cART INITIATION DURING PREGNANCY AMONG HIV-INFECTED WOMEN IN LUSAKA, ZAMBIA: THE IMPACT OF DURATION OF cART ON PREGNANCY OUTCOMES AND PREDICTING POSTPARTUM LOSS TO FOLLOW UP

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

Chapel Hill
2015

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ABSTRACT

Angela Marie Bengtson: cART initiation during pregnancy among HIV-infected women in Lusaka, Zambia: the impact of duration of cART on pregnancy outcomes and predicting postpartum loss to follow up
(Under the direction of Audrey Pettifor)

Treatment guidelines for HIV-infected pregnant and breastfeeding women have shifted in recent years towards lifelong maternal cART. These changes have led to important reductions in MTCT and are expected to improve maternal HIV outcomes. However, the increasing use of cART during pregnancy has led to challenges. cART's role in causing adverse pregnancy outcomes has been of concern, as has improving postpartum retention in care. In Aim 1a, we evaluated duration of cART during pregnancy's association with LBW due to growth restriction. We found no evidence of an increased risk of LBW for women receiving cART for ≤8 weeks (RR 1.22, 95% CI: 0.77, 1.91), 9-20 weeks (RR 1.23, 95% CI: 0.82, 1.83), or 21-36 weeks (RR 0.87, 95% CI: 0.22, 3.46), compared to women who never initiated treatment. In Aim 1b, we examined the association between duration of cART during pregnancy through 32 weeks gestation with SGA and preterm birth. We used MO to address measurement error in gestational age. In the complete-case analysis, there was no evidence of an association between duration of cART and SGA or preterm birth. When MO was performed, RRs for SGA moved closer to and past the null, but remained imprecise. For preterm birth, RRs for 9-32 weeks of cART moved away from the null as the variance due to measurement error increased. In Aim 2, we developed a risk score to predict LTFU at 6 months postpartum among women who initiated cART during pregnancy. We observed that 25% of women were LTFU by 6 months postpartum. A risk score

cut-point of 11, (42nd percentile) had 85% sensitivity (95% CI 0.82, 0.88) and 22% specificity (95% CI 0.20, 0.24) to detect women LTFU. A risk score cut-point of 18, (69th percentile) identified the 23% of women with the highest probability of LTFU and had sensitivity 32% (95% CI 28%, 36%) and specificity 80% (95% CI 78%, 82%). As lifelong cART increasingly becomes the standard of care, efforts to reduce postpartum LTFU and understand the mechanisms by which cART impacts pregnancy outcomes are essential to sustain improvements in PMTCT and maternal HIV outcomes.

For my grandmothers, Clare Whites and Grace Bengtson, who fought for their right to an education so that I wouldn't have to.

ACKNOWLEDGEMENTS

There were so many people who made this dissertation successful. I am grateful to my dissertation committee for their dedication, insight and guidance throughout this process. To my adviser and chair Audrey Pettifor, who made my transition to the Department of Epidemiology possible, and has supported me in countless ways throughout my time at UNC - thank you. To Bill Miller, Daniel Westreich, Ben Chi, Allen Wilcox and Brian Pence, thank you for your input, time and encouragement. I have learned so much from each of you. I would also like to thank colleagues at the Centre for Infectious Disease Research in Zambia (CIDRZ) who collected the data I used for my dissertation research and helped me to understand it during my eight months in Zambia.

My dissertation research was supported by the HIV/STD training grant, which has been an incredible source of support and learning over the past two years – both from Bill Miller and my fellow trainees. To the faculty and staff in the Department of Epidemiology, particularly Nancy Colvin, Carmen Woody and Valerie Hudock, thank you for making this department an incredible place to be for the last five years.

Finally, I want to thank my family and friends for their unconditional love and support. In particular my parents, who taught me to believe I could do anything I put my mind to, and have always been there to encourage me when I set out to take their advice. Thank you to my siblings, nephews, niece and extended family for your support along the way. And to the many amazing friends, both near and far, who have been a part of this journey with me – you have inspired to

do things I never thought I could do, supported me when I was sure that I couldn't do them and celebrated with me when it turned out that I could. Thank you.

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ANC	antenatal care
ART	antiretroviral therapy
BMI	body mass index
cART	combination antiretroviral therapy
CI	confidence interval
CIDRZ	The Center for Infectious Disease Research in Zambia
ECS	The European Collaborative Study
LBW	low birthweight
LTFU	lost to follow-up
MI	multiple imputation
MO	multiple overimputation
MTCT	mother to child transmission
NRTI	nucleoside reverse transcriptase inhibitor
PI	protease inhibitor
PMTCT	prevention of mother to child transmission
RR	risk ratio
SGA	small for gestational age
SSA	Sub-Saharan Africa
WHO	World Health Organization
ZEPRS	Zambian Electronic Perinatal Record System

INTRODUCTION

A. Significance

Nearly 8 million people are now accessing cART in the developing world.[1] In Zambia, antiretroviral coverage for HIV-infected pregnant women is near 80%.[2] Access to cART during pregnancy has long been recognized as effective for reducing MTCT.[3-6] The maternal health impact of increasing cART use during pregnancy is less clear. Earlier cART initiation during pregnancy may impact pregnancy outcomes and reduce LTFU from HIV care. In light of recent policy shifts towards lifelong treatment for all HIV-infected pregnant women, evidence is needed on how expanding cART use during pregnancy will impact pregnancy and retention in HIV care outcomes.

The impact of cART during pregnancy on adverse pregnancy outcomes.

Pregnant women are increasingly a priority population for early cART initiation. Starting in 2010, the WHO recommended initiation of lifelong cART for pregnant women with CD4 count ≤350. Women with higher CD4 counts receive either cART throughout pregnancy and breastfeeding or an abbreviated prophylactic antiretroviral regime to prevent vertical transmission.[7, 8] In 2013 the WHO endorsed a strategy known as *Option B+*, where all HIV-infected pregnant and breastfeeding women initiate lifelong cART, irrespective of CD4 count.[9] Several countries in SSA are in the process of implementing Option B+.[10-12] Option B+ holds promise for reducing MTCT, but has been associated with high LTFU after cART initiation.[13] As policy makers expand cART access to all HIV-infected pregnant women, there is an urgent

need for evidence on how proposed policy changes are likely to impact pregnancy outcomes, including LBW, preterm birth and SGA.

Scaling up cART access for pregnant women will, over time, lead to cART exposure earlier during gestation for fetuses. In SSA, presentation to ANC and treatment initiation for HIV-infected women typically occurs after the first trimester. In Zambia, 56% of women present to ANC during their second trimester.[14] As lifelong cART is expanded to all pregnant women, over time more women will begin subsequent pregnancies already on cART. Additionally, interventions aimed at earlier entry into ANC and cART initiation during pregnancy are likely to be a part of public health efforts to scale up cART access under Option B+. Longer duration of cART use during pregnancy has been associated with a reduced risk of vertical transmission,[15] but may pose risks for pregnancy outcomes.[16-18] How duration of cART impacts pregnancy outcomes and postpartum LTFU from HIV care remains unclear.

Preterm birth, LBW and SGA are major contributors to childhood mortality in the developing world. Preterm birth is defined as delivery prior to 37 weeks gestation, LBW as birthweight <2,500 grams and SGA as birthweight below the 10^{th} percentile for a given gestational age.[19-21] Globally, preterm birth is the leading cause of death in children under 5 years of age and contributed to nearly 1 million deaths in 2013.[21] In SSA, LBW infants account for 40% of all neonatal deaths.[22] SGA constituted 27% of live births in 2010 [20] in low and middle income countries and has also been identified as a major contributor to perinatal mortality.[23] In addition to an increased risk of mortality, preterm birth, LBW and SGA are associated with increased morbidity, inhibited growth and cognitive development and chronic disease later in life.[23, 24]

HIV-infected women are at a higher risk of having an adverse pregnancy outcome, including LBW, preterm birth and SGA. HIV-infected women have consistently been shown to have a higher incidence of LBW, preterm birth and SGA, compared to HIV-uninfected women.[25-28] Advanced HIV disease, indicated by lower CD4 count and higher viral load, has been associated with an even greater risk of poor pregnancy outcomes.[28-31]

The impact of cART during pregnancy on the risk of an adverse pregnancy outcome remains unclear. The biologic mechanism by which cART use may affect pregnancy outcomes is not clear and associations between cART and pregnancy outcomes have been reported on both sides of the null.[32-40] cART use has more consistently been linked to an increased risk of preterm birth.[32, 33, 35, 36, 38] However, being born too early is also one reason a baby may be LBW. Therefore hypothesized mechanisms for cART's impact on preterm birth may also be relevant for LBW or SGA. cART has been hypothesized to increase the risk of preterm birth by disrupting the maternal cytokine environment and elevating the Th1 immune response during pregnancy.[41, 42] Elevated levels of cytokines associated with the Th1 immune response during pregnancy have been associated with a range of adverse pregnancy outcomes,[43, 44] including preterm birth among HIV-infected women.[41] Changes to the cytokine environment and Th1 response due to maternal cART use could impact fetal growth and length of gestation,[41] however little evidence exists how maternal immunologic changes impacts pregnancy outcomes for HIV-infected women.

Earlier initiation of cART during pregnancy may impact pregnancy outcomes. High viral load and low CD4 count are important predictors of poor pregnancy outcomes among HIV-infected women.[28-31, 45] Time to viral suppression after cART initiation depends on initial viral load, but may take a month to several months.[46-48] Earlier initiation of cART during

pregnancy increases the likelihood of viral suppression by delivery. Low BMI is also an indicator of advanced HIV disease and is associated with LBW.[49-52] Initiation of cART leads to improvement in maternal nutritional status and BMI over time.[53, 54] The impact of nutritional gains associated with cART initiation may be even greater for women in SSA, many of whom may be undernourished.[55, 56] The immunological and nutritional benefits resulting from longer duration of cART may have the potential to translate into reductions in LBW and SGA.

