whom initiated HAART (78.4%) during follow-up, were followed for a median of 31.2 months (IQR: 10.3, 53.6) and had a median of 30 HIV care visits (IQR: 11, 57). Of those who started treatment, 110 (17.8%) switched to an alternative regimen due to an adverse event or treatment failure. At baseline, compared to those who remained untreated, children who later initiated HAART had greater degree of immunodeficiency ($p<0.01$) with a corresponding lower median CD4 percentage ($p<0.01$), more advanced HIV clinical stage ($p<0.01$), and were more likely to have at least one symptom or condition ($p<0.01$). Those who initiated HAART were similar to those who did not in terms of gender ($p=0.05$) and median age ($p=0.17$) as well as cotrimoxazole initiation at the beginning of follow-up ($p=0.05$). The median duration of observation for children who started HAART, 36.9 months (IQR: 14.0, 55.7) with 31.3 of those months (IQR: 11.4, 52.0) during receipt of HAART, was longer than the median 11.5 months (IQR: 3.0, 27.0) observed for untreated children ($p<0.01$), and there was a parallel difference in median number of visits (40 versus 9, $p<0.01$).

Eighty children (10.1%) died during the 2089.8 accrued person-years of follow-up, an overall mortality rate of 3.8 deaths per 100 person-years (95% CI: 3.1, 4.8). The

unadjusted mortality rate ratio comparing HAART to the lack thereof was 0.54 (95% CI: 0.34, 0.85). There were 51 deaths during the 1620.9 person-years (77.6% of total follow-up) contributed by children receiving HAART, a rate of 3.2 deaths per 100 person-years (95% CI: 2.4, 4.2), and 29 deaths during the 468.9 non-HAART person-years, a rate of 6.0 deaths per 100 person-years (95% CI: 4.1, 8.6). The mortality rates per 100 person-years during and after the first 90 days of HAART were 16.4 (95% CI: 11.0, 24.5) and 1.8 (95% CI: 1.3, 2.7), respectively. The proportion of HAART-untreated children who died (17.0%) was higher than the 8.2% of HAART-initiating children who died ($p<0.01$), and the absolute three-year risk of death for children receiving HAART was 0.14 compared with 0.48 for children not receiving treatment. Thus, the unadjusted HAART mortality risk ratio was 0.31 (95% CI: 0.23, 0.43). Six children (0.8%) transferred care and 76 (9.6%) were lost to follow-up. A smaller proportion of children who initiated HAART (7.9%) than untreated children (19.3%) either transferred care or were lost to follow-up ($p<0.01$), suggesting that HAART with its associated more frequent visit schedule improved retention in care, and reflecting that children who were in care for a shorter period of time had less opportunity to begin treatment. Figure 1, in addition to showing the reduction in population size over time due to death, transfer of care, or loss to follow-up, illustrates the timing of HAART initiation. Of the 619 children who initiated treatment, 325 (52.5%) did so during the first 30 days of follow-up.

Estimates of the effect of HAART on mortality are presented in Table 2. Because patients with advanced disease are most likely to begin treatment, an unadjusted model that did not account for this confounding by indication suggested no effect of HAART on mortality, relative to no therapy (HR: 1.38, 95% CI: 0.84, 2.27). An unweighted model

that adjusted for baseline confounders only (HR: 0.73, 95% CI: 0.41, 1.31), as well as that from an unweighted model that additionally but improperly adjusted for time-varying confounders (HR: 0.67, 95% CI: 0.37, 1.21), shifted the estimates in the direction of the null but also failed to clearly suggest a protective effect of HAART on survival.. After appropriately accounting for time-dependent confounders affected by exposure using MSMs, HAART was strongly protective against mortality. The HR from an IPTC-weighted model was 0.17 (95% CI: 0.05, 0.64), while that from an IPTCV-weighted model was 0.25 (95% CI: 0.06, 0.95). The HR from an IPTCV-weighted model before the median event time of 2.8 months, 0.29 (95% CI: 0.09, 0.95), was similar to the HR of 0.18 (95% CI: 0.03, 0.99) after the median (interaction p=0.32). Advanced and severe immunodeficiency at baseline, compared to not significant immunodeficiency, were the only baseline factors independently associated with mortality in an IPTCV-weighted model (HR: 3.16, 95% CI: 1.18, 8.47, and HR: 5.08, 95% CI: 1.49, 17.39, respectively). IPTCV-weighted cumulative incidence curves depict the survival benefit of HAART, not evident in the unweighted curves that do not adjust for confounding and selection bias (Figure 2).

Discussion

This study, which uniquely uses observational data to estimate the effect of HAART on mortality in a pediatric population in a resource-deprived setting, revealed that treatment markedly improved the survival of HIV-infected children in Kinshasa, DRC. Using a marginal structural model, we estimated that HAART reduced the hazard (rate) of mortality during follow-up, relative to no therapy, by 75% (HR: 0.25, 95% CI:

0.06, 0.95). Although it was less precise, our result was essentially identical in magnitude to the HR of 0.24 (95% CI: 0.11, 0.51) from the only other study in children[63] to use a method that could overcome barriers to unbiased effect estimation in non-randomized data, such as time-dependent confounders affected by exposure. This is particularly noteworthy since the earlier study included children enrolled in a multicenter prospective cohort study in the United States.

The equivalence of HRs offers evidence that HAART is as effective in improving the survival of HIV-infected children in the DRC, a severely resource-deprived nation still recovering from its recent history of poor governance and civil wars,[159] as it is in resource-privileged settings. This result parallels the consistency of effect observed between adults in Europe and the United States[61] and South Africa,[62] and is important because virtually all children receiving HAART globally, due to scale-up of antiretroviral provision in low- and middle-income countries, live in resource-poor areas.[149] Pediatric HIV programs in sub-Saharan Africa, like ours in Kinshasa, often face challenges that could adversely affect patient survival, including limited drug options and diagnostic capacity, delayed healthcare seeking and poor retention in care, and prevalent comorbidities and undernutrition.[124] This emphasizes why a homogeneous effect of HAART on survival in children could not be assumed across distinct contextual milieus. Evidence on the interaction between HAART and nutrition is limited, but it has been speculated that micronutrient deficiencies may decrease the effectiveness of antiretroviral drugs.[160] If true, this might have resulted in effect estimates that were attenuated compared to had the children been better nourished overall, although it makes the results applicable to similar populations. The higher mortality rate observed during the first 90

days of HAART, compared to the rate after 90 days of therapy, was consistent with findings in multiple prior pediatric studies.[152-156]

The validity of results is contingent upon methodological assumptions, one of which is the inclusion of all important confounders. Although HIV RNA was infrequently assessed, it is arguable that this factor when available was prognostic for and affected by HAART, as well as associated with survival. While not including viral load is a limitation that may have introduced some amount of bias into our estimate, failing to account for this factor may have only had minimal influence. In the work by Patel et al.,[63] the HR of 0.30 (95% CI: 0.11, 0.82) in the 36% of children with HIV RNA measurements was similar to the overall results which were not adjusted for viral load: 0.24 (95% CI: 0.11, 0.51). Limited availability of second- and third-line drug options, and only being able to occasionally measure virological suppression, also meant that some children who were recorded as receiving treatment could have actually been failing therapy. This may have diluted the estimated effect of HAART if these patients were more likely to die. However, because children were frequently evaluated both clinically and immunologically, and because adherence was routinely assessed, it is expected that inadequate virological control was uncommon.

As suggested by several recent pediatric studies,[152,155,161] it is conceivable that some children classified as lost to follow-up actually died, which may have affected both the validity and precision of our estimate. If this was more common amongst children not receiving HAART, possible because there was less opportunity to discern deaths in these children due to their less frequent clinic visits, the strength of effect could have been underestimated. Because children not receiving HAART had fewer appointments, their

data were correspondingly carried forward to a greater degree, which also may have affected results. On a related note, covariate data were quite complete. The variable most often missing, because it required laboratory testing and not just simple collection onto forms during clinic visits, was CD4 cell percentage. Assuming that children were on average halfway to their next scheduled test when follow-up concluded, 83% of the expected number of results was available. Data on other time-varying factors were close to fully complete. It is important to emphasize that the methods used will not yield a fully unbiased measure unless all underlying assumptions are met. If study imperfections occurred (e.g., misclassification of HAART status, or the incorrect recording of HIV-related symptoms or conditions due to the limited capacity in Kinshasa to definitively diagnose certain conditions such as cryptococcal meningitis), this is a possibility.

Strengths of our study include precise ascertainment of dates of HAART initiation and death, regular patient follow-up, which mitigated the influence of the carrying forward data, and no treatment discontinuation by any active patient, which allowed for the intent-to-treat assumption and straightforward effect interpretation. In our setting, patients who missed visits were actively tracked, which decreased the possibility that children who died were misclassified as lost to follow-up. This is supported by the fact that during the first 90 days of HAART, a period when the rate of mortality was high (16.4 per 100 person-years; 95% CI: 11.0, 24.5), the rate of loss to follow-up was comparatively much lower (3.4 per 100 person-years; 95% CI: 1.4, 8.2). No child had ever received therapy prior to participation in our program, which adds to the generalizability and relevance of our results because most children initiating HAART worldwide are treatment-naïve. Furthermore, given the brief history and limited

availability of vertical transmission prevention services in Kinshasa, it is unlikely that many children had been previously exposed to prophylactic nevirapine. This means that nevirapine-resistant HIV variants[162,163] were probably rare, and increases the likelihood that the nevirapine-based regimens most children received were effective. Patel et al.[63] sensibly speculate that a stronger HR than 0.24 would have been estimated had their population been naïve to treatment rather than experienced. If true, because our HR of 0.25 was comparable despite arising from treatment-naïve children, it is possible that the aforementioned underlying contextual factors may have moved our estimate modestly towards the null.

The large difference between the HRs from the unweighted and weighted models indicates that there was considerable time-dependent confounding, and validates the analytical approach. The importance of accounting for dynamic visit schedules is highlighted by the distinct HRs from the IPTC- and IPTCV-weighted models.

In summary, HAART had a substantial, beneficial impact on the survival of HIV-infected children in Kinshasa, DRC. Along with supplementing the limited knowledge base on the quantitative effects of pediatric treatment, this study presents the first valid estimate to date of the extent by which therapy decreases mortality in a highly relevant population of HIV-infected children living in a resource-deprived setting. Our estimate, which represents the expected outcome had HAART been evaluated via randomized assignment, provides compelling evidence that the accelerating rollout of antiretrovirals is a profoundly lifesaving endeavor. Pooled data from pediatric cohorts in less developed areas should be used in future research not only to generate more precise effect estimates,

but also to focus on the effects of HAART within particular groups such as undernourished children, which remain incompletely understood.

Table 3. Characteristics of 790 HIV-infected children initiating HIV care in Kinshasa, DRC, between December 2004 and May 2010.

	Total (n=790)	Initiated HAART (n=619)	No HAART (n=171)	*p*-value[a]
BASELINE[b]				
Median age, years (IQR[c])	5.9 (2.7, 9.8)	5.9 (2.6, 9.6)	5.9 (3.4, 10.2)	0.17
Age [n (%)]				
<1	63 (8.0)	52 (8.4)	11 (6.4)	0.76
1–4	277 (35.1)	218 (35.2)	59 (34.5)	
5–9	265 (33.5)	208 (33.6)	57 (33.3)	
10–17	185 (23.4)	141 (22.8)	44 (25.7)	
Female sex [n (%)]	415 (52.5)	314 (50.7)	101 (59.1)	0.05
HIV clinical stage (WHO[c]) [n (%)]				
1	153 (19.4)	80 (12.9)	73 (42.7)	<0.01
2	232 (29.4)	176 (28.4)	56 (32.7)	
3	369 (46.7)	331 (53.5)	38 (22.2)	
4	36 (4.6)	32 (5.2)	4 (2.3)	
Median CD4 percentage (IQR[c])	15 (9, 22)	13 (7, 20)	22 (16, 28)	<0.01
Severity of immunodeficiency (WHO[c]) [n (%)]				
Not significant	174 (22.0)	100 (16.2)	74 (43.3)	<0.01
Mild	88 (11.1)	56 (9.0)	32 (18.7)	
Advanced	76 (9.6)	57 (9.2)	19 (11.1)	
Severe	452 (57.2)	406 (65.6)	46 (26.9)	
HIV symptoms or conditions [n (%)]	157 (19.9)	139 (22.5)	18 (10.5)	<0.01
Started cotrimoxazole at first visit [n (%)]	726 (91.9)	575 (92.9)	151 (88.3)	0.05
FOLLOW-UP				
Total person-years accrued	2,089.8	1,832.8	257.0	N/A
HAART person-years accrued	1,620.9	1,620.9	0.0	N/A
Median months of follow-up (IQR[c])	31.2 (10.3, 53.6)	36.9 (14.0, 55.7)	11.5 (3.0, 27.0)	<0.01
Median number of program visits (IQR[c])	30 (11, 57)	40 (16, 61)	9 (4, 18)	<0.01

Lost to follow-up or transferred care [n (%)]	82 (10.4)	49 (7.9)	33 (19.3)	<0.01
Died [n (%)]	80 (10.1)	51 (8.2)	29 (17.0)	<0.01

[a] *p*-values are for the comparison of children who received HAART to children who did not receive HAART.
[b] Baseline was shifted from enrollment to date of first CD4 percentage result for 41 of 790 children (5.2%). For these 41 children, the median number of months from enrollment to first CD4 percentage was 2.3 (IQR: 1.1, 5.3).
[c] IQR, interquartile range; WHO, World Health Organization.

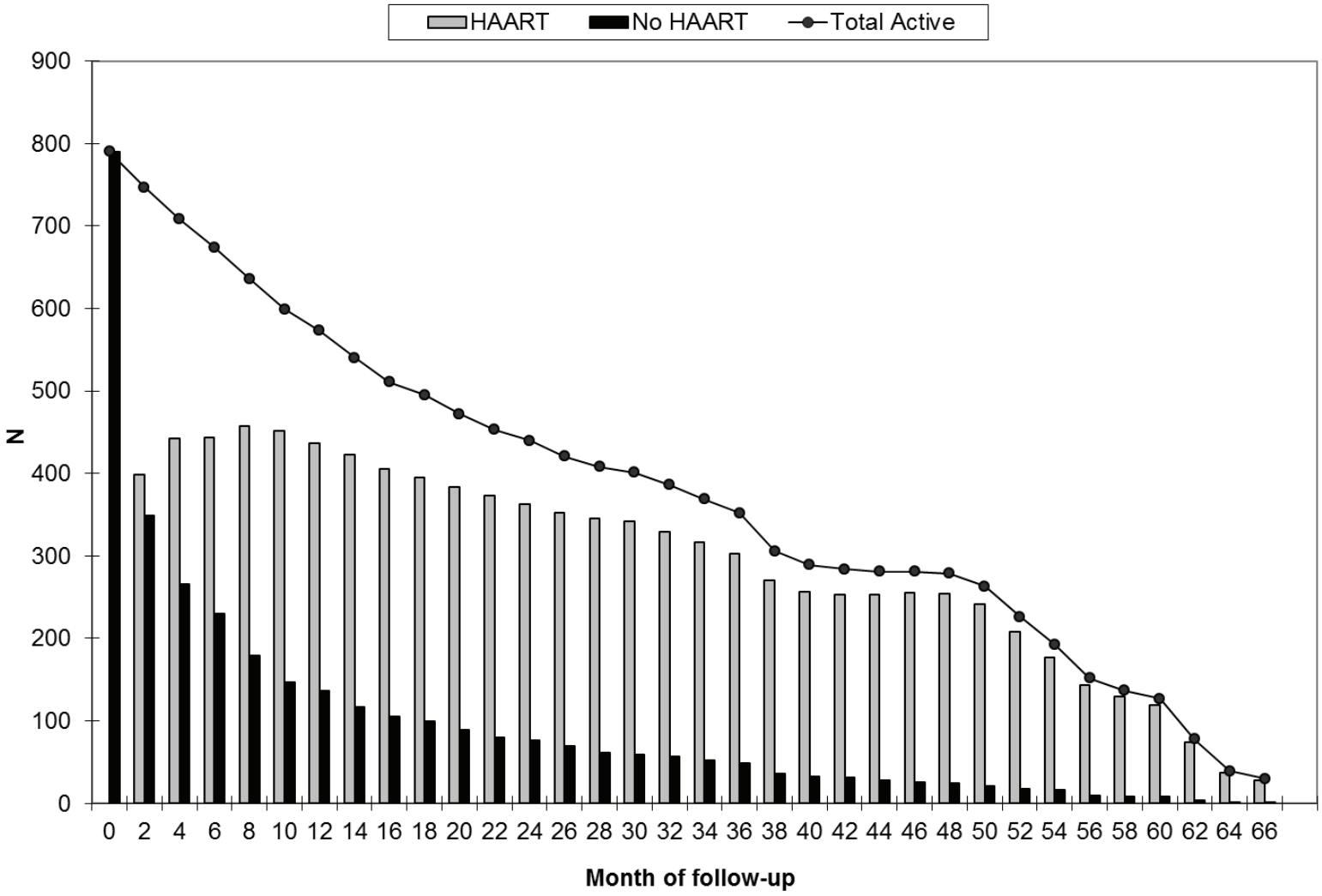
Table 4. Estimated effect of HAART on mortality among 790 HIV-infected children initiating HIV care in Kinshasa, DRC, between December 2004 and May 2010.[a]

	HR[b]	Robust 95% CI[b]
Unweighted, unadjusted (no confounders)	1.38	0.84, 2.27
Unweighted, adjusted (baseline confounders only)	0.73	0.41, 1.31
Unweighted, adjusted (baseline and time-varying confounders)	0.67	0.37, 1.21
IPTC-weighted[a]	0.17	0.05, 0.64
IPTCV-weighted[a]	0.25	0.06, 0.95

[a] All estimates are derived from pooled logistic models that include time modeled as a restricted cubic spline with four knots. Comparing HAART to no HAART, the unadjusted mortality rate ratio was 0.54 (95% CI: 0.34, 0.85) while the unadjusted ratio of three-year mortality risks was 0.31 (95% CI: 0.23, 0.43).

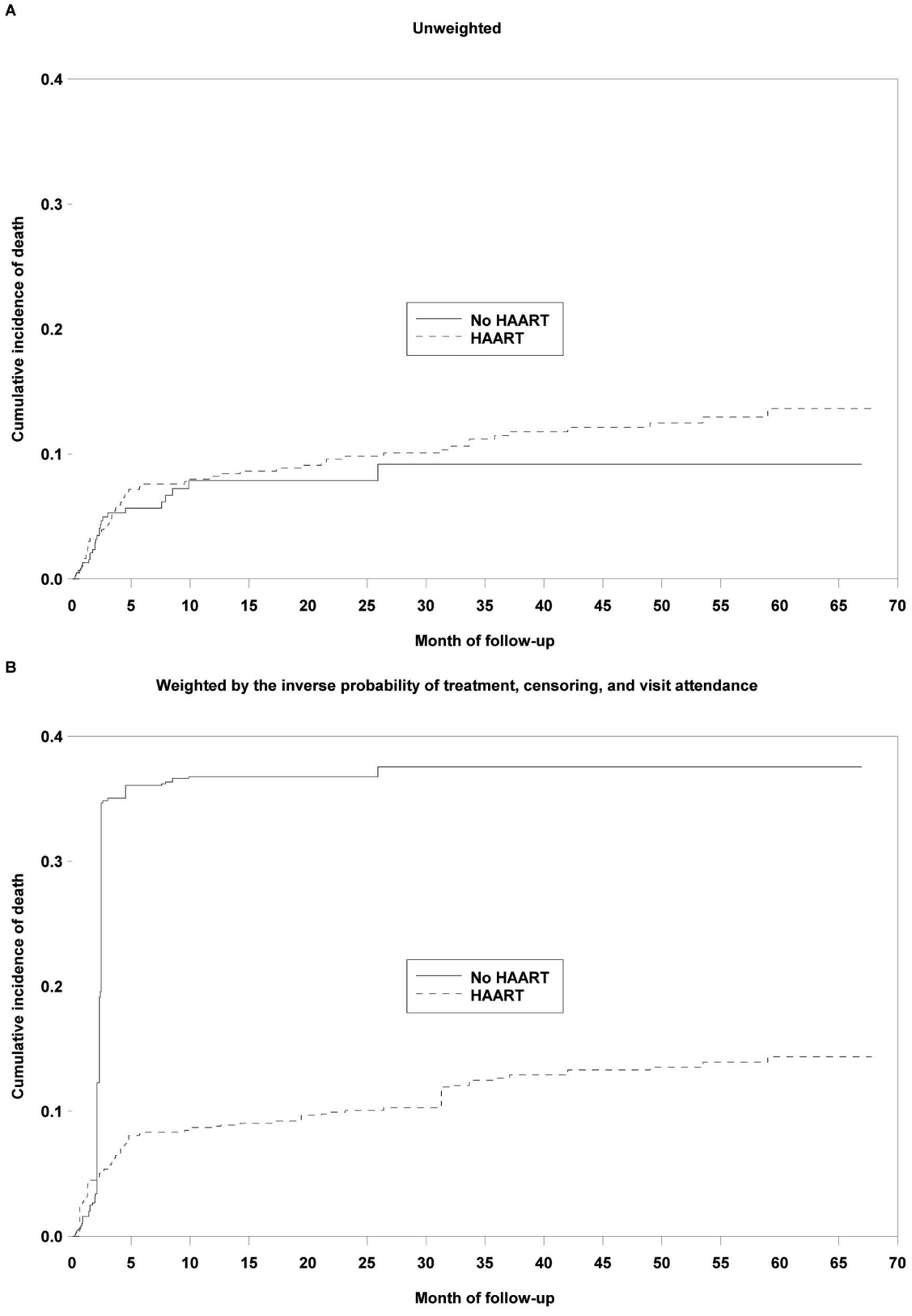
[b] CI, confidence interval; HR, hazard ratio; IPTC, inverse-probability-of-treatment-and-censoring; IPTCV, inverse probability of treatment, censoring, and visit attendance.

Figure 3. Number of active children by month of follow-up and HAART status.



The timing of HAART initiation, in addition to the reduction in population size over time due to death, transfer of care, or loss to follow-up, is illustrated.

Figure 4. Cumulative incidence curves depicting the effect of HAART on survival among 790 HIV-infected children.



V.

QUANTIFICATION OF CD4 RESPONSES TO HIGHLY ACTIVE ANTIRETROVIRAL THERAPY OVER FIVE YEARS AMONG HIV-INFECTED CHILDREN IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

Abstract

Background

In HIV-infected children, the long term effects of highly active antiretroviral therapy (HAART) on CD4 responses are incompletely understood. Information from resource-deprived areas is particularly scarce, despite the fact that most children with HIV live in such settings.