The association between cART and multiple pregnancy outcomes has been investigated in several studies comparing women initiating cART during pregnancy, to women initiating less efficacious regimens (i.e. mono or dual therapy) or to women who began cART before pregnancy.[16, 32, 33, 35, 39, 57, 58] Women initiating cART during pregnancy tend to have more advanced HIV than women receiving mono or dual therapy, introducing the possibility of confounding by indication for treatment.[33, 57, 58] Women who initiate cART before pregnancy represent survivors only of early cART treatment, and therefore are also not directly comparable to women initiating cART during pregnancy.[59] It is possible that reported adverse associations between cART and poor pregnancy outcomes are biased due to confounding by indication for treatment or survivor bias.[33]

Restricting to a population of women eligible to initiate cART reduces bias by design. Examining cART's impact on pregnancy outcomes among a population of new cART users with the same indication for treatment [7, 60] avoids confounding by indication and survival bias. By utilizing appropriate comparison groups, such an approach offers new insight into the relationship between duration of cART and LBW, preterm birth and SGA.

Retention in care among HIV-infected pregnant women

Successfully retaining individuals in HIV care is critical to optimizing HIV outcomes. As the proportion of HIV-infected individuals on lifelong treatment increases, HIV associated mortality and transmission are expected to decline over time, provided those individuals remain in care.[61-64] Substantial LTFU after cART initiation has also contributed to growing concerns over antiretroviral drug resistance and the need for second line drug regimes.[65, 66] Drug resistance testing is expensive and second line regimes are not widely available in most developing country settings.[67] LTFU in care is highest within the first 12 months of initiating treatment for HIV-infected individuals.[68] Expanding access to cART in SSA will only translate into improved clinical outcomes if individuals are retained in HIV care,[69] particularly through the first year of treatment.[70]

Pregnancy is associated with LTFU up among HIV-infected women.[71-73] The reasons for higher LTFU after pregnancy are not well understood and may be related to late presentation to ANC and inadequate preparation before starting cART. ANC presentation after 20 weeks gestation has been associated with LTFU after delivery.[74] Having to transfer HIV care from a maternity clinic to an HIV clinic after delivery,[75] may also contribute to LTFU. Reducing LTFU among postpartum women has been recognized as key component to successfully scaling-up cART treatment.[70, 73, 76]

Earlier cART initiation during pregnancy also corresponds to earlier entry into supportive HIV care services, which may reduce LTFU. Women who test positive for HIV at ANC in Zambia are enrolled in comprehensive HIV care and seen monthly during pregnancy.[60] HIV care services include addressing medication toxicity, adherence support and couples counseling to encourage disclosure and partner testing.[60] Difficulty with tolerating drugs and adherence

issues are predictors of being lost to care.[77-81] Disclosure of HIV status has been linked to improved retention in care.[82-84] Women who initiate treatment and HIV care earlier during pregnancy may have more time to address barriers to care during pregnancy and may be less likely to be LTFU after delivery.

B. Innovation

This dissertation expands our current understanding of HIV and pregnancy by investigating how duration of cART during pregnancy impacts important pregnancy and HIV outcomes. Preventing vertical transmission has been the focus of much of the research on cART use during pregnancy. Our understanding of cART's impact on pregnancy outcomes and strategies to reduce postpartum LTFU remain limited. This project fills important gaps in our knowledge on the association between duration of cART and pregnancy outcomes, as well as options for targeting women most likely to be LTFU after delivery, in several innovative ways:

First, this dissertation expands our knowledge of cART's impact on pregnancy outcomes by focusing on duration of treatment. Whether different types of antiretroviral drugs impact the risk adverse pregnancy outcomes has been the focus of several previous studies. However, less attention has been paid to how timing or duration of treatment during pregnancy may impact pregnancy outcomes.

Second, this work provides new insight into the relationship between duration of cART during pregnancy and LBW due to growth restriction. The association between cART and LBW has been evaluated in several studies; however analyses have typically included both term and preterm births. By restricting to a population of term births, we provide some of the first evidence from SSA on the association between duration of cART during pregnancy and LBW due to growth restriction.

Third, we demonstrate the use of a novel method, multiple overimputation, to address missing data and measurement error. This work not only introduces and demonstrates the use of multiple overimputation to an epidemiologic audience, but provides an example investigating how associations between duration of cART and preterm birth and SGA change under differing assumptions about measurement error in gestational age.

Finally, this project develops the first risk score to identify women who initiated cART during pregnancy with a high likelihood of postpartum LTFU. As lifelong cART is scaled up, the increased costs to health systems of providing treatment to HIV-infected pregnant and breastfeeding women must be balanced with prevention efforts to retain these women in care. Risk scores may offer a novel strategy to identify women most likely to be LTFU to target for retention interventions.

CHAPTER I. REVIEW OF THE LITERATURE

A. Aim 1 – Duration of cART during pregnancy and pregnancy outcomes

The impact of ART during pregnancy has long been studied due to toxicity and teratogenicity concerns. Early animal studies suggested that zidovudine, or AZT, may have detrimental effects to the development of fetuses, but that deficits disappeared over time.[85] Beginning in 1994, AZT, and later neverapine, were recognized as a safe and effective for reduced the risk of MTCT.[86-88] After the introduction of antiretroviral drugs during pregnancy, no significant differences in prevalence of birth defects, physical growth, immunologic parameters or cognitive and developmental function were seen among children exposed in-utero to ART, compared to HIV-exposed children who did not receive prenatal ART exposure.[89-93] Maternal toxicities associated with ART use during pregnancy have also been low; anemia being the most prominent.[16, 94] Limited evidence suggests that efavirenz may increase the risk of neural tube defects if used during the first trimester of pregnancy.[95-97] However in 2012 the WHO published a technical update reviewing the evidence on efavirenz use in early pregnancy and concluded it was safe and effective to use.[98] There is ongoing monitoring for teratogenicity or toxicities associated with ART use in pregnancy in both the developed and developing world.[93, 99-101]

The association between cART use during pregnancy with LBW, preterm birth, and SGA

Preliminary evidence from Europe

ART is largely considered to not impair physical or cognitive development; however controversy remains about its impact on pregnancy outcomes. The possibility of an increased

risk of LBW (<2,500 grams), SGA (birthweight <10th percentile) and preterm birth (delivery prior to 37 weeks gestation) has been of greatest concern.

A possible adverse association between ART and pregnancy outcomes was first suggested by results published from a small Swiss cohort (hereafter Swiss Cohort) in 1998. The results indicated that 29 out of 37 women on ART therapy during pregnancy containing at least two reverse transcriptase inhibitors experienced an adverse event.[36] The largest proportion of adverse events was attributable to anemia; however 10 of the events were preterm births. Comparing women receiving cART (3+ antiretroviral drugs), to HIV-infected women not receiving therapy, an OR of 2.30 for preterm birth (95% CI 1.17-7.10) was observed after adjustment for confounders, including HIV clinical stage. Birthweight for all 30 infants in the study was in the normal range.[36]

In contrast to the results from the Swiss Cohort, in 1998 results of AZT's impact on birthweight and preterm birth were published by the European Collaborative Study (ECS).[102] Among 2,229 mothers across seven sites in Europe, women who took AZT during pregnancy were significantly less likely to have a LBW baby (OR 0.55; 95% CI 0.39–0.79) or a preterm birth (OR 0.76; 95% CI 0.53–1.09).[102] These results suggested the AZT used during pregnancy may improve pregnancy outcomes. However residual confounding by disease status could not be ruled out. High viral load and CD4 count are associated with an increased risk of LBW and preterm birth.[103, 104] While the study attempted to control for CD4 count, only about 50% of women had a CD4 count available.[102]

In an effort to further evaluate whether ART use during pregnancy posed a risk for LBW or preterm birth, the ECS and Swiss Cohorts combined their data. In the combined analysis, the impact of both monotherapy (1 antiretroviral drug) and cART on the risk preterm birth and LBW

were considered.[35] An increased odds of preterm birth associated with cART (OR 1.82; 95% CI 1.13, 2.92), compared to no treatment, was observed. The OR for preterm birth for cART with a PI was 2.60 (95% CI 1.43, 4.75), compared to no treatment, after adjustment for CD4 count. There was no increased odds of preterm birth associated with monotherapy, compared to no therapy (OR 1.03; 95% CI 0.70, 1.50). There were also no significant differences in birthweight by type of ART. However, in a separate ecological analysis the authors note that the prevalence of LBW and very LBW rose between 1985 and 1999, when ART use during pregnancy was being scaled up.[38]

The ECS and Swiss Cohort study were among the first to highlight the possible increased risk of preterm birth associated with PIs. This relationship was again examined using data from a population-based surveillance system of HIV-infected pregnant women in the UK and Ireland. cART (with or without a PI), compared to monotherapy, was associated with preterm birth (OR 1.39; 95% CI1.05–1.83), among the 81% of women with a CD4 count available.[58] The authors note that the proportion of preterm births was higher among pregnancies with missing CD4 cell count, compared to those with CD4 count (17% vs 12%). A German/Austrian cohort also noted an association between PI-based cART and preterm birth (OR for preterm birth of 3.40; 95% CI 1.13-10.2), compared with monotherapy.[105] While the link between cART and preterm birth was concerning, the possibility of confounding by indication for treatment was noted by authors of the UK/Ireland study. During the study period (1990-2005), no clear guidelines for treatment of pregnancy women existed.[58]

In addition to evaluating preterm birth and LBW outcomes, several studies examined the risk of SGA with ART use during pregnancy. In the UK/Ireland cohort, cART was associated with lower mean birthweight z-scores standardized for gestational age, when compared to mono

or dual therapy (2 antiretroviral drugs): cART –0.06 and mono/dual therapy 0.06.[58] In the German/Austrian cohort, the mean birthweight z-score for children exposed to PI-based cART (+0.46; 95% CI 0.01-0.92) and dual therapy (+0.43; 95% CI 0.03-0.82) was higher, compared with those exposed to monotherapy.[105] A French study of 8,192 mother-infant pairs also found no associations between ART of any type (e.g. cART, mono or dual therapy) and SGA. Infants exposed to cART had lower mean birthweight z-scores (-0.09, 95% Cl -0.15, -0.02), compared to infants exposed to monotherapy. However, when time was taking into account, there was no difference in mean birthweight z-scores during periods of predominate cART exposure (2005-2006) compared with periods of monotherapy (1999-2004).[106]

Contradictory evidence from the US

As evidence was growing in Europe that cART during pregnancy increased the risk of preterm birth, and possibly LBW, several studies in the United States (US) came out with contradictory findings. In 2002, a study of 1,598 women in the US and found that cART (with or without a PI – both groups combined) was not associated preterm birth or LBW (OR 1.08 and 1.03, respectively), compared to monotherapy. cART (without a PI) compared to monotherapy was slightly protective against LBW, although not statistically significant (OR 0.86; 95% CI (0.51–1.42).[39] Comparisons of cART with a PI compared to monotherapy, or to cART without a PI were above the null for both preterm birth and LBW, although none of the results were significant. An association between cART with a PI, compared to cART with no PI was observed for very LBW (<1500 g) (OR 3.56; 95% CI 1.04, 12.19), however there were only 16 very LBW events. The authors note that women with more advanced HIV were more likely to get cART with a PI. As in the ECS and Swiss Cohort study, the authors did control for CD4

count, but did not have information on HIV clinical stage or viral load, again raising the possibility of confounding by indication for cART.

The lack of an association between either cART or monotherapy with preterm birth or LBW birthweight among women in the US was again confirmed by the Women and Infants Transmission (WITS) study.[16] cART with a PI did not significantly predict preterm birth or LBW in multivariable analyses. Bivariable analyses showed several regimens, including cART with a PI, with associations below the null (suggesting a possible protective effect).[16].

The findings from the WITS were confirmed by Schulte et al., who reported no association between cART during pregnancy and preterm birth or LBW for women in the US. In fact, reporting data from 14,464 infants who were enrolled in Pediatric Spectrum cohort, LBW among HIV-exposed infants decreased from 35% to 21% between 1989 and 2004 and preterm birth decreased from 35% to 22%.[107] In multivariable analysis, no form of treatment (mono, dual or cART) predicted LBW. Having symptomatic HIV (OR 1.43; 95% CI 1.18–1.72) and having an HIV-infected child 1.34 (1.12–1.60) were associated with LBW. cART with a PI was the only type of combination therapy associated with preterm birth (OR 1.21; 95% CI 1.04–1.40), as was having an HIV infected child (OR 1.65 (1.38–1.97) and symptomatic HIV infection (OR 1.34; 95% CI 1.10–1.62).[107] These results suggest that, with possible exception of PI's, advanced HIV disease, often an indication for cART use, rather than cART use itself may cause LBW and preterm birth. Further, as ART use scaled-up over time, the proportion of LBW and preterm births decreased. However, it is important to note that inference at the individual level cannot be drawn from an ecological (aggregate) analysis.

In 2006, the first study in the US was published indicating a positive association between cART and preterm birth. cART with a PI, compared to cART without a PI, was associated with

an OR 1.8 (95% CI 1.1-3.0), after adjustment for multiple confounders. No other comparison of ART (mono, dual or cART) was associated with preterm birth, nor was any ART use versus no ART use. For LBW, no type of ART was associated with LBW and ORs were below the null (range 0.7-0.9), although not statistically significant.[33]

To try to resolve the disparate results between European and US studies, in 2010 data was pooled to investigate the association between cART and preterm birth. Pooling across two European studies and one US study, cART was associated with increased odds of preterm birth (OR 1.5; 95% CI 1.19, 1.87), compared to dual therapy.[108] Due to heterogeneity in studies, the investigators were not able to pool data comparing cART to monotherapy. In individual analyses, an increased odds of preterm birth was observed in the two European studies (OR 2.40; 95% CI 1.49, 3.86 and OR 1.43; 95% CI 1.10, 1.86), but not the US-based study (OR 0.92; 95% CI 0.67, 1.26).[108]

In response to the ongoing controversy over ART use during pregnancy, in 2007 Kourtis et al. published a meta-analysis on the relationship between ART use and preterm birth.[109] Fourteen studies were included ART use during pregnancy was not associated with an increased risk of preterm birth (OR 1.01; 95% CI 0.76, 1.34). However, monotherapy (predominantly AZT), compared with no therapy, decreased the odds of preterm birth (OR 0.86; 95% CI 0.73, 1.01), while cART, compared with no therapy, slightly increased the odds (OR 1.13; 95% CI 0.79, 1.63). cART with a PI, compared to without a PI, also increased the odds of preterm birth (OR 1.24; 95% CI 0.76, 2.02). An elevated OR for preterm birth was also found for cART initiated prior to pregnancy or within the first trimester, compared to later (OR 1.71, 95% CI 1.09, 2.67), however this may result from women with more advanced HIV initiating therapy

before becoming pregnancy. The authors also note the large degree of heterogeneity between studies and several researchers have questions the validity of their results.[110, 111]

Pregnancy outcomes for women in the developing world

As cART use during pregnancy has expanded, conflicting evidence has been reported about its impact on pregnancy outcomes among women in the developing world. CART with a PI, compared to mono or dual therapy, has been associated with an elevated odds of LBW (OR 1.5; 95% CI 0.7, 3.2) and but not preterm birth (OR, 1.1; 95% CI, 0.5–2.8) among women in Latin America and the Caribbean.[37] Similar results were seen in a study in India, where cART (predominately with PIs) was associated with in increased odds of LBW (OR 1.46 (0.75, 2.87), as well as preterm birth (OR 3.35; 95% CI 1.52, 7.38).[112] Among women in Cote D'Ivoire, cART initiated before pregnancy (OR 2.88; 95% CI 1.10, 7.51) and cART initiated during pregnancy (OR 2.12; 95% CI 1.15, 4.65) were both associated with an increased odds of LBW, compared to monotherapy.[34] However, another study in South Africa found that cART use, regardless of type (PI-based, neverapine-based or efavirenz-based) or timing of initiation (early <28 weeks gestation or late ≥28 weeks gestation), was largely protective against LBW (OR range: 0.38-1.01).[40] In the same study, cART use, especially early cART use, was associated with preterm birth (OR range: 3.00-5.64). In a study in Malawi and Mozambique, the proportion of LBW's among 3,273 HIV+ women was 11.5%; similar to proportion among HIV-uninfected women. However, longer duration of cART, compared to no treatment, reduced the odds of preterm birth (OR 0.93; 95% 0.92, 0.94), after adjustment for CD4 count and viral load.[113] Among HIV-infected women in Botswana, cART initiated before pregnancy increased the odds of SGA (OR 1.8; 95% CI 1.6, 2.1) and preterm birth (OR 1.2; 95% CI 1.1, 1.4). Among women initiating treatment during pregnancy, cART was associated with an increased odds of SGA (OR

1.5; 95% CI 1.2, 1.9) and preterm birth (OR 1.4; 95% CI 1.2, 1.8), compared with monotherapy.[32] Among women in Brazil, pre-conception cART was associated with an increased odds of LBW (OR 3.6; 95% CI 1.7, 7.7) and preterm birth (OR 5.0; 95% CI 1.5, 17.0), compared with monotherapy.[114]

Timing of cART initiation

The WITS was the first study to look at timing of cART initiation during pregnancy. In the WITS, timing was categorized as early initiation (≤25 weeks gestation study visit) or late initiation (≥32 weeks gestation study visit or delivery). Late use of ART without AZT significantly predicted preterm birth (OR 7.86; 95% CI 1.39–44.58), however this result included only 10 events. Late use of ART with AZT was protective against preterm birth (OR 0.53; 95% CI 0.34–0.83).[16] Among HIV-infected women in the Netherlands, 44% who initiated cART before 13 weeks gestation experienced a preterm birth, compared with 21% who initiated cART at ≥ 13 weeks gestation.[17] A later study by Watts et al. found an increased odds of preterm birth among women who used cART with a PI in the first trimester (OR 1.55), compared to mono or dual therapy in the first trimester or women who started PI-based cART after the first trimester.[18] cART, with or without a PI, used after the first trimester was not associated with an increased odds of preterm birth, nor was cART used at any time associated with an increased odds of SGA.[18] More broadly, HIV-infected women experiencing a preterm birth more often had longer duration of cART prior to pregnancy, compared to those with term births, in an Italian cohort.[115] These studies raise questions about whether the timing or duration of cART might impact pregnancy outcomes.

cART and fetal growth: Special concern for women in sub-Saharan Africa?

For women in SSA, cART use during pregnancy may have a greater impact on fetal growth than for women in the US and Europe. An association between cART and birthweight has more consistently been reported in the developing world, than among women in developed countries.[32, 34, 40] One reason for this may be the different nutritional and HIV disease status of women during pregnancy in SSA, compared with the US or Europe. Women in SSA may be more likely to be anemic or be underweight at the start of pregnancy or not gain sufficient weight during pregnancy.[49-52, 116, 117] Such factors could increase the risk of intrauterine growth restriction, which has been associated with an increased risk of iatrogenic preterm birth in HIV-infected women.[118] Women in developing countries may also be less likely to be virally suppressed and have lower CD4 counts coming into pregnancy, than women in the US and Europe.[7, 8, 119] Low BMI, low CD4 count and detectable viral load are all predictors of poor pregnancy outcomes.[45-47, 120, 121] However, cART use is associated with an increase in BMI and CD4 count, and decrease in viral load.[54, 122] Therefore while women in SSA may have a higher risk of an adverse pregnancy outcome coming into pregnancy, the use of cART during pregnancy may also confer greater advantages for improving fetal growth than for women in in the developed world.