Methods

We analyzed observational data from HAART-naïve children enrolled between December 2004 and May 2010 into an HIV care and treatment program in Kinshasa, Democratic Republic of Congo. To estimate the effect of HAART on CD4 percentage while accounting for time-dependent confounders affected by prior exposure to HAART, a marginal structural linear mean model was used.

Results

790 children contributed 2090 person-years with a median of 31 months of follow-up. The median age at the start of follow-up was 5.9 years (interquartile range: 2.7, 9.8), 405 (51%) were in HIV clinical stage 3 or 4, and 528 (67%) had advanced or severe immunodeficiency. During observation, 80 children (10%) died, 76 (10%) were lost to follow-up, six (1%) transferred care to another facility, and 619 (78%) initiated HAART. Compared to no HAART, the estimated absolute rise in CD4 percentage was 6.8% (95% CI: 4.7%, 8.9%) after six months of HAART, 8.6% (95% CI: 7.0%, 10.2%) after 12 months, and 20.5% (95% CI: 16.1%, 24.9%) after 60 months. CD4 percentage gains were slowest but greatest among HAART-treated children who had a baseline CD4 percentage <15. The cumulative incidence of recovery to "not significant" WHO age-specific immunodeficiency was lower if HAART was started when immunodeficiency was severe rather than mild or advanced.

Conclusions

HAART increased CD4 percentages among HIV-infected children in a resource-deprived setting, as previously noted among children in the United States. The more gradual and protracted recovery observed in children with lower baseline CD4 percentages supports the earlier initiation of pediatric therapy.

Introduction

The CD4 cell count and percentage are key markers of HIV disease progression, since throughout the course of infection CD4 cells are generally depleted in the absence of highly active antiretroviral therapy (HAART).[164,165] In children, initiation of HAART at low CD4 levels is associated with a reduced likelihood of immune reconstitution[114-116,166] and greater likelihoods of growth failure,[166,167] clinical progression,[167,168] and mortality.[102-104,131] HIV treatment guidelines therefore recommend HAART at lower CD4 cell counts and percentages.[157,169] Multiple randomized controlled trials[89,170] and prospective cohort studies[108,171,172] have demonstrated that HAART effectively suppresses viral load in children. However, the effect of HAART on immune reconstitution in pediatric populations, particularly in resource-deprived settings, remains incompletely understood.

While randomized trials in the United States (U.S.)[88] and Europe[89] demonstrated that children receiving HAART had greater CD4 percentage increases than those receiving less potent antiretroviral therapy, the profound survival and clinical progression benefits of HAART[48,49] restricted subsequent investigations of immunological responses to therapy to observational contexts. Prior pediatric studies have generally been of short duration[91,102-104,107,108,116,117,166,167,172-179] with limited available sample sizes[91,103,104,107,108,115,117,166,167,173-175,177-183] and were unable to contrast the effect of HAART in comparison to no HAART. One study estimated the effect of HAART versus no HAART on CD4 percentage, finding that the mean CD4 percentage of 1236 U.S. children rose by 2.3% after one year of HAART compared to no HAART, with the difference increasing to 4.4% by five years.[95] Of the studies in low resource areas,[102-

[104,107,108,166,172,173,175-177,184] just two[27,41] appraised CD4 responses at more than two years after HAART initiation with none quantifying the effect of HAART on immune reconstitution relative to no HAART.

Global access to HAART is expanding,[30] including to the over 90%[1] of children living with HIV infection in areas of the world where poverty and undernutrition are common and may affect HAART outcomes.[124,185] Since HAART reduces morbidity and mortality among HIV-infected children in resource-deprived settings,[133,186,187] these children may be on HAART for decades. Hence understanding immune reconstitution over longer periods of time in this population is essential. Further work on responses to HAART in this group may also inform the optimal timing of HAART initiation in children greater than two years of age.[123,157,188,189] Therefore, we estimated the effect of HAART on mean CD4 percentage in an observational clinical cohort of HIV-infected children in the Democratic Republic of Congo (DRC), using marginal structural models (MSMs) to account for confounders affected by prior exposure while adjusting for time-dependent confounding by indication.

Methods

Study Population, Measurements, and Follow-up

We used data from a comprehensive, family-centered HIV care and treatment program at two sites in Kinshasa, DRC: Bomoi Healthcare Center and Kalembe Lembe Pediatric Hospital.[133,186] The study population was restricted to HAART-naïve children less than 18 years of age at baseline with HIV infection confirmed by serology if at least

18 months of age and by DNA PCR or HIV viral load otherwise. If a child's CD4 percentage was obtained at time of program enrollment, baseline was defined as the date of HIV care initiation (between December 2004 and May 2010); otherwise, baseline was defined as the date of first CD4 percentage result. As in previous studies,[63,93] this allowed children to be included despite missing baseline data. Follow-up continued until either the date of death or the clinic visit preceding loss to follow-up, transfer of care, or August 2010. Children were classified as lost to follow-up if they withdrew from care or were not located by three tracking attempts after a missed visit. All children who initiated HAART contributed no HAART person-time prior to HAART initiation.

Laboratory monitoring, diagnosis and treatment of opportunistic infections, and prescription of cotrimoxazole prophylaxis and HAART were based on national[135] and World Health Organization (WHO)[105,122,134,157] guidelines. The first-line HAART regimen consisted of of zidovudine or stavudine, lamivudine, and nevirapine or efavirenz and could be initiated at any visit after enrollment. Infants less than two years of age were not always started on therapy immediately, as recommended by WHO in 2009,[157] because virological diagnostics were only intermittently available. Physicians documented clinical data using standardized forms during routine visits (scheduled monthly for children receiving HAART and quarterly for children not receiving HAART) and unscheduled visits (for children needing acute care). If a child died, program personnel collected the date and suspected cause of death. CD4 cell count and percentage were assessed biannually at the DRC National AIDS Reference Laboratory. HIV viral load was only occasionally available.

Parental informed consent for the HIV care program was obtained for all children, with minors at least 12 years of age additionally providing their assent. The University of North Carolina at Chapel Hill Institutional Review Board and the Ethics Committee of the Kinshasa School of Public Health approved the study.

Statistical Analysis

Estimation of the total effect of HAART on change in mean CD4 percentage at time *t*, the primary aim of analysis, entails adjustment for factors that are common causes, or confounders, of both exposure and outcome. Whereas some confounders are measured at baseline only, others, known as time-varying confounders affected by prior exposure, are measured throughout follow-up and are causal intermediates between HAART and CD4 percentage at time *t* while concurrently common causes of subsequent HAART status and CD4 percentage at time *t*. Specifying time-varying confounders affected by prior exposure, for example CD4 percentage at times prior to *t*, as time-varying covariates in a standard regression model will fail to yield the total effect of HAART on change in mean CD4 percentage at time *t* because the effects of HAART are mediated in part through the time-varying factors. Therefore, to appropriately estimate the immunological effect of HAART in the presence of such factors, we used inverse probability weighting.

Our dataset was configured into a one row per person-day structure, with missing covariate data carried forward from last measurement. We used covariate histories and six logistic regression models to predict child and time-specific probabilities of treatment, censoring, and visit attendance, which were then multiplied to calculate composite

stabilized inverse probability weights. Specifically, time and baseline confounders were predictors in the models for the treatment weight numerator and denominator, with time-varying confounders affected by prior exposure also included in the latter. The censoring weight numerator and denominator models, as well as those for the visit attendance weight numerator and denominator, were identical to the treatment weight models except for the additional inclusion of time-varying HAART as a predictor in the two censoring models, and both time-varying HAART and time since last visit in the two visit attendance models. The combined weights were then applied in a linear regression model with cumulative HAART exposure and baseline confounders as independent variables and CD4 percentage as the outcome, using only observations with a measured CD4 percentage result, to estimate the parameters of a linear repeated measures MSM. To account for within-subject correlation induced by weighting and resulting from repeated CD4 percentage measurements in individuals, and to yield 95% confidence intervals (CIs) based on robust variance, the model was fitted using generalized estimating equations with an independent working covariance matrix. If children received uninterrupted HAART during follow-up, plausible in our program because physicians routinely assessed adherence and never discontinued treatment for active patients, and assuming correct model specification along with no unmeasured confounding or informative censoring, the MSM yields the difference in mean CD4 percentage had children started HAART immediately compared to had they never started HAART during follow-up.

A posited directed acyclic graph[140] and previous studies informed confounder selection. Age, gender, and WHO HIV clinical stage and severity of immunodeficiency

were baseline confounders, while time-varying confounders affected by prior exposure were cotrimoxazole prophylaxis, HIV-related symptoms or conditions, and CD4 percentage. Age and CD4 levels were used to calculate age-specific severity of immunodeficiency (not significant, mild, advanced, or severe), based on WHO guidelines.[105] For all children, including those for whom follow-up started at first CD4 result rather than HIV care initiation, HIV clinical stage (1-4) was that at enrollment because it was assessed only at first visit. A child had HIV-related symptoms or conditions if diagnosed with one or more of the following: Kaposi's sarcoma, oral or esophageal candidiasis, severe weight loss, tuberculosis, fever or diarrhea of one month or more, lymphocytic interstitial or *Pneumocystis jirovecii* pneumonia, chronic herpes simplex, oral hairy leukoplakia, cryptococcal meningitis, toxoplasma or HIV encephalopathy, or HIV-associated nephropathy. Gender, cotrimoxazole, and symptoms or conditions were coded dichotomously, as was HAART in logistic models. In linear models, cumulative HAART exposure was coded into indicators for six month categories, that is, no HAART, >0 to 6 months, >6 to 12 months, and so on through a maximum of 66 months. Age and time were modeled as restricted cubic splines with four knots at the 5^{th}, 35^{th}, 65^{th} and 95^{th} percentiles, as was CD4 percentage when a time-varying confounder affected by prior exposure. CD4 percentage, which was examined because it is a more stable metric than absolute CD4 count in children,[190] was expressed as a whole number when an outcome in linear models.

Results from unadjusted as well as adjusted but unweighted linear repeated measures models were examined for comparison to elucidate reductions in bias by use of weighted models. To estimate the effect of HAART on CD4 percentage by baseline

categories (CD4 percentage <15%, 15%–24%, and ≥25%), models were fitted within those strata. We used the log-rank test after constructing Kaplan-Meier plots and Cox proportional hazards regression to assess whether time to reaching "not significant" WHO age-specific immunodeficiency differed by degree of immunodeficiency (mild, advanced, severe) at HAART initiation. The proportionality of hazards was verified by visual inspection of log-negative-log survival estimates. All analyses, including a macro[146] to generate cumulative incidence curves[191] to visually depict time to immunological recovery, were completed in SAS version 9.2 (SAS Institute, Cary, NC).

Results

Characteristics of the 790 children at the start of and during follow-up are presented in Table 1. At baseline, the median age was 5.9 years (interquartile range [IQR]: 2.7, 9.8) and more than half (405, 51.3%) had clinically evident stage 3 or 4 HIV disease. As the median CD4 percentage was 15 (IQR: 9, 22), immunodeficiency was either advanced (76, 9.6%) or severe (452, 57.2%) in most patients. Cotrimoxazole was initiated by almost all children (726, 91.9%) at first visit.