Methodological considerations and challenges to causal inference

Key challenges to interference about the impact of cART during pregnancy on pregnancy outcomes are confounding by indication for treatment and flawed comparison groups. As discussed above, many of the initial studies on cART use during pregnancy were conducted at a time when clear guidelines for treatment during pregnancy did not exist. Measures of advanced HIV disease, including detectable viral load and low CD4 count, are associated with poor

pregnancy outcomes.[28-31, 45] To the extent that sicker women are more likely to receive cART, particularly PI-based cART, adverse associations may reflect bias from confounding by indication for cART treatment.[58, 123, 124] Controlling for maternal HIV disease stage could mitigate such bias, however many studies have been limited to information on clinical stage or CD4 count,[58] and did not have information on viral load.[16, 35, 39]

Including women who initiated cART treatment before pregnancy has also complicated inference.[34, 125] Women who initiate cART before pregnancy represent survivors only of early cART treatment, and therefore may differ from women initiating cART during pregnancy. The differences between two such groups is known as survivor bias.[59] Patel et al. attempted to limit survivor bias and confounding by indication by restricting their study population to women who initiated therapy during pregnancy and by controlling for viral load, CD4 count and clinical stage. They found slightly elevated odds of both preterm birth (OR 1.22; 95% CI0.70, 2.12) and LBW (OR: 1.36, 95% CI: 0.76, 2.44).associated with cART with a PI compared to mono or cART without a PI.[57] The attenuation of the ORs for cART use associated with preterm birth and LBW in that study, compared to earlier published European studies.[35, 36, 38, 58, 108] may suggest fundamental differences between US and European cohorts or suggest confounding by indication and survivor bias in effect estimates.

Gaps in Knowledge

The relationship between cART and adverse pregnancy outcomes involves many unanswered questions. Chief among them is an understanding of the biological mechanism(s) by which cART influences pregnancy outcomes. Several mechanisms have been proposed, including mitochondrial toxicity, [126-129] placental insufficiency, [118, 130] inhibited progesterone production [131] and a TH2 to TH1 cytokine shift.[41, 42] From a clinical

perspective, whether timing or duration of treatment during pregnancy affects pregnancy outcomes has been an important concern. However, answering questions related to how timing of treatment impacts pregnancy outcomes is challenging, because length of treatment and length of gestation are correlated. Women who initiate cART later during pregnancy may appear to be less likely to have a preterm birth, for example, when in actuality they simply were closer (and therefore more likely to reach) term by the time they began treatment.

One way to mitigate the dependency between timing of treatment and length of gestation has been to assess LBW as an outcome, instead of preterm birth. However, infants may be LBW because they were born too early (preterm) or they were born to small (intrauterine growth restriction). Consequently, evaluating how timing or duration of cART impacts LBW (when both term and preterm births are included) does not distinguish whether cART affects fetal growth, length of gestation, or both.

Summary and Conclusions

Despite receiving considerable research attention, whether cART increases the risk of an adverse pregnancy outcome remains unclear. Hypothesized reasons for disparate results between studies include: differences in study population characteristics, confounding by indication or survivor bias. On the whole, cART has most consistently been associated with an increased risk of preterm birth. There is less evidence that cART increases the risk of SGA or LBW for women in the developed world, but may increase the risk of LBW or SGA for women in the developing world.

In light of the limited and inconclusive evidence on the relationship between cART and pregnancy outcomes for women in the developing world, there is an urgent need for further investigation. Whether cART regimens increase or decrease the risk of adverse pregnancy

outcomes, as well as if duration of cART during pregnancy impacts pregnancy outcomes remains unclear. Preterm birth, LBW and SGA are an important infant and public health outcome in the developing world. As cART use during pregnancy is rapidly being scaled up throughout SSA, it is important to assess how these changes are likely to impact pregnancy outcomes.

B. Aim 2 – Predicting postpartum LTFU among HIV-infected women who initiate cART during pregnancy

With over 8 million people on cART in SSA, retaining individuals in HIV care has emerged as a critical public health issue.[4, 62] LTFU along the HIV treatment cascade between HIV testing and viral suppression is well documented in the United States. According to the Centers for Disease Control, only 51% of HIV-infected individuals are estimated to be retained in ongoing HIV care and only 28% are virally suppressed.[132] In SSA, limited healthcare infrastructure, resources and personnel pose further challenges to reducing LTFU along the HIV treatment cascade. A systematic review of retention in care in SSA estimated that only 66% of individuals eligible for treatment initiate cART and that overall only 24% of individuals eligible for treatment are engaged in ongoing care.[80] Women staring HIV treatment during pregnancy are at an ever greater risk of LTFU. In a study in South Africa, 57.5% of HIV-infected pregnant women were LTFU between HIV testing and six months after delivery.[72] Increasingly, effective engagement and retention of individuals in HIV care has been recognized as critical to improving HIV outcomes on a population level.[133, 134]

The importance of retention in care to improve HIV outcomes

Successfully retaining individuals in HIV care is critical to reducing mortality and HIV transmission on a population level.[63, 64, 79, 135] LTFU is critical issue for cART programs in SSA and accounts for 56% of attrition.[136] LTFU after initiation of cART has contributed to growing concerns over antiretroviral drug resistance and the need for second line drug

regimes.[65, 66] Drug resistance testing is expensive and second line regimes are not widely available in most developing country settings.[67] The WHO has recognized improved retention in care as critical to improving clinical outcomes as cART is scaled up throughout SSA.[69]

Factors associated with loss to follow-up and improved retention in HIV care

LTFU in HIV care has been associated with a number of factors at both the individual and structural level. At the individual level, having a CD4 count ≥200 at treatment initiation has been associated with an increased risk of LTFU (HR 1.74; 955 CI 1.09, 2.78), as has being <25 years of age (HR 1.87; 95% CI 1.15, 3.05) and initiating treatment as an inpatient in the hospital (HR 1.89; 95% CI 1.06, 3.38).[71] Side effects due to medication toxicity have been cited as a reason for LTFU among patients on cART.[137, 138] At the structural level, barriers to care, including inconvenient clinic hours, long lines at the clinic and difficulty in scheduling an appointment, have been associated with LTFU.[77, 79] Stigma associated with being HIV-infected is also a barrier for many individuals to remaining in HIV care.[77, 139, 140]

Disclosure of HIV status has been shown to reduce LTFU. Having disclosed one's HIV status to a member of their social network was the strongest predictor of retention in HIV care (OR 1.5; 95% CI 1.1, 1.9) in a study of Latino and African American men who have sex with men.[84] Disclosure was strongly associated with retention in care at 2 years in another US-based study (OR 16.0; 95% CI 1.7, 148), although the confidence intervals reflect the small sample size (N=36).[141] Among HIV-infected adolescents in West Africa, disclosure of status to HIV-infected adolescents was associated with a reduced risk of LTFU or death (HR 0.23; 95% CI: 0.13–0.39).[82] However, in Nigeria disclosure of HIV status was associated with a reduction in cART adherence (OR 0.85; 95% CI 0.76, 0.94), [83] which is closely linked to retention in care.[142, 143]

Early enrollment in HIV care has also been shown to reduce LTFU. Among individuals in a rural South African, being in pre-ART care for at least 6 months before starting cART reduced the risk of LTFU by half (HR 0.49; 95% CI 0.24, 1.00).[71] In addition to pre-ART care, initiating cART at a higher CD4 counts has also been shown to reduce LTFU. In a study in Lesotho, individuals who initiated cART at CD4 count >200, compared to ≤200, were 39% less likely to be LTFU (HR0.61; 95% CI 0.43, 0.87).[144]

Pregnancy and loss to follow-up

Pregnancy is associated with loss to follow-up after cART initiation among HIV-infected women. In a cohort of HIV-infected individuals in South Africa, women who initiated cART during pregnancy were three times as likely to be LTFU over a 4 years study period, compared to all other individuals who initiated cART (HR 3.20; 95% CI 1.86, 5.51).[71] A separate study in South Africa found similar results for the association between pregnancy and retention in HIV care at 6 months (HR 3.75; 95% CI 1.53,9.16).[73] Pregnancy at the time of cART initiation has also been associated with LTFU at 3 years after initiation (32% of pregnant women vs. 13% of non-pregnant women; p-value <0.001).[70] Reducing LTFU among postpartum women has been recognized as a key component to successfully scaling-up cART treatment.[73, 145]

The reasons for high LTFU after pregnancy are not well understood and may be related to late presentation to antenatal care, delays in clinical HIV assessments or inadequate preparation for cART.[146] In a study in India, women who presented to antenatal after 20 weeks gestation were significantly more likely to be LTFU, compared to women who presented within the first 20 weeks (OR 1.75; 95% CI: 1.12, 2.73).[74] Similar results were seen among South African and Kenyan women, where initiation earlier during gestation decreased the risk of LTFU.[147, 148] In a Swiss cohort, having a detectable viral load at delivery (which could

correlate with late presentation to ANC), predicted LTFU during the first year postpartum.[149] Delays between clinical staging or CD4 assessment and cART initiation may also contribute to LTFU during pregnancy. Among HIV-infected pregnant women in Malawi, the median time between CD4 count assessment and cART initiation fell from 41 days to 15 days between 2006 and 2009; during the same time period retention in care at 6 months after delivery improved from 17% to 65%.[75] Women who initiate cART while pregnant may also have only recently tested positive for HIV at antenatal care and therefore may be unprepared to initiate lifelong treatment.[70, 150] However, rapid initiation of lifelong cART was acceptable to women diagnosed with HIV during pregnancy in a pilot study in South Africa.[151]

As lifelong cART for pregnant and breastfeeding women is scaled up across SSA, there has been concern about the ability to retain women in care.[63, 64, 69]. Unfortunately, LTFU during and after pregnancy is common [152]. In Malawi, 17% of women initiating lifelong cART during pregnancy were LTFU by 6 months after treatment initiation and retention at 12 months was lower among pregnant women than other adults initiating cART [153, 154]. In South Africa, 32% of women initiating cART during pregnancy were lost by 6 months postpartum [147]. Early evidence from Option B+ implementation in several countries suggests that more women are initiating cART during pregnancy under option B+ [154, 155], but that those initiating treatment at CD4 counts >350 are at a substantially higher risk of LTFU.[153, 156] However among non-pregnant HIV-infected adults, initiating treatment at CD4 counts >350 has not been shown to increase LTFU or decrease viral suppression by 48 weeks after initiation.[157]

Interventions to reduce loss to follow-up in HIV care

Numerous interventions to reduce LTFU in cART programs have been proposed, including improving patient preparation, community-based care and adherence support, technological innovations and structural interventions to reduce barriers to attending care. Despite this range of proposed interventions, few have focused exclusively on reducing LTFU among pregnant women. However, a number of trials are currently underway to evaluate different packages of services to improve retention in care among HIV-infected pregnant women.[158-163] No one strategy alone will improve retention in care, but rather the interventions described below are often part a package of services designed to reduce LTFU.

Improved patient preparation

Patient preparedness for cART initiation has been recognized as important to retaining individuals in HIV care. Coetzee et al. reported on a patient-centered preparation program for individuals initiating cART at a primary care clinic in South Africa, where individuals were enrolled into comprehensive HIV care before starting cART and developed relationships with the clinical care team. The program led to an overall survival probability of 86.3% and viral suppression of 69.7% at 24 months after initiation.[164] In a separate cART preparation program in rural South Africa, patients underwent a minimum of 3 individual counseling sessions and participated in ongoing support groups before starting cART. Of the 1,803 individuals who initiated cART between 2005 and 2009, 82.4% were in care or had transferred and only 6.5% were LTFU by the end of the study period.[71]

Community-based care and adherence support

Programs that use community health workers (CHWs) or community-based nurses to delivery HIV services and adherence support in patient's homes have been successful in

reducing LTFU. In rural Malawi, a program that included home-based care for opportunistic infections, adherence support from CHWs and defaulter tracing when patients missed their HIV clinic appointments significantly reduced to risk of LTFU (RR 0.02; 95% CI 0.00, 0.12), compared to those that did not receive the intervention.[165] In another program that included 13,391 patients initiating cART across 27 treatment facilities in 8 countries, only 1% of patients were LTFU at clinics that offered home visits, supervision by a community nurse, adherence counseling and other services, compared with 14% LTFU at clinics that only offered adherence counseling.[142] In Rwanda, patients who received daily home visits from CHWs who watched them take their medication, nutritional support and transportation stipends were more likely to be retained in care and virally suppressed 1 year after cART initiation (RR 1.15; 95% CI, 1.03, 1.27).[78] Finally, in Zambia training community volunteers to provide adherence support at the health facility and community level led to a 15% drop in LTFU in the 12 months after the intervention was implemented.[143]

Technological innovations

Technological innovations, including using mobile phones and electronic medical records systems to contact patients also reduces LTFU. With mobile phone use rapidly increasing in SSA, text messaging interventions have led to improvements in cART adherence in SSA,[166] and hold promise for also improving retention in care.[162, 163] In one study in Uganda, over 64% of HIV-infected patients on cART had access to a mobile phone. Patients enrolled in the intervention were contacted by mobile phone or text message if they missed a clinic appointment. After missing a clinic visit, 79% of patients presented for treatment within an average of 2.2 days (SD 1.2 days) after being contacted on their mobile phone.[167] A randomized controlled trail of a mobile-based intervention that consists of a weekly text message

that requires patients to ‘check-in’ with a healthcare provider within 48 hours is now underway in Kenya to see if it improves retention in care during the first year on cART.[168] Electronic medical record systems that can quickly flag patients who have missed their HIV appointments for tracing are also critical to reducing LTFU in cART programs.[71, 169]

Reducing structural barriers to remaining in HIV care

Reducing structural barriers to HIV care, such as financial constraints, lack of transport and distance from cART services, has also been the focus of several interventions to reduce LTFU. In one study of LTFU in South Africa, of 182 patients who had missed a HIV appointment, 34% cited financial limitations as the reason, with transportation being the largest monetary cost.[170] Reducing patient costs or providing cART free of charge in resource-constrained settings has been identified as key to linking and retaining patients in HIV care.[133, 170] One strategy to reduce cART program costs has been to decentralize cART services from district hospitals to community clinics. Decentralizing cART services has the added benefit of reducing transportation time and costs for patients.[71]

Once patients initiate cART, interventions that help them navigate the healthcare system and address mental health and substance abuse may further help to reduce LTFU. A review of interventions to improve retention in care in the United States found that having a peer navigator accompany patients through HIV care improved retention in care.[79] Addressing other healthcare needs, such as mental health or substance abuse issues, also improves retention in care. An intervention that provided mental health and substance use services, as well as other supportive services, to HIV-infected adolescents improved HIV care appointment attendance from 7% to 73%.[171] In another study of men who have sex with men, depressed patients were

twice as likely to miss an HIV care appointment within a 12 month period, compared to patients who were not depressed (OR 2.01; 95% CI 1.01,3.99).[172]

Methodological issues in measuring loss to follow up

Defining loss to follow-up

A central methodological challenge to evaluating cART programs is that no universal definition for LTFU exists.[173] For example, in one cART program in rural South Africa, LTFU was defined as no patient contact for >6 months.[71] In another study in Nigeria, LTFU was defined as any patient who did not return to the clinic >60 days from their last scheduled visit.[83] In an analysis from Malawi and Zambia, LTFU was defined as not having attended a cART clinic visit for >90 days from the last scheduled appointment.[68] Defining LTFU includes several dimensions, including whether it is measured prospectively (from last scheduled appointment) or retrospectively (within a number of days from study end), includes only clinic visits or clinic and pharmacy visits or whether it is considered the date of a missed appointment or the date that definition of LTFU is met.[174]

How LTFU is defined can lead to dramatic variation in the cumulative incidence of LTFU within a given program. In one analysis that assessed the sensitivity of results to 17 different definitions of LTFU, the proportion of patients lost by 2 years after treatment initiation varied from 22% to 84%.[174] In another, LTFU at 12 months after initiation ranged from 14% to 34%, depending on the definition of LTFU.[175]

The rate of LTFU also varies over time and is often highest during the first 6-12 months of treatment. High rates of LTFU at the beginning of treatment may reflect challenges in adhering to medication or reflect high mortality in the first year of treatment.[68] In an evaluation of cART programs in Zimbabwe and Malawi, the proportion of patients retained

through the first 12 months of treatment was 84.1% and 79.9% respectively. However, among patients who were retained through the first year, retention at 24 months was 95.3% and 95.2%.[68] Similarly in a study in South Africa, patient retention increased from 80% at 6 months after treatment initiation, to 95% between 6 and 12 months.[176]

A number of universal definitions for LTFU have been proposed. Based on empirical work from Zambia, ≥60 days since the last scheduled clinic appointment has been suggested as a reasonable definition for LTFU.[177] Analyzing data from 180,718 patients from 111 health facilities in 19 countries, Chi et al. recommended ≥180 days since last patient encounter as a universal definition for LTFU to standardize monitoring and reporting efforts globally.[178] Shepherd et al. point out that while guidance on a universal definition for LTFU is helpful, no one definition of LTFU will fit every cART program and definitions should be guided by how often patients are scheduled to receive HIV care.[174]

Competing Risks

When LTFU is the outcome of interest, competing risks due to death need to be taken into account, since patients who die can no longer be LTFU. In time-to-event analyses where competing risks are present, estimation of the cumulative incidence of an event (i.e. the cumulative incidence function (CIF)), rather than the survival function, is often desirable.[179] In time-to-event analyses without competing events, the CIF is simply the complement of the survival curve (i.e.1-survival function). Standard Kaplan-Meier survival curves treat competing risks as censoring events and assume the probability of LTFU is the same for those who experience a competing event (i.e. mortality), as for participants remain under observation. However, when the competing event is death, LTFU can no longer occur and 1-traditional Kaplan-Meier survival curves overestimates of the CIF.[180] Particularly in developing country

settings where mortality after cART initiation may be high, failing to account for competing risks can appreciably bias estimates of LTFU. For example, the cumulative incidence of LTFU at 3.5 years among individuals starting cART with a CD4 count <100 in Zambia was 29.3% with traditional Kaplan-Meier analysis, but only 22.9% when competing risks were taken into account.[180]

Cause-specific proportional hazard models and Fine and Gray's subdistribution proportional hazards model are two methods that can be used to account for competing risks.[181-183] Cause-specific proportional hazards models allow for the estimation of cause specific hazard ratios for both the primary event of interest and the competing event by removing competing events from subsequent risk sets for the primary event. This approach is analogous censoring competing risks in traditional survival analysis.[183] Unlike traditional survival analysis however, the cause-specific hazard is not directly related to the CIF, unless independence between the primary outcome and competing event is assumed.[184-186] Cause-specific hazard ratios therefore cannot be directly interpreted as increasing or decreasing the probability of an event, but rather as an association of two 'instantaneous rates' (hazards) for a specific outcome in the presence of a competing risk and are better suited to studying the etiology of disease.[183]

Developed by Fine and Gray, subdistribution proportional hazard models directly estimate the CIF, accounting for competing events.[182] These models are therefore a better choice when the overall probability of an outcome or an estimate of the risk an event associated with exposure is desired.[184] Subdistribution proportional hazard models take into account competing risks by allowing participants with competing events to remain at risk and continue to contribute person time, weighting their remaining time at risk by the inverse probability of

censoring due to a competing event.[182] While it may seem counterintuitive for individuals who had a competing event to remain in the risk set, these individuals can be thought of as representing those individuals in the population who can no longer experience the primary event of interest.[183]

Gaps in Knowledge

In order to improve retention in care among postpartum HIV-infected women, a number of important gaps in knowledge need to be filled. First, information on what interventions or group of interventions, effectively reduce LTFU among postpartum women is urgently needed. A number of trials [158-163] are ongoing that will help to shed light on the supportive services are necessary to help retain HIV-infected women in care after the birth of a child. Second, as health systems consider the dual costs of scaling up cART to all pregnant and breastfeeding women and interventions to support retention in care – in a context of resource constraint – approaches that target retention services towards women with the highest likelihood of LTFU may be helpful. Risk scores are such an approach that have been used to identify persons with acute HIV-infection [187-190], partners unlikely to seek HIV-testing [191] and to develop selective screening guidelines for sexually transmitted infections [192, 193]. In resource-limited settings, risk score may provide a useful tool for targeting retention in care interventions to groups of women most likely to be LTFU. In order to be effective, a risk score would need to effectively and reliably identify women LTFU after delivery. Whether such a targeted approach would translate into reductions in HIV-related mortality and transmission at a population level is unknown and would additionally need to be evaluated.