During the 2089.8 person-years accrued, the median duration of follow-up was 31.2 months (IQR: 10.3, 53.6), and the median number of clinic visits was 30 (IQR: 11, 57). The number and proportion of patients active in HIV care for at least 12, 24, 36, 48, and 60 months were 573 (72.5%), 440 (55.7%), 352 (44.6%), 279 (35.3%), and 127 (16.1%), respectively. Eighty children (10.1%) died, 76 (9.6%) were lost to follow-up, and six (0.8%) transferred to another care facility. HAART was started by 619 children (78.4%) at a median of 4.0 months (IQR: 2.2, 15.3) after baseline. At initiation, the

median CD4 percentage was 12 (IQR: 7, 18) and 454 children (73.3%) were severely immunosuppressed. Durations of HAART during follow-up and the number of CD4 percentage measurements during each of the six month periods are depicted in Figure 5. HAART was received by 167 children (27.0%) for >0-12 months, 177 (28.6%) for >12-36 months, and 275 (44.4%) for >36 months. Those receiving HAART, 110 (17.8%) of whom changed regimens because of treatment failure or an adverse event, were followed for a median of 31.3 months (IQR: 11.4, 52.0) and contributed 1620.9 person-years (77.6% of the total) while on HAART. Of the 3137 total CD4 percentage measurements, 2485 (79.2%) were obtained during receipt of HAART.

According to the MSM, HAART resulted in a 6.8% (95% CI: 4.7%, 8.9%) absolute rise in mean CD4 percentage in the first six months and a 8.6% (95% CI: 7.0%, 10.2%) gain at 12 months, relative to no HAART (Table 2, model 4; Figure 6, panel A). The rate of increase was less in subsequent months and years. Not properly accounting for time-varying confounders affected by prior exposure yielded attenuated effects (Table 2, models 1-3). For comparison to the appropriately adjusted model-based results, we also plotted the observed, unadjusted CD4 percentage evolutions for children receiving HAART and children not receiving HAART (Figure 6, panel B).

The effect of HAART on CD4 percentage differed by category of CD4 percentage at baseline (Figure 7). In children with a baseline CD4 percentage ≥ 25%, CD4 percentage rose by almost 10% (95% CI: 7.4%, 12.4%) in the first 12 months, to a mean of 40%, and stabilized. In children with a baseline CD4 percentage of 15%–24%, CD4 percentage increased rapidly in the first 12 months, to a mean of 29%, with more gradual

increases thereafter. Gains were slower in children with a baseline CD4 percentage <15%.

Only 83 of 619 children (13.4%) had "not significant" WHO age-specific immunodeficiency at time of HAART initiation. There was a reduced hazard of recovery to "not significant" immunodeficiency (unadjusted hazard ratio: 0.62; 95% CI: 0.40, 0.96) and a corresponding longer time to recovery (log-rank p=0.03) if HAART was initiated when immunodeficiency was severe rather than mild or advanced. In the 82 children who initiated HAART with either mild or advanced immunodeficiency, the cumulative incidences of recovery to "not significant" immunodeficiency at 12, 24, and 36 months were respectively 28.5%, 36.3%, and 42.7%, while the proportions in the 454 children who started HAART with severe immunodeficiency were 17.5% by 12 months, 26.0% by 24 months, 27.7% by 36 months, and 29.9% by 48 months (Figure 8).

Discussion

HAART markedly increased the mean CD4 percentage in a cohort of HIV-infected children in Kinshasa, DRC compared to no HAART, by 8.6% (absolute) at 12 months and 20.5% at 60 months of HAART. Our study provides the first estimate of the effect of HAART on CD4 percentage relative to no HAART among antiretroviral-naïve children in a resource-deprived setting and just the second estimate of this informative contrast overall.[95] This was achieved by using inverse probability weighting to account for evident biases. Additionally, our study is one of the few providing evidence of sustained CD4 percentage improvements following HAART initiation in children.[63,186]

Our findings that HAART increased CD4 percentages in children over the short and long term, both overall and within baseline CD4 percentage categories, were generally concordant with those from the methodologically similar U.S. study.[95] That study reported lesser gains throughout follow-up (e.g., 2.3% and 4.4% at 12 and 60 months, in contrast to 8.6% and 20.5%). This could be due to the large difference in the baseline CD4 percentage distributions of the study populations, as the median baseline CD4 percentage was approximately 25% in the U.S. cohort compared to 15% in the Kinshasa cohort. A higher baseline CD4 percentage results in lower potentials for gains. The difference was also present within strata, and may contribute to the greater and more rapid change we noted among children with a baseline CD4 percentage ≥25%.

Among children with a baseline CD4 percentage <15%, the CD4 percentage increases at 12 and 24 months of HAART (4.0% and 10.0%) were close to those noted in the U.S. study (4.4% and 8.0%). In these children, the mean CD4 percentage recovered by 42 months to "normal" levels (>25%).[95,168,176] While this indicates that recovery is possible for children initiating HAART with advanced immunodeficiency, CD4 percentages remained below 20% even after 24 months. The prolonged period of immunodeficiency likely contributes to prolonged morbidity and mortality during HAART. The decreased probability of immunological treatment success following initiation at low CD4 levels revealed in our and prior studies,[95,114-116,166] as well as high mortality rates in children initiating HAART at low CD4 percentages,[102-104,131] provide compelling evidence that pediatric HAART should not be delayed until CD4 percentages drop below 15%. The newly revised WHO guidelines are in accordance with this evidence.[157]

Not being able to include HIV viral load as a confounder was a limitation, as was the lack of diagnostic capacity in Kinshasa to identify all HIV-related symptoms and conditions. The appropriateness of the methodological approach, including accounting for the dynamic visit schedule and its potential to influence result validity,[143] was supported by the dissimilar results from weighted and standard unweighted models. Exposure misclassification was minimized through accurate recordkeeping of initiation dates and frequent monitoring of adherence. However, because virological monitoring was infrequent and drug options were limited in our setting, the possibility exists that some children recorded as receiving HAART were actually failing therapy, which may have biased our estimates downward. We also examined immunological recovery by severity of immunodeficiency and not just CD4 percentage categories.

Our results build upon prior evidence showing the positive immunological impact of pediatric HAART across CD4 levels and supporting therapy initiation prior to immune system degradation. By contributing unique, relevant information on long term responses in a resource-deprived setting, this study widens the breadth of understanding on a relationship that is central to the morbidity and mortality outcomes of HIV-infected children. Future efforts should focus on pooling data from similar cohorts in order to more precisely estimate effects within immunological subgroups and even longer after HAART begins.

Table 5. Characteristics of 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.

BASELINE [a]	
Median age, years (IQR[b])	5.9 (2.7, 9.8)
Age [n (%)]	
<1	63 (8.0)
1–4	277 (35.1)
5–9	265 (33.5)
10–17	185 (23.4)
Female sex [n (%)]	415 (52.5)
WHO[b] HIV clinical stage [n (%)]	
1	153 (19.4)
2	232 (29.4)
3	369 (46.7)
4	36 (4.6)
Median CD4 percentage (IQR[b])	15 (9, 22)
WHO[b] age-specific severity of immunodeficiency [n (%)]	
Not significant	174 (22.0)
Mild	88 (11.1)
Advanced	76 (9.6)
Severe	452 (57.2)
Started cotrimoxazole at first visit [n (%)]	726 (91.9)
FOLLOW-UP (ALL CHILDREN)	
Total person-years accrued	2089.8
Median months of follow-up (IQR[b])	31.2 (10.3, 53.6)
Median number of program visits (IQR[b])	30 (11, 57)
Died [n (%)]	80 (10.1)
Lost to follow-up [n (%)]	76 (9.6)
Transferred care [n (%)]	6 (0.8)
Initiated HAART	619 (78.4)
Number of CD4 percentage results	3137
FOLLOW-UP (CHILDREN INITIATING HAART)	
Median weeks of follow-up prior to HAART initiation	4.0 (2.2, 15.3)
Severe immunodeficiency at HAART initiation [n (%)]	454 (73.3)
Median CD4 percentage at HAART initiation (IQR[b])	12 (7, 18)
HAART person-years accrued	1620.9
Median months on HAART (IQR[b])	31.3 (11.4, 52.0)
Switched HAART regimen [n (%)]	110 (17.8)
Number of CD4 percentage results during HAART	2488

[a] Baseline was shifted from enrollment to date of first CD4 percentage result for 41 of 790 children (5.2%). For these 41 children, the median number of months from enrollment to first CD4 percentage was 2.3 (IQR: 1.1, 5.3).

[b] IQR, interquartile range; WHO, World Health Organization.

Table 6. Estimated effect of HAART on CD4 percentage, 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.[a]

	Absolute change in CD4 percentage from baseline (95% CI[b])					
MODEL	>0 to 6 months of HAART	>6 to 12 months of HAART	>18 to 24 months of HAART	>30 to 36 months of HAART	>42 to 48 months of HAART	>54 to 60 months of HAART
1. Unweighted, unadjusted (no confounders)	0.3 (-1.5, 2.2)	1.2 (-0.1, 2.6)	4.0 (2.3, 5.6)	3.4 (1.2, 5.6)	3.6 (0.7, 6.5)	3.7 (-0.5, 7.8)
2. Unweighted, adjusted (baseline confounders)	4.2 (2.2, 6.1)	5.7 (4.1, 7.3)	9.9 (7.9, 11.9)	10.8 (8.2, 13.4)	12.6 (9.6, 15.7)	16.3 (12.0, 20.5)
3. Unweighted, adjusted (baseline and time-varying confounders)	8.4 (6.7, 10.2)	8.4 (7.3, 9.4)	5.0 (3.8, 6.1)	3.6 (2.2, 5.1)	4.2 (5.8, 4.2)	4.8 (2.6, 6.9)
4. Weighted, adjusted[c] (baseline confounders)	6.8 (4.7, 8.9)	8.6 (7.0, 10.2)	12.7 (10.7, 14.7)	13.4 (10.9, 15.9)	15.9 (12.8, 19.0)	20.5 (16.1, 24.9)

[a] All estimates are derived from repeated measures linear models, fit with generalized estimating equations, that include time modeled as a restricted cubic spline with four knots.

[b] CI, 95% confidence interval.

[c] Marginal structural model. Weighting appropriately accounts for time-varying confounders affected by prior exposure.

Figure 5. Duration of HAART (bars) and number of CD4 percentage measurements during each of the six month periods (lines) in 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.