Summary and Conclusions

Reducing LTFU in cART programs is an important public health priority. A number of clinical and sociodemographic characteristics are associated with being LTFU, including being pregnant at the time of cART initiation. Interventions ranging from providing community-based care and adherence support to text-messaging programs have been implemented to help reduce LTFU and improve retention in HIV care. However few interventions have focused specifically on pregnant women. A number of methodological challenges hamper the measurement and comparison of LTFU results, including the lack of a universal definition for LTFU and the need to account for competing risks due to mortality when analyzing LTFU as an outcome. Using appropriate statistical methods to account for competing risks and carefully considering how LTFU is defined are essential for evaluating interventions to reduce LTFU and improving HIV outcomes for individuals initiating cART, including pregnant women.

CHAPTER II. STATEMENT OF SPECIFIC AIMS

A. Rationale

In SSA, nearly 14 million women are living with HIV and the prevalence of HIV among pregnant women remains above 20% in many countries.[194, 195] Preventing mother to child transmission of HIV requires early initiation of cART during pregnancy.[7, 8] However, cART initiation during pregnancy also has implications for maternal health. Longer duration of cART during pregnancy may impact pregnancy outcomes and help to improve postpartum retention in HIV care.

Longer duration of cART use during pregnancy reduces the risk of vertical transmission and may improve pregnancy outcomes.[15, 16, 40, 108] Measures of advanced HIV disease status, such as low CD4 count, unsuppressed viral load and poor nutritional status are associated with an increased risk of adverse pregnancy outcomes.[40, 45] Early initiation of cART during pregnancy may improve immune function and nutritional status prior to delivery,[46, 47] and lead to improved pregnancy outcomes. Reducing LBW, preterm birth and SGA among HIV-exposed infants would significantly improve neonatal mortality and infant health outcomes in SSA.[22, 24]

At 6 months postpartum women often transition from receiving HIV care at a maternity clinic to an HIV clinic and many are lost from HIV care.[63, 71, 73] Shorter duration of cART during pregnancy may serve as a marker for poor engagement in HIV care and help to predict postpartum LTFU.[74, 196] A number of other factors, including CD4 count, employment status, and age also predict LTFU.[71, 150] Risk scores offer one strategy to combine information on

predictors of LTFU to identify women at delivery with the highest likelihood of postpartum LTFU to target for retention in care interventions.

Currently, many countries in SSA have revised national guidelines to place all HIV-infected pregnant and breastfeeding women on lifelong cART.[12] Policymakers face a paucity of information on how these changes are likely to impact pregnancy and retention in care outcomes. The proposed study uses existing data collected under a policy of cART initiation during pregnancy for women with a CD4 count ≤350 to provide insight into how scaling up lifetime cART treatment for HIV-infected pregnant women in SSA may impact future pregnancy and retention in HIV care outcomes:

B. Specific aims and hypotheses

Specific Aim 1a: To estimate the association between duration of cART use during pregnancy and low birthweight (< 2500g), among HIV-infected women with a CD4 count ≤350 with term pregnancies who deliver in a healthcare facility.

Hypothesis 1a: We hypothesize that for women in SSA with a CD4 count ≤350 who deliver at term, longer duration of cART will be associated with a decreased risk of LBW.

Specific Aim 1b: To estimate the associations between duration of cART use during pregnancy with preterm birth (<37 weeks gestation) and SGA (birth weight below the 10^{th} percentile), and to assess the sensitivity of these associations to various assumptions about measurement error in gestational age, among HIV-infected women with a CD4 count ≤350 and who deliver in a healthcare facility.

Hypothesis 1b: We hypothesize that that longer duration of cART may increase the risk of preterm birth, but not SGA. Further, we hypothesize that effect estimates may appreciably vary under different assumptions about measurement error in gestational age.

Specific Aim 2: To develop a risk score that can be used at delivery to identify women who initiated cART during pregnancy with a high likelihood of LTFU by 6 months postpartum.

Hypothesis 2: We hypothesize that, in addition to other factors, duration of cART during pregnancy will be a strong predictor of LTFU at 6 months postpartum.

CHAPTER III. METHODS

A. Aim 1 – Duration of cART before delivery and pregnancy outcomes

A1. Overview and Study Design

This study draws on the existing clinical data collected by the Zambian Ministry of Health in collaboration with CIDRZ to define a unique study population and statistical analysis for each sub-aim and aim. CIDRZ maintains ZEPRS, a clinical obstetric database in operation since 2007 with information on over 115,000 pregnancies[86], as well as a clinical HIV database. Data collected between 2007 and 2011 can be linked between the obstetric and HIV databases. Together these databases represent a rich and existing resource with longitudinal, sociodemographic, obstetric, HIV clinical and treatment information to investigate the maternal health impact of cART use during pregnancy.

Table 3.1. Maternal and Obstetric Characteristics of 115,552 Pregnancies recorded in ZEPRS in Lusaka, Zambia: 2007-2010

Characteristic	N (%)	Characteristic	N (%)
Age, years		Gestational at enrollment visit, weeks	
<15 years	515 (0.4)	<20 weeks	29,390 (25.4)
15–19 years	19,827 (17.2)	20-27 weeks	64,081 (55.5)
20–24 years	37,113 (32.1)	28-31 weeks	15,416 (13.3)
25–29 years	30,892 (26.7)	32-35 weeks	5,048 (4.4)
30–34 years	18,607 (16.1)	≥36 weeks	1,617 (1.4)
35–39 years	7,227 (6.3)	*Missing*	-
≥40 years	1371 (1.2)	Birthweight, grams	
Missing	-	<2500	12,401 (10.8)
Education		≥2500	102,171 (89.2)
None	4,070 (4.1)	*Missing*	*980*
Primary	43,580 (43.7)	Agreed to HIV testing	111,108 (96.2)
Secondary	47,883 (48.0)	Among 23,392 HIV+ women:	
Tertiary	4,122 (4.1)	CD4 Screening	18,928 (79.1)
Missing	*15897*	CD4 Count	
Marital status		≤350	9,419 (49.8)
Single	10,053 (9.0)	>350	9,509 (50.2)
Married or cohabitating	100,542 (90.5)	*Missing*	*5004*
Divorced or widowed	545 (0.5)	HIV+ women prescribed	
Missing	*4412*	HAART	4,384 (18.4)

Adapted from Chi et al. 2011.

The associations between duration of cART during pregnancy and pregnancy outcomes (Aim 1a and Aim 1b) were assessed using a retrospective cohort design. Among women who delivered at a health facility

between 2009 and 2013, duration of cART during pregnancy and preterm birth, LBW and SGA status was assessed at birth.

A2. Study Population

Starting in 2007, women presenting for ANC care to public health clinics in the Lusaka district of Zambia were entered into the ZEPRS electronic medical records system. In Lusaka, coverage of ANC is near universal (94%),[197] as is HIV testing (96%) and CD4 count screening (79%) at ANC (Table 3.1), suggesting that ZEPRS has good population coverage of HIV-infected pregnant women in Lusaka.[14] For the purposes of Aim 1a and Aim 1b, data from ZEPRS was used to investigate the association between duration of cART during pregnancy and pregnancy outcomes.

A2a. Eligibility criteria

For the purposes of investigating associations between duration of cART during pregnancy with LBW, preterm birth and SGA, HIV-infected women with singleton pregnancies at gestational age ≥28 weeks, a valid CD4 count measure of ≤350 during pregnancy, who are not on cART at the time of presentation to ANC, deliver in a health facility between 2009- 2013, and who have no major health conditions, such as diabetes, heart disease or hypertension, were eligible for inclusion.

Aim 1a – Duration of cART during pregnancy and LBW. cART use during pregnancy could affect LBW through two different pathways: shortening gestation length (leading to preterm birth) or restricting fetal growth. Duration of cART during pregnancy is structurally linked to length of gestation, making it difficult to meaningfully assess the association between duration of treatment and preterm birth. Therefore, we limited our study population for Aim 1a

to women delivering term (>37 weeks gestation) infants. This approach allowed us to focus on the potential relationship between duration of cART and LBW due to growth restriction.

Aim 1b – Duration of cART during pregnancy and preterm birth and SGA. In order to evaluate preterm birth and SGA outcomes, women delivering both term and preterm infants were included in the analysis for Aim 1b.

Women who initiate cART before pregnancy were not included in either Aim 1a or Aim 1b to avoid comparing survivors of ongoing cART therapy to new users of cART. Women who were eligible to initiate cART treatment but do not do comprised a substantial proportion of women with CD4 counts $\leq$ 350 and therefore were included in the study populations for both Aim 1a and Aim 1b.

Generalizability – Findings from Aim 1a are generalizable to HIV infected pregnant women with a CD4 count $\leq$350 and who delivered a term infant at >37 gestational weeks at a health facility in SSA. Findings from Aim 1b are generalizable to a similar population of women, but additionally include preterm infants. Lusaka is an urban area where CD4 screening, ANC use and delivering in a health facility are high. Results may be less generalizable to settings with low CD4 screening, ANC uptake, fewer facility-based deliveries or a lower risk of preterm birth.

Limitations – Women eligible to initiate cART who do not deliver in a health facility were not included in our analysis because they did not have pregnancy outcome information available. In Lusaka, nearly 25% of women who attend at least one ANC visit do not deliver in a health facility.[14] Women who initiated cART at CD4 counts >350 were not included in the primary analysis, but were included in a sensitivity analysis (see section A6).

A3. Exposure, Outcome and Confounder Assessment

A3a. Exposure Assessment

For Aim 1a, the exposure of interest was duration of cART before delivery, measured in completed weeks by delivery and categorized as never initiated (referent), ≤8 weeks, 9-20 weeks, and 21-36 weeks. Category cut-points were based on the functional form of the relationship between the exposure and outcome, as well as clinical considerations to approximately correspond with cART initiation in early, mid and late pregnancy. We also considered duration of cART before delivery as a continuous measure of weeks on cART.

For Aim 1b, the exposure of interest was duration of cART before delivery, measured in completed weeks and assessed at 32 weeks gestation. Duration of cART during pregnancy is intrinsically linked to length of gestation. Consequently, longer duration of treatment may appear protective against preterm birth or SGA simply by being a marker of longer gestation. To mitigate the connection between duration of treatment and duration of gestation and allow the majority of women to complete their exposure duration before delivery, we assessed cART duration at 32 weeks. Duration of cART was categorized into never initiated or did not initiate by 32 weeks gestation (referent), 1-8 weeks and 9-32 weeks. Category cut-points were based on the functional form of the relationship between the exposure and outcome, as well as clinical considerations to approximately correspond with early and mid-to-late pregnancy.

For both Aims 1a and 1b, gestational age was calculated by last menstrual period (LMP) for pregnancies less than 20 weeks at time of enrollment into ANC, following standard clinical practices in resource-limited settings.[198] For those ≥20 weeks at enrollment, both LMP and symphysis-fundal height were used. If these two methods yielded gestational ages within 3

weeks of each other, the date based on the LMP was used. If not, the fundal height–derived gestational age was used.

A3b. Outcome Assessment:

For Aim 1a, the primary outcome was a binary measure of LBW, defined as <2,500 grams.[199] Mean birthweight as a continuous measure was assessed as a secondary outcome.

For Aim 1b, SGA was considered the primary outcome and defined as birthweight below the 10th percentile for each week of gestational age at birth (weeks 28-41). The reference curve used to define SGA was based on fetal weight and adjusted to account for lower overall birthweights in a Zambian population.[200] Preterm birth (delivery <37 weeks gestation) was considered as a secondary outcome.

A3c. Confounder Assessment:

For Aim 1a, confounding variables were identified using directed acyclic graphs[201, 202] and included age, baseline BMI, baseline CD4 count, baseline hemoglobin, education, parity, previous preterm birth (<37 weeks gestation), number of ANC visits and gestational age at birth. Number of ANC visits and education were used as proxies for health seeking behavior.[203-205] The functional form of the relationship between continuous confounders and outcome was assessed and confounders were modeled either as linear terms (age, CD4 count and gestational age at birth) or using restricted quadratic splines (BMI and hemoglobin).[206] Information on viral load, WHO clinical stage, drug regimen and antiretroviral adherence was not available in ZEPRS.

For Aim 1b, the same methods for confounder selection were used and the same confounders considered, with the addition of intermittent presumptive therapy (IPT) for malaria, syphilis screening/treatment and self-reported tuberculosis status. Gestational age at birth was

not considered a confounder in the Aim 1b analysis because it is used to define both preterm birth and SGA outcomes.

A4. Statistical Analyses

A4a. Preliminary data exploration

The distribution of all exposure, outcome and confounding variables was assessed for outliers, implausible values and proportion of missing data before statistical analyses began. Potential confounders were identified from literature review and based on known biologic mechanisms. To ensure appropriate and efficient coding of all covariates, the functional forms of each covariate's relationship with the outcome was assessed. Continuous, ordinal, categorical, polynomials and splines coding schemes were considered.

A4b. Approach and model choice

For Aim 1a, In the primary analysis Poisson models with robust variance estimators [207] were used to estimate RRs for the association between duration of cART and LBW (<2500 grams; binary outcome). As a secondary analysis, linear regression was used to estimate mean changes in birthweight (continuous outcome) associated with category of cART duration and duration of cART as a continuous measure. We note that effect estimates are interpretable as associations and not causal effects because not all women are under observation from a uniform time point at the beginning of pregnancy (e.g. there is left truncation in the data) and due to unmeasured confounding.

The goal of Aim 1a was two-fold: 1) to identify women whether women with longer durations of cART during pregnancy and who deliver at term may be at a higher risk of sub-optimal birthweight outcomes, to target for infant nutritional interventions following birth and 2)

to identify any associations between duration of cART during pregnancy and birthweight, which may be seen when cART is scaled up to all HIV-infected pregnant women in Zambia.

For Aim 1b, We used Poisson models with robust variance estimators,[207] to estimate RRs and 95% CIs, for the associations of duration of cART with SGA and preterm birth in three separate analyses. First, we conducted a complete-case analysis using only the observed data (naïve-analysis). Second, we used MI to impute missing data for all confounders, the exposure and the gestational age as a continuous measure (used to define both outcomes). The imputation model included predictors of any missing data (not only missing gestational age), all confounders, the exposure and the outcome. Third, we used MO to impute missing confounder and exposure information and to overimpute gestational age. Due to the fact that the data arise from routine clinical care and not all women are under observation from a uniform time point in pregnancy and the likely presence of unmeasured confounding, we note that reported effect estimates are interpretable as associations and not causal effects.

In the MO analysis, Bayesian observation-level priors were specified for all measured values of gestational age, with a mean of w_i and a variance due to the measurement error in gestational age. The mean of the prior distribution (w_i) was calculated by taking the observed value of gestational age (x_i) and adding an off-set (a_i), equal to the difference between the observed and expected values of gestational age. A range of variances due to measurement error were considered, corresponding to 15%, 30% and 60% of the variance in gestational age. MO was performed using the Amelia II package in R (R Development Core Team Vienna, Austria).[208]

The goal of Aim 1b was also twofold: 1) to attempt to correct the measurement error in gestational age in ZEPRS and assess how missing data and measurement error may have biased

associations 2) to identify any associations between duration of cART during pregnancy and preterm birth and SGA, which may be seen when cART is scaled up to all HIV-infected pregnant women in Zambia.

Multiple Overimputation – an overview

MO is a recently proposed general-purpose method from political science literature [208-210] to address missing data and measurement error. Briefly, with MO missing data are addressed in the same way as multiple imputation. Missing values are multiply imputed based on observed covariates. Observed, but mismeasured, values are handled slightly differently. Mismeasured values are overimputed (replaced) with multiply imputed values based on observed covariates, with the additional step of observed mismeasured value serving as observation-level Bayesian priors in the imputation model.[210] In this way, the available prior information (in the form of the observed value x_i) about the true value x^*_I is incorporated into the imputation model. Whereas multiple imputation rests on the assumption that that data are missing at random, conditional on observed covariates, MO rests on a weaker assumption that measurement error for an observed value is random, conditional on the observation-level prior and observed covariates.[210]

The goal of specifying observation-level Bayesian prior is to incorporate prior knowledge, as well as appropriate uncertainty, about the true value of x (x^*_i) into the imputation model[210]. In the case of MO, the observed value of x_i provides considerable prior knowledge about the value of x^*_i, however other sources of information can be incorporated to provide additional information about x^*_i, such as information from a validation study or reference standard.

Specifying observation-level Bayesian priors involves two parts: specifying the mean of prior distribution and the variance due to measurement error. The mean of the prior distribution is set to a proxy value (w_i) most likely to reflect x^*_i, since x^*_i is unknown.[210]. After the mean of the prior distribution has been specified, to appropriately account for uncertainty about x^*_i, we must specify a variance around w_i. The variance around w_i can be conceptualized as the variance due to measurement error, and is chosen or estimated in one of three ways. The value of the variance due to measurement error may be chosen based on published estimates.[211] If repeated measures of the mismeasured covariate are available, the variance due to measurement error can be directly estimated from the data.[210] Finally, if repeated measures or published information is not available, the variance due to measurement error can be conceptualized as the proportion of the observed covariate's (x) variance due to measurement error and estimated from the data. The proportion of variance due to measurement error is an arbitrary value assigned by the investigator and therefore is best considered as a range of values (e.g. 30%-70%).

A4c. Descriptive Analyses

For Aim 1a, in addition to presenting descriptive statistics for duration of cART and confounders in tabular format, we investigated the following descriptively:

1. Frequency and proportion of women eligible to initiate cART who do not initiate during pregnancy.
2. Frequency and proportion of women eligible to initiate cART, who do not deliver in a facility.
3. Compared the distribution of covariates between women who deliver at a health facility and those who do not deliver at a health facility.

4. Compared descriptive statistics for LBW, birthweight and preterm birth between HIV-infected women included in the analysis and HIV-uninfected women in ZEPRS.

For Aim 1b, in addition to presenting descriptive statistics for duration of cART and confounders in tabular format, we investigated the following descriptively:

1. Frequency and proportion of women who had a SGA, preterm and both SGA and preterm infant.
2. Frequency and proportion of women that did not initiate cART during pregnancy and the frequency and proportion that did not initiate cART by 32 weeks gestation.
3. Compared the mean, 25^{th} and 75^{th} percentile birthweights for each week of gestational age from a reference curve to observed birthweight and gestational age values to assess measurement error in gestational age.
4. Examined histograms of birthweights within weeks of gestational age to assess possible digit preference in recorded birthweight values.
5. Examined the proportion of missing data for the exposure and all confounders.