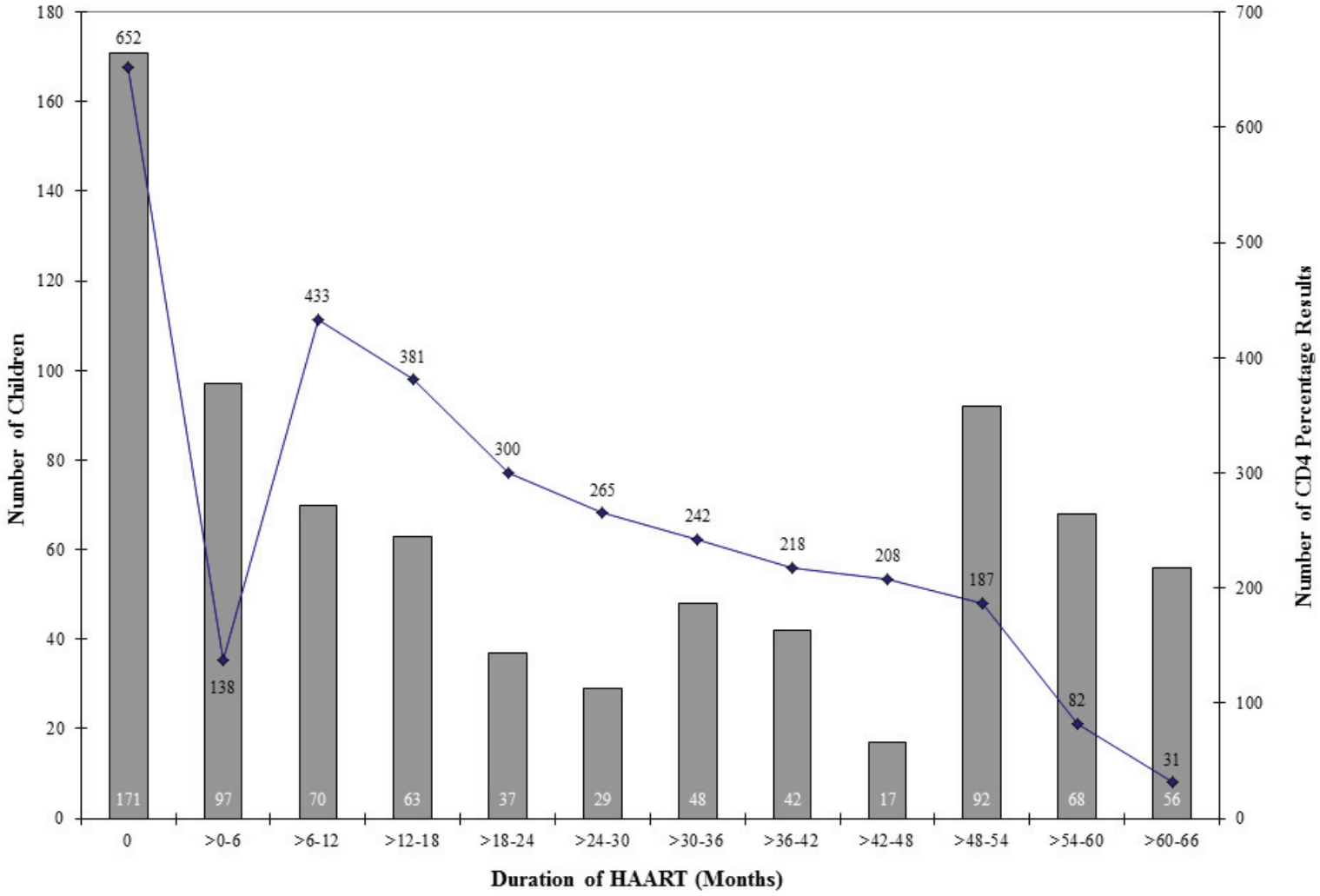
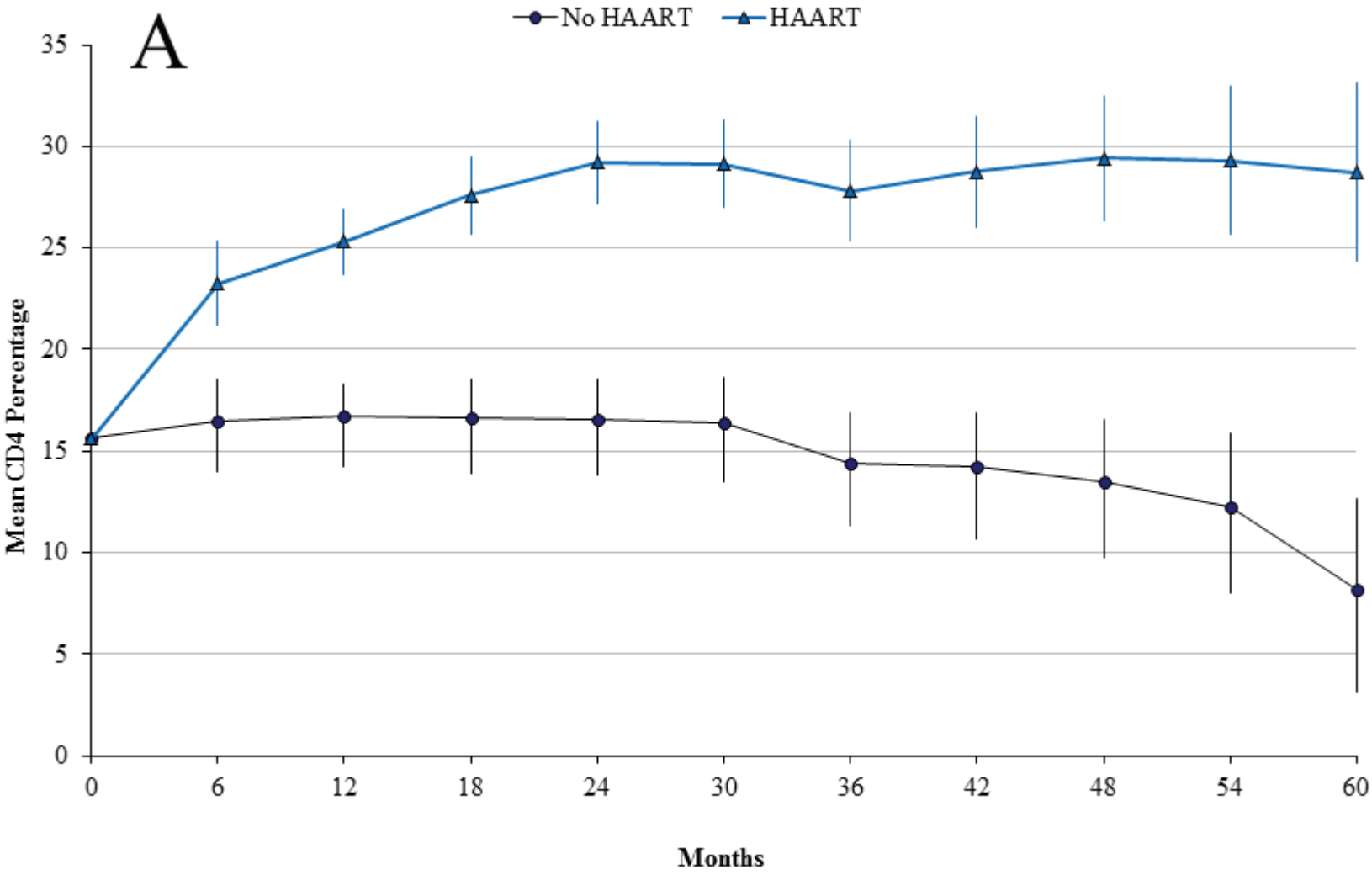
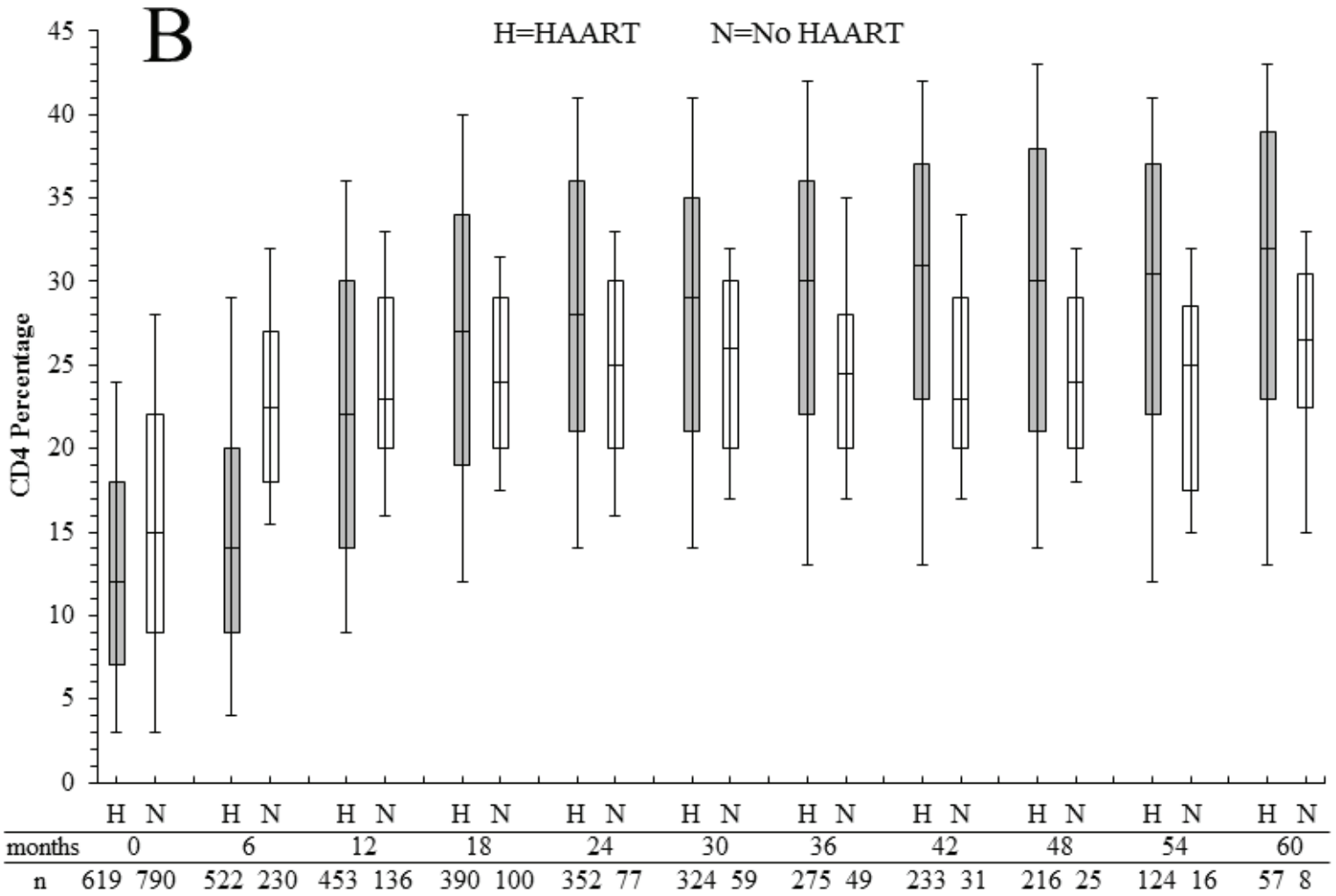


Figure 6. Estimated effect of HAART on CD4 percentage from marginal structural model (Panel A)[a] and observed, unadjusted CD4 percentage evolutions among children receiving HAART and children not receiving HAART (Panel B)[b],790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.

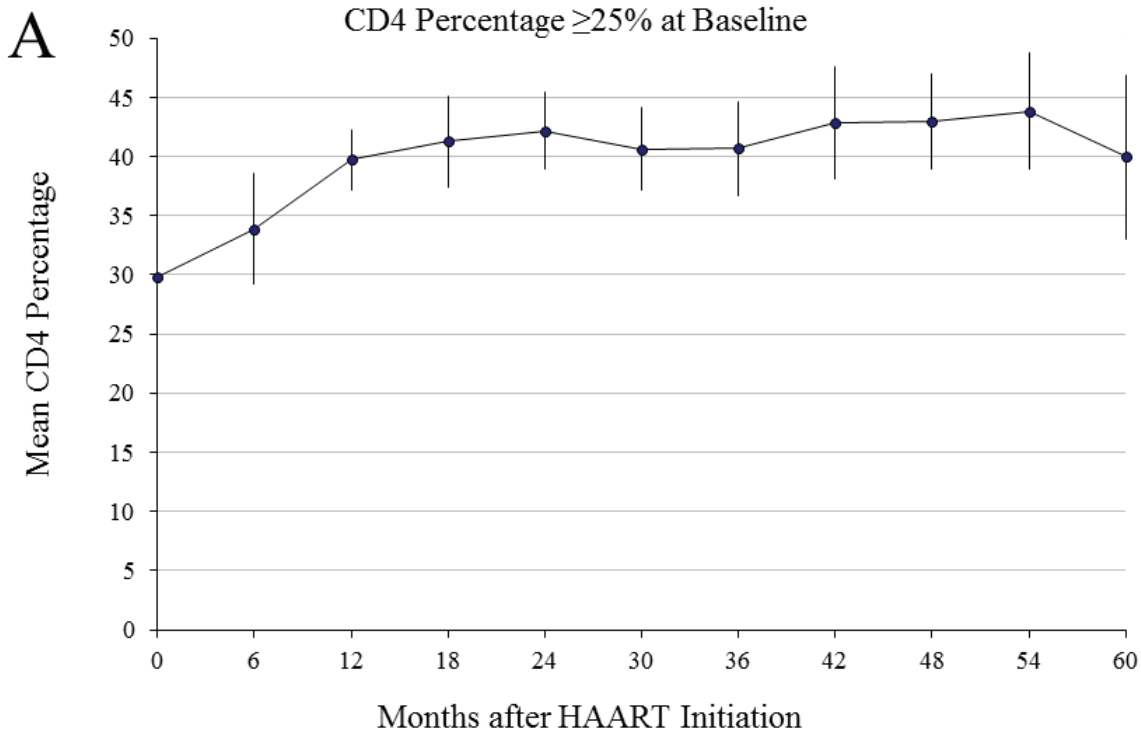


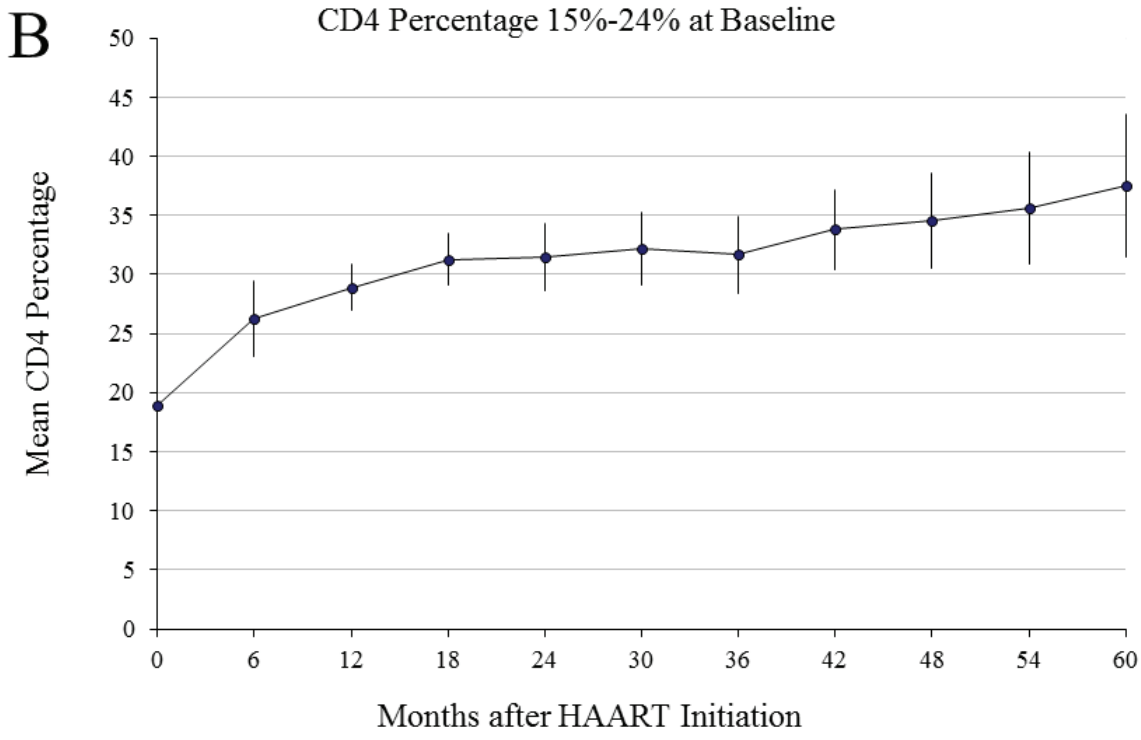


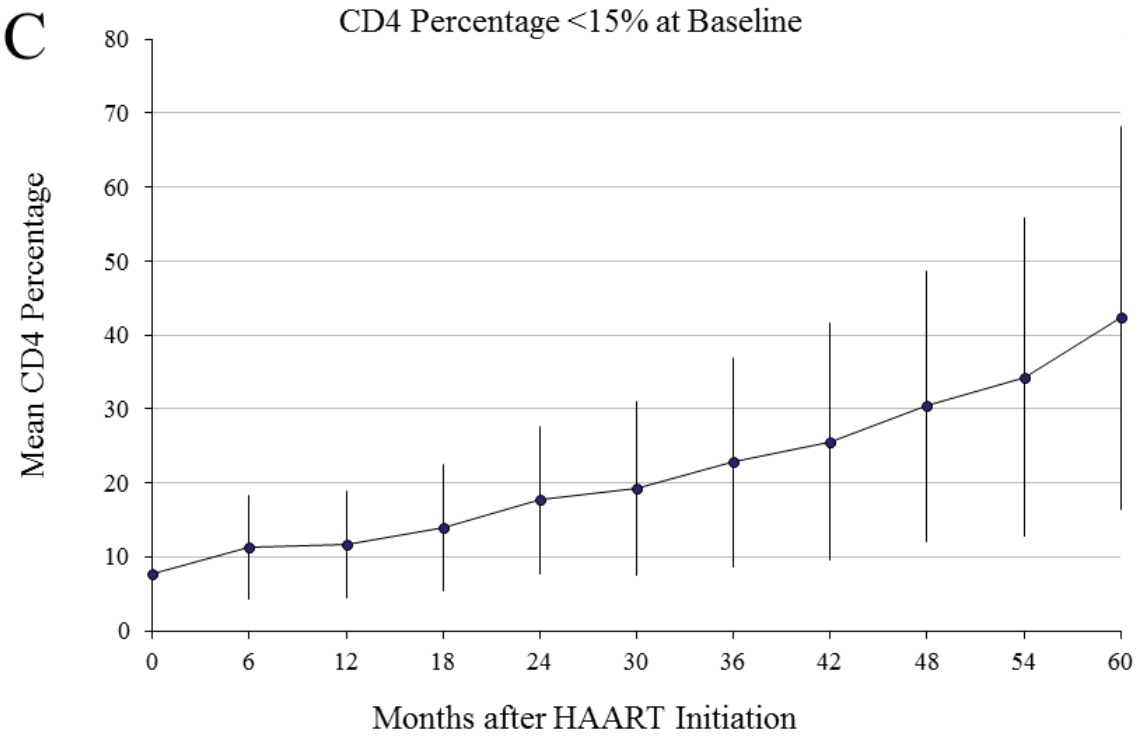
[a] To plot CD4 percentages among children not receiving HAART, time was coded categorically (six month periods).

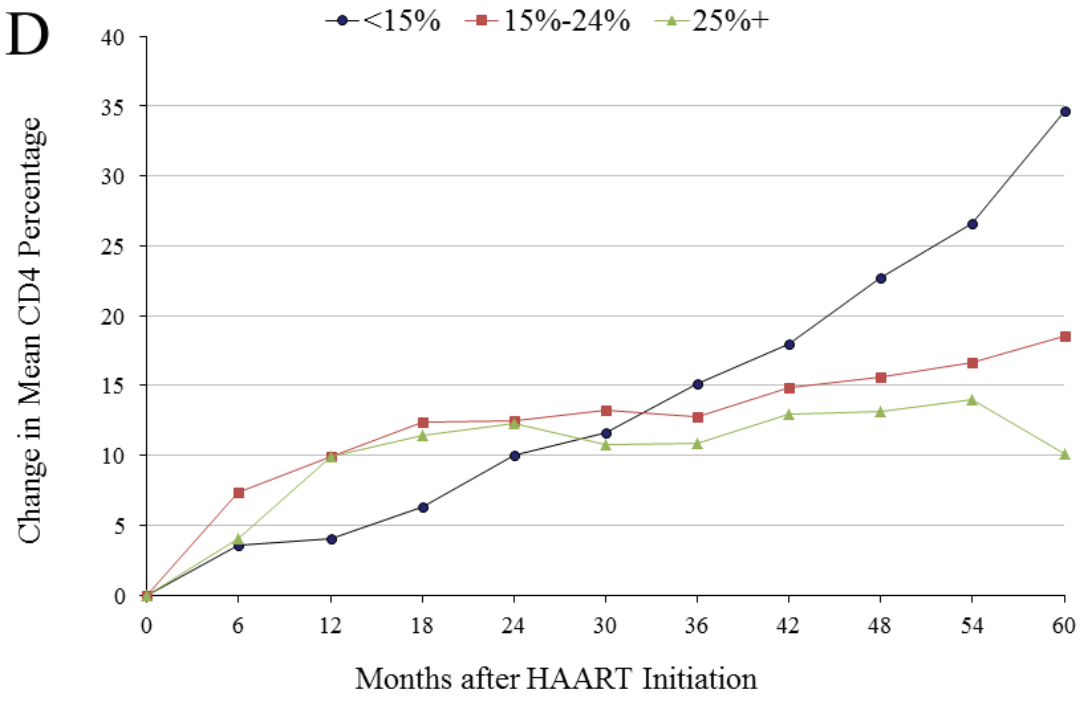
[b] The time scale for children not receiving HAART is time since the start of follow-up, while the time scale for children receiving HAART is time since HAART initiation. Each box plot depicts the median and interquartile range, with the error bars marking the 10th and 90th percentiles. The most recent CD4 percentage was carried forward to each six month cut point.

Figure 7. Estimated effect of HAART on CD4 percentage, by category of CD4 percentage at baseline, 790 children initiating HIV care between December 2004 and May 2010 in Kinshasa, DRC.[a]



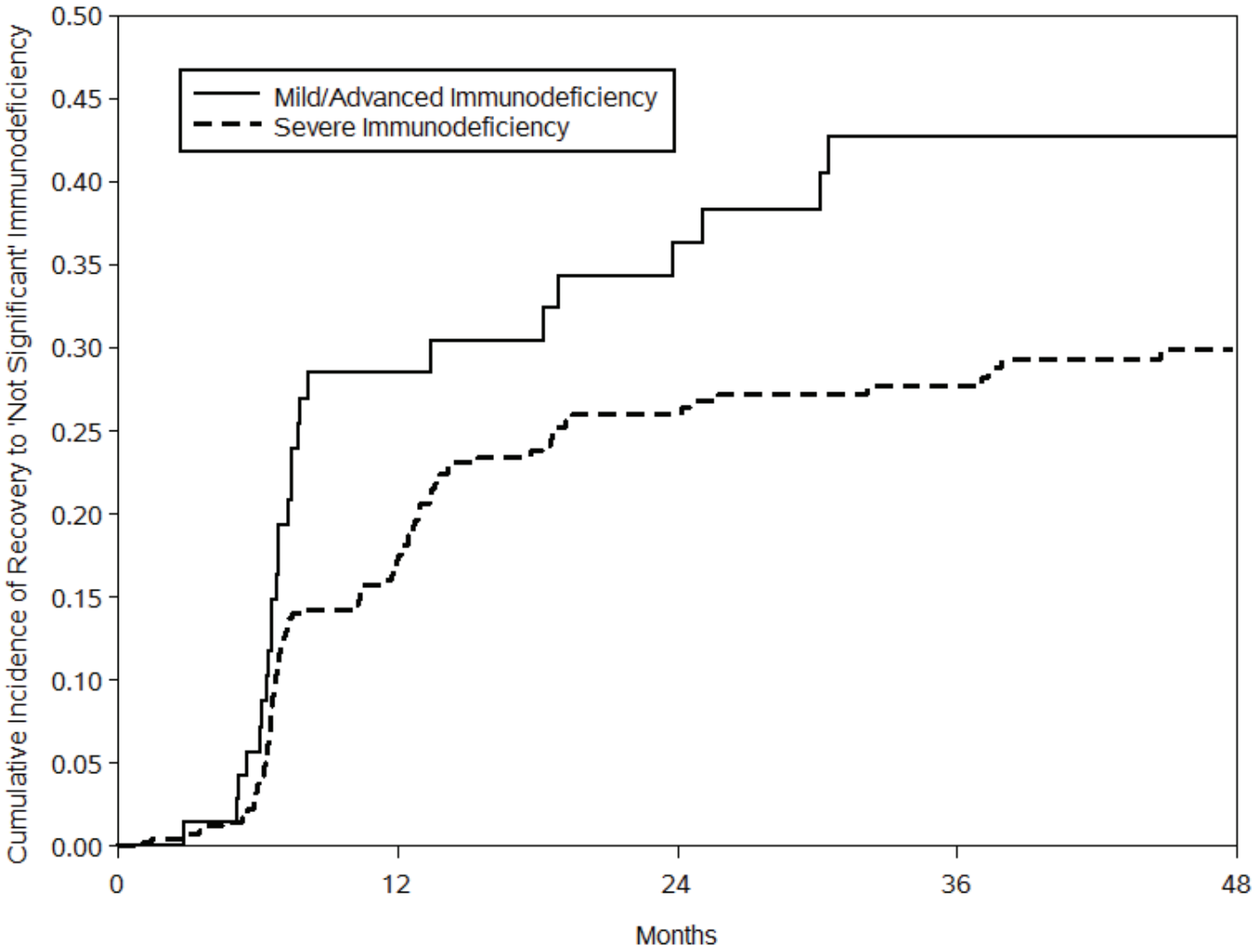






[a] Estimates are from repeated measures linear models, fit with generalized estimating equations, that include time modeled as a restricted cubic spline with four knots. As there were no discernable upward or downward trends in CD4 percentage over time among children not receiving HAART when spline terms were included as predictors in the stratum-specific models, it is assumed that the cumulative HAART exposure parameters represent change from baseline. Error bars represent 95% confidence intervals.

Figure 8. Cumulative incidence curves of recovery to “not significant” WHO age-specific immunodeficiency, by category of suppression at the start of HAART, 536 children initiating HAART with mild, advanced, or severe immunodeficiency between December 2004 and May 2010 in Kinshasa, DRC.



VI.

DISCUSSION

Summary of Findings

Data from an observational cohort of HIV-infected children participating in a care and treatment program in Kinshasa, DRC were used to address three specific aims. The first two aims focused on estimation of the effects of HAART. The outcome examined in the first aim was survival, while the goal of the second aim was to quantify the effect of HAART on CD4 percentage, a marker of immunological progression, both overall and by baseline category of CD4 percentage. The third aim was to determine whether the degree of immunodeficiency upon commencement of HAART was associated with eventual recovery to "not significant" WHO age-specific immunodeficiency.

The effects of HAART on survival and CD4 percentage among children in the resource-deprived setting of Kinshasa were similar to those previously noted among children in the United States. Contrasting HAART to no HAART, the HR for mortality was 0.25 (95% CI: 0.06, 0.95), and the estimated absolute CD4 percentage improvement was 6.8% (95% CI: 4.7%, 8.9%), 8.6% (95% CI: 7.0%, 10.2%), and 20.5% (95% CI: 16.1%, 24.9%) after six, 12, and 60 months of HAART, respectively. Across baseline CD4 percentage categories, HAART led to short and long term immunological gains, although the rise was slower among children who had a baseline CD4 percentage <15. If HAART was started when immunodeficiency was severe rather than mild or advanced,

there was a lower cumulative incidence of recovery to "not significant" WHO age-specific immunodeficiency at 12, 24, and 36 months after HAART initiation.

Contributions of Findings

There are several ways that this work extends the scope of the literature on the effects of HAART on HIV disease progression. The study is unique not only because of its pediatric focus; it is also highly and increasingly relevant because of its focus on children who, like most individuals receiving HAART globally, are treated in a resource-deprived setting. The study examined survival as well as short and longer term immunological outcomes, and improved upon prior research by using an antiretroviral-naïve rather than a fully or partially non-HAART comparison group. Given that answers to questions concerning the effects of HAART in children are ethically unobtainable via randomized trial, the specific aims were assessed by utilizing observational data in concert with appropriate epidemiological methods such as MSMs estimated by inverse probability weighted regression.

The provision of HAART in resource-deprived environments is fraught by distinct challenges that may impact outcomes. This study, which was timely and pertinent because HAART rollout in the developing world will continue to increase in coming years if current trends continue,[30] helped to mitigate context-specific substantive gaps. For instance, given that findings emerged from a program with limited diagnostic and laboratory capacity that serves a population faced by prevalent comorbidities and undernutrition, the research informs expectations for the effects of HAART on survival and CD4 percentage in such environments. In doing so, it may help to maintain the

momentum for the provision of pediatric HIV care and treatment in areas where it is most urgently needed. For example, the general finding that the effectiveness of HAART in Kinshasa, according to the metrics examined, was similar to that in more developed settings serves as empirical evidence to support the scale-up of programs in areas that are, relatively speaking, less resourced. This is particularly important given the current global economic downturn and the 2010 UNAIDS report[1] which states that HIV has "halted" and "begun to reverse," which together combine as an unfortunate pretext for the rationalization of decreased financial commitment to fight the epidemic. The UNAIDS report[1] notes that "growth in investment for the AIDS response has flattened for the first time," and a report published by *Médecins Sans Frontières* (MSF) entitled "Punishing Success: Early Signs of a Retreat from Commitment to HIV Care and Treatment" warns that the Global Fund to Fight AIDS, Tuberculosis and Malaria funded 35% fewer proposals in 2009 than in 2008 and that PEPFAR has capped spending despite increasing outlays during the past five years.[192] An HIV/AIDS policy adviser for MSF cites increasing stock-outs of antiretroviral drugs and a "significant gap" between the calculated needs of countries over the next decade and pledged donor contributions.[193]

While no single factor can stem the tide of decreasing financial support for anti-HIV initiatives, evidence that HAART can be effective in settings where it is most widely required, like that from this study, plays an essential beneficial role. This work, due to the simple fact it included nearly 1,000 children who were not receiving care before their enrollment into the Kinshasa program, is illustrative of the reality that profound needs endure even while HIV incidence and prevalence is stabilizing or declining in many areas.[1] In the DRC alone, there are an estimated 60,000 women each year needing of

prophylactic antiretroviral drugs to decrease the risk of vertical transmission, though access remains extremely limited.[194] Worldwide, the annual number is nearly 1.4 million, with only half of women having access to services.[30] Approximately three in four of the 1.3 million children in low-and middle income countries who need HAART are not receiving it.[30] For the foreseeable future, pediatric HIV will remain a pressing public health issue that demands a sustained and systematic response.

This study meaningfully contributes to the ongoing debate on when pediatric HAART should be started. It was revealed that the immunological response to HAART was unequal across baseline CD4 percentage categories, with recovery more gradual in the lowest CD4 percentage group, and that greater immunodeficiency at HAART initiation was associated with impaired recuperation to the "not significant" level. These findings suggest that HAART should be started earlier rather than later, and could be used to inform antiretroviral guidelines. Thus, they could have downstream effects on clinical practice and the delivery of care, in addition to policy.

There are several related points worth noting. First, it is possible to investigate the question via other observational means such as the application of methods to mimic trials of immediate versus deferred HAART, as previously described. However, it is still informative, as emphasized by the multitude of cited studies that have done so, to examine whether experiences differ across baseline CD4 strata. Second, this study bolsters a knowledge base in children that is narrow, particularly in resource-deprived settings. The WHO pediatric antiretroviral guidelines[105,157] are based on observational data from resource-privileged contexts that examined how CD4 levels influenced survival outcomes among children who were untreated or receiving AZT monotherapy,[195,196] and

call for research in resource-deprived settings to evaluate the appropriateness of their thresholds for starting HAART. This study responds to that call, and augments the currently available evidence which is described as "very low quality" by WHO. Third, longer term immunological recovery was examined, an aspect that is crucial because this endpoint, in addition to clinical outcomes, is an important marker of the success of HAART. Simply stated, this work helps to widen the breadth of what is known about an issue that remains only partially understood.

These findings may also contribute to future cost-effectiveness and mathematical modeling analyses. The authors of a recent study of the causal effect of HAART on mortality in adults[61] express that it "would be highly desirable to have a reliable estimate of the effect of cART on the overall survival for public health planning and to inform HIV modeling and cost/effectiveness calculations." The estimates from Kinshasa could be used for these purposes as well. For example, the survival results have utility in years of life lost calculations, and if the finding that it takes longer for children with lower baseline CD4 percentages to attain "safe" levels is assumed to be true, the risks of death and clinical progression while immunodeficiency is high could be weighed against the risk of adverse events arising from receiving HAART for a period of many years.

Strengths and limitations

This study, like any other, featured various strengths and limitations. One limitation was that data on HIV viral load, a factor that fits the definition of a time-dependent confounder affected by exposure, were extremely sparse as a result of logistical and infrastructural constraints in the DRC, and therefore could not be

incorporated into the analysis. While some studies had the luxury of including this variable,[55,58-61,93,94] many were unable to do so because they used data from an era[54,63,92,95] or setting[62,133] where this biomarker was not measured. While it is acknowledged that the effects of HAART on survival and CD4 percentage may have been underestimated if the few available virological results were used to inform HAART initiation decisions, it is encouraging that the methodologically strongest previous study of children juxtaposed their overall findings with those from a sub-analysis that accounted for viral loads in the 36% of the study population that had virological data, and found that the effect of HAART on survival was virtually unchanged.[63]

On a related note, it can also be argued that clinical status (e.g., WHO HIV clinical stage) is a time-dependent confounder affected by exposure. Similar to viral load, some studies account for this factor,[55,59,63,94,95] while most others (including some that account for viral load) do not.[54,60-62,92,93,133] In the Kinshasa care and treatment program, WHO HIV clinical stage is assessed only at enrollment. While it theoretically would have been possible to assign a clinical status value to every visit by abstracting and coding physicians' past observations, this was not practically feasible given that more than 20,000 records would have needed to be reviewed.

It is also worth putting into perspective the amount of person-time that this study featured. While most of the highlighted MSM analyses included more person-time than did this study, it is worth noting that several studies involved a comparable amount of collective follow-up,[58,132] and several others were published with considerably less.[60,94,133] The only other study from a sub-Saharan African setting, of adults, included many more individuals (14,267) than this cohort, but the median follow-up duration was

only four months.[62] In examining longer term effects of HAART in a resource-deprived context, this study was unique, and also featured the first investigation in children.

Other related studies have lacked exact information on when HAART was initiated (leading to assumptions on actual start dates, and possible biases), featured infrequent follow-up (leading to speculation that data may be misclassified), and included individuals who started HAART but later discontinued it (leading to unclear interpretation of effect estimates). This study improved upon these shortcomings. First, children visited the program no less than quarterly; in fact, most visited monthly. These frequent visits mitigated the downsides of the "carry forward" approach to missing data. Specifically, having subsequent observations that were temporally closer made it easier to tell if a particular value for a given variable, for example HIV-related symptoms or conditions, was coded incorrectly and required correction. Second, the precise dates of HAART initiation were available. Third, barring any undisclosed lack of adherence as counseling is provided at each visit, children remained on HAART once initiated.

The data used in this study were remarkably complete. While the carry forward approach did obviate any gaps in time-dependent covariates, there was very little such filling of missing data collection during follow-up. Data were almost invariably complete on variables including WHO HIV clinical stage, age, and gender, which was vital because complete baseline covariate data was a universal inclusion criterion, as noted in the *Study population* section. To account for the fact that a small percentage of children were not immunologically assessed at enrollment, baseline was reassigned to the first CD4 percentage result when necessary, as in prior related studies.[55,63,93,95,132]

It should be noted that some other similar studies adjusted for additional baseline and time-dependent covariates, such as total white and red blood cell counts and hemoglobin, platelet, and CD8 cell levels. If this analysis were to have included these factors, which may not have been prognostic for HAART in this setting, some individuals would have been excluded due to unavailability of baseline data. For example, as approximately 10% of enrolled children to date do not have an initial hemoglobin measurement, the decision to include hemoglobin would have reduced the proposed study population by a corresponding degree unless missing baseline data were predicted via imputation or assumed to be equivalent to a subsequent measurement, two approaches that are imperfect. No factors highly suspected as confounders were excluded from analysis, even if the amount of missing data at baseline was considerable.

Although featuring many excellent qualities, as described, the data were insufficiently rich to address all possible questions about the effects of HAART on survival and clinical and immunological progression. For instance, the size of the study population and the total amount of person-time was not enough to examine the effects of HAART on survival by levels of baseline immunodeficiency or undernutrition, or the effects of HAART on immunological outcomes within categories of baseline CD4 percentage and undernutrition. Investigating a clinical progression outcome such as AIDS was not possible not only because a non-negligible proportion of children had an advanced disease state at enrollment, and hence would have been excluded from such an analysis, but also because the diagnostic capacity in the DRC is less than that in resource-privileged settings. This means that all qualifying conditions in the AIDS case definition[19] (e.g., cytomegalovirus retinitis, chronic intestinal isosporiasis) could not have

been identified, meaning that the study would have been adversely affected by at least some degree of outcome misclassification. Additionally, it would have been necessary to complete a comprehensive review of physicians' notes for tens of thousands of visits in order to conclude with a high degree of confidence whether a patient had AIDS, a process that is substantially more involved than determining if a checkbox for at least one HIV-related symptom or condition was marked.

Future Research Directions

The above limitations inspire directions for future research, which will be possible if data from pediatric HIV care and treatment programs are pooled, as has been done with adult data from the United States and Europe,[61] or if a program accrues sufficient individuals and person-time. Repeating the same analyses using such a dataset would improve the precision of all estimates and would allow for even longer term assessment of the immunological effects of HAART, both important goals in their own right. Data pooled from other programs, however, would also allow for the investigation of new questions: specifically, those that cannot be pursued with the Kinshasa data alone. These include the effects of HAART on adverse events, on clinical progression, on survival by baseline immunodeficiency and by baseline undernutrition, and on immunological progression by both baseline CD4 percentage and undernutrition.

Undernutrition has been repeatedly alluded to as an important factor in resource-deprived settings, and as a target for future research in terms of its connection with the effects of HAART on survival and clinical and immunological progression. The influence of undernutrition on those associations is only partially understood, even though it is both

common among HIV-infected children and well known to be a determinant of health outcomes. A review of the interplay between HIV and nutrition is thus warranted.

There is little doubt that the connection between nutritional status and HIV is synergistic. The basis for this claim is twofold. First, the course of HIV infection – that is, loss of cell-mediated immunity followed by the development of opportunistic infections – leads to undernutrition, a reality reflected by the classification of wasting syndrome as an AIDS-defining condition.[19] As immunodeficiency worsens, HIV patients often experience chronic diarrhea caused by opportunistic pathogens including *Cryptosporidum parvum*, *Mycobacterium avium* complex, and cytomegalovirus,[197,198] which results in weight loss,[199,200] malabsorption of sugar and fats,[201-203] and small bowel morphology abnormalities that exacerbate malabsorption.[204] Couple these effects with reduced oral intake during acute episodes of opportunistic disease[205,206] in addition to an increased metabolic rate[205,207] and greater protein and micronutrient needs[208] during infection, and it is clear how HIV can lead to undernutrition. Second, even in the absence of the virus, undernutrition results in several of the same clinical and immunological consequences as HIV. On its own, undernutrition is a cause of mortality[209,210] and leads to impairments in immune function.[211,212] Considering these facts collectively, there is a substantive foundation for nutritional status impacting survival as well as clinical and immunological progression within the context of HIV infection, and these relationships have been studied.

There is a growing body of evidence on the associations between nutritional status and outcomes in HIV-infected individuals. The endpoint that has been examined in relation to nutritional status in HIV infection is survival, and this connection is better

understood than the association between nutritional status and immunological outcomes. Several studies focused on patients not receiving ART. In an unadjusted analysis of 514 untreated Zambian children less than 15 years of age who were followed for an average of two years, the hazard of death increased if the baseline weight-for-age Z-score (WAZ), a marker of nutritional status,[213] was lower (i.e., was indicative of more severe undernutrition).[214] In multivariable analysis, for every one standard deviation increase in WAZ, the hazard of death decreased by approximately 20% (HR: 0.83; 95% CI: 0.76, 0.91). Among 48 untreated South African infants, those who died during follow-up (mean, 28.5 months) were more undernourished beginning at three months of age than those who survived.[215] A 1989 chart review of 32 deceased AIDS patients showed that the degree of undernutrition was more severe closer to the time of death;[216] another study in the pre-antiretroviral era followed 39 AIDS patients for a mean of 212 days and found that survival was greatly prolonged if the body cell mass was greater than 30% of body weight.[217] A meta-analysis of 2,510 untreated children from 10 cohorts in resource-deprived settings revealed that low WAZ was predictive of death over 12 months of follow-up, across strata of immunodeficiency.[218]

The influence of nutritional status on survival in the context of HAART has also been evaluated. One study followed 394 treated Singaporean adults, 35% of whom received HAART, for a median of 2.4 years – in multivariable analysis, those with a body mass index (BMI) indicative of moderate or severe undernutrition (less than 17 kg/m^2) at time of HAART initiation, relative to no undernutrition (BMI above 18.5 kg/m^2), were at a higher hazard of death (HR: 2.19; 95% CI: 1.29, 3.73).[219] This finding was corroborated in an analysis of 1,507 Malawian adults, which revealed that individuals were more likely

to die during the first three months of HAART if they were more severely undernourished at time of HAART initiation.[220] Compared to no undernutrition, the adjusted OR of death given mild undernutrition (BMI between 17 and 18.5 kg/m^2) was 2.1 (95% CI: 1.2, 3.8), with adjusted ORs of 2.4 (95% CI: 1.7, 6.3) given moderate undernutrition (BMI between 16 and 17 kg/m^2) and 6.0 (95% CI: 4.6, 12.7) given severe undernutrition (BMI less than 16 kg/m^2). Results were similar in a study that followed 320 HAART-initiating Tanzanians aged 15 years or more for a median of 11 months – relative to no undernutrition, the hazard of death was elevated given severe undernutrition at HAART initiation (HR: 2.12; 95% CI: 1.06, 4.24).[221] Compared to normal BMI, low BMI at HAART initiation has been independently linked to mortality in the first six months of HAART among adults in Malawi (HR: 2.92; 95% CI: 2.04, 4.17)[222] and Zambia (HR: 2.4; 95% CI: 1.8, 3.2).[97] Initial body weight in the lowest gender-specific quartile was associated with death among 910 Haitians aged 13 years or more who started HAART and were followed for a median of 13 months (HR: 3.3; 95% CI: 2.9, 3.7).[223]

In children, a previously described observational study to compare HAART with less potent combination ART suggested that for each one standard deviation increase in WAZ at time of initiation, the hazard of death was reduced by approximately 20% (HR: 0.82; 95% CI: 0.61, 1.10).[75] Severe wasting was associated with mortality during the first three months of HAART in 439 Malawian children,[224] and each one standard deviation increase in baseline WAZ decreased the hazard of death during the first year of HAART by over half among 299 children in the DRC (HR: 0.39; 95% CI: 0.26, 0.57).[125] An increasing hazard of death across four levels of decreasing baseline WAZ was noted

among 2,398 Zambian children who started HAART and were followed for a median of 378 days,[102] and in 236 Haitian children initiating HAART who were followed for a median of 20 months, a baseline WAZ more than three standard deviations below the median, compared to less undernourishment, was associated with an increased hazard of death (HR: 2.07; 95% CI: 1.39, 3.10).[103]

Evidence on the influence of nutritional status on immunological outcomes is more sparse. One study that examined the impact of nutritional status on survival also evaluated changes in CD4 counts at six and 12 months after initiation of HAART or less potent ART, and found that baseline BMI was not associated with immunological response.[219] This result was echoed in a study of 1,458 children in the United Kingdom, Ireland, and Uganda in which WAZ at time of HAART initiation was unrelated to a CD4 percentage change of at least 10% six months later.[225] However, among adults in South Africa, low baseline BMI was associated with poor immunological response one year after initiation of HAART.[96]

All told, the preponderance of evidence points towards a deleterious effect of undernutrition on survival in both the absence and presence of ART, with far less known about how nutritional status influences CD4 levels. While studies to measure the clinical progression and immunological effects of HAART among children in resource-deprived areas are nonexistent, the impacts of nutritional status on survival have been examined in such a setting. However, it should be noted that these investigations, like the majority of observational studies to assess the immunological response to HAART, have focused only on treated subsets – the effects of differential nutritional status in the context of HAART have been quantified, but the effects of HAART in the context of differential

nutritional status have not. This is a subtle distinction, but quantifying how HAART works across degrees of nutritional status, in addition to the existing knowledge of how individuals with varying nutritional status progress during HAART, would substantively contribute to the debate on when to start HAART with regards to nutritional status.

In its pediatric antiretroviral guidelines,[105,157] the WHO suggests delaying HAART until severe undernutrition is stabilized but admits that this recommendation is based on expert opinion and not actual data. If studies indicate that HAART is less effective in the presence of undernutrition, this would support calls for adjuvant nutritional supplementation for HIV-infected children[226,227] and perhaps even the delay of HAART in the presence of poor nutritional status, a path at odds with the conclusion of a recent study in untreated Zambian children that revealed substantial CD4 losses during successful nutritional rehabilitation.[228] Such information could valuably inform policy, especially since a trial in severely malnourished children to compare immediate HAART without nutritional supplementation to immediate or delayed HAART plus nutritional supplementation is ethically untenable. The WHO[105,157] and the Blantyre Working Group[227] have stated that further research on the effectiveness of ART in severely undernourished children is urgently needed.

The information presented in this section collectively informs hypotheses for future studies – it is speculated that children with greater undernutrition at HAART initiation would have an increased hazard of death, clinical progression, and not attaining immune recovery. While no studies have examined how baseline nutritional status influences the survival, clinical progression, and immunological effects of HAART, substantive knowledge of the consequences of undernutrition (e.g., deleterious survival

and immunological impacts) informs the hypotheses that lesser undernutrition would be associated with a greater reduction in the hazard of death and clinical progression by HAART, and that greater undernutrition would be associated with a reduced increase in CD4 percentage at all time points. If future studies reveal, for example, that HAART unequally affects progression across levels of undernutrition and that undernourished children have poorer post-HAART outcomes, they would not only encourage care and treatment programs to prioritize and provide nutritional supplementation. They would also offer essential empirical evidence that HAART initiation decisions should consider nutritional status, and support the expert opinion that HAART might best be delayed in the presence of undernutrition.

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