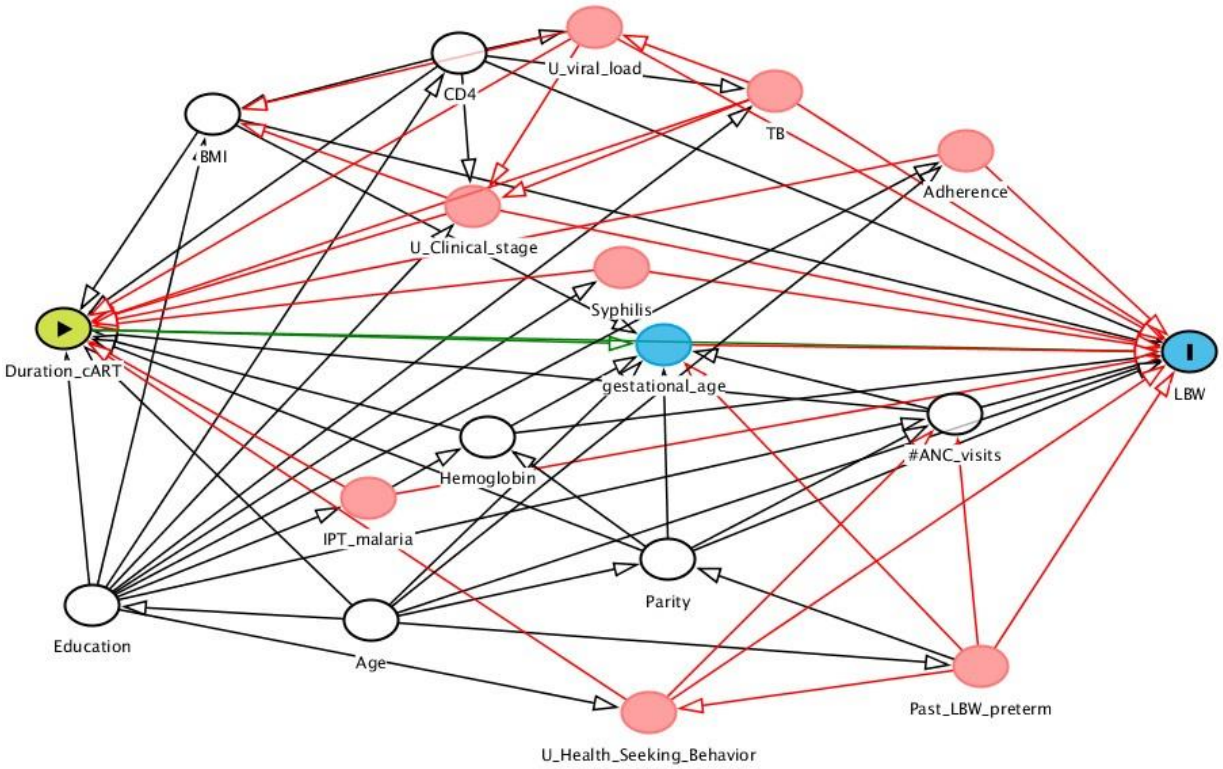
A4d. Confounder selection

For Aim 1a, directed acyclic graphs (DAG) were used to identify a minimally sufficient adjustment set of confounders that need to be controlled for in order to close all non-causal pathways between duration of cART and LBW will be included in the full multivariable model (Figure 3.1). Confounders in the minimally sufficient adjustment set included: number of ANC visits, age, BMI, CD4 count, WHO clinical stage, cART adherence, viral load, education, hemoglobin, intermittent presumptive therapy (IPT) for malaria, parity, past adverse pregnancy outcome, syphilis screening/treatment, self-reported tuberculosis status and health seeking behavior. Due to model convergence issues past adverse pregnancy outcomes, syphilis

screening/treatment during pregnancy and tuberculosis status were not included in the final multivariable model. Additionally, information on viral load, adherence, and WHO clinical stage were not available in ZEPRS. We therefore note there is likely unmeasured confounding in our analysis.

Gestational age is on the causal pathway between duration of cART and LBW and therefore is a mediator, and not confounder, of the relationship between duration of cART and LBW. Since we are unable to estimate causal effects (due to left truncation and unmeasured confounding), we dealt with gestational age by restricting our analyses to term births in an effort to avoid the inherent dependency between duration of treatment and length of gestation.

Figure 3.1. Directed Acyclic Graph for Relationship between Duration of cART and LBW



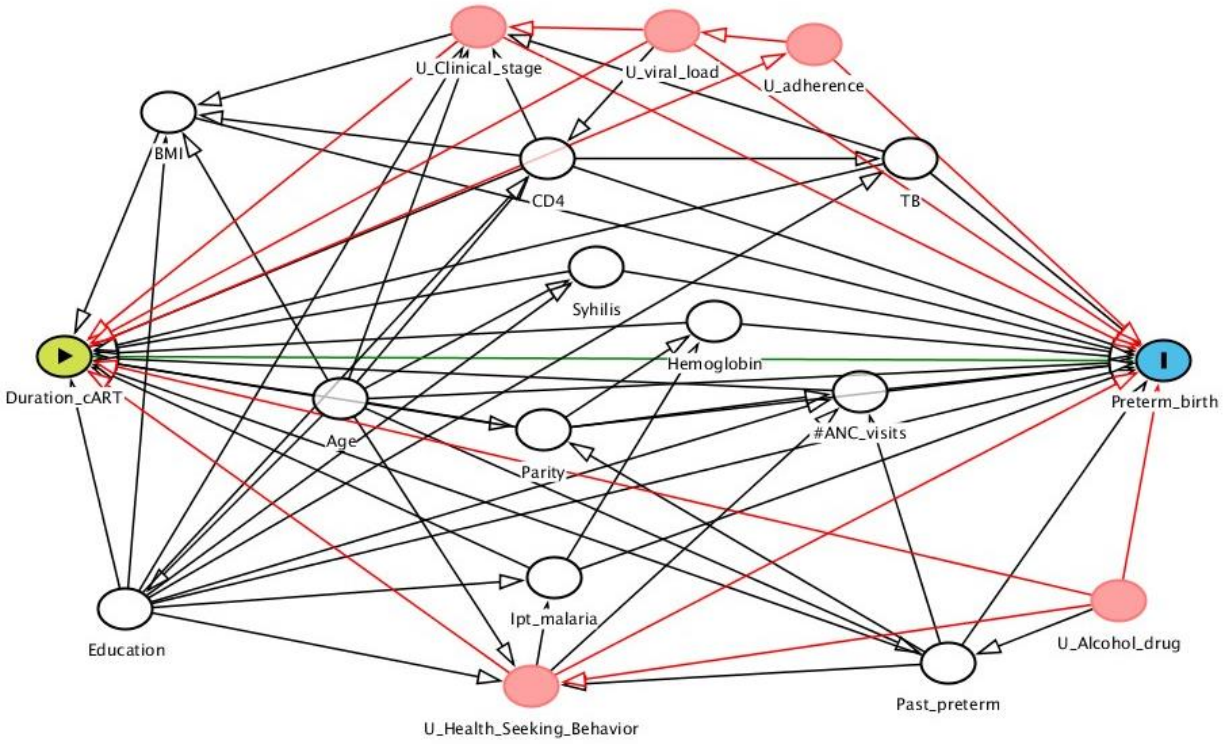
For Aim 1b, confounders were identified in the same manner as in the Aim 1a. The minimally sufficient adjustment set (Figure 3.2), included: included age, baseline BMI, baseline

CD4 count, baseline hemoglobin, education, IPT for malaria, parity, previous preterm birth (<37 weeks gestation), syphilis screening/treatment, self-reported tuberculosis status, number of ANC visits, viral load, antiretroviral adherence and drug, WHO clinical stage, alcohol use and health seeking behavior. Information on adherence, viral load, WHO clinical stage and drug and alcohol use was not available in ZEPRS. We therefore note the presence of unmeasured confounding.

A4e. Multivariable analyses

For both Aim 1a and 1b, measured confounders in the minimally sufficient adjustment set that could be included and still ensure model convergence were included in the final models. In both sub-aims, we attempted to use log binomial models to estimate RRs for the associations of duration of cART with LBW (Aim 1a), preterm birth and SGA (Aim 1b). In both analyses, log-binomial models did not converge and so Poisson models with robust variance estimators were used.[207] For Aim 1a, as a secondary analysis linear regression was used to estimate mean changes in birthweight (continuous outcome) associated with each duration of cART category and duration of cART as a continuous measure.

Figure 3.2. Directed Acyclic Graph for Relationship between Duration of cART and Preterm Birth



A4f. Sensitivity Analyses

For Aim 1a, we conducted the following sensitivity analyses:

1. **Inclusion of women who initiate cART with CD4 count >350:** The retrospective data used in this analysis was collected when the Zambian Ministry of Health's policy was to initiate women on cART during pregnancy who had a CD4 count ≤350.[60] In practice, women with a CD4 count >350 were sometimes initiated on cART during pregnancy for clinical reasons. Currently, Zambia is in the process of implementing and scaling up lifelong cART for all pregnant and breastfeeding women.[154] In an effort to make our results as generalizable as possible to the current cART initiation policy in Zambia, we conducted a sensitivity analysis including all women who initiated cART during pregnancy with a CD4 count >350.

2. Multiple imputation for missing data: As with many sources of routinely collected clinical information, missing data was a concern. To address possible bias from missing data, in a sensitivity analysis we assessed predictors of missing 1) CD4 counts, 2) cART initiation dates 3) confounders and 4) missing data 1-3 combined (all missing data imputed) and performed multiple imputation (n=50 imputations)[212] for the missing data.

Assumptions of multiple imputation: Multiple-imputation provides unbiased estimates of effect if data are missing completely at random (MCAR) or missing at random (MAR) conditional on measured variables.[212, 213] When data are MCAR observed data are a simple random sample of complete data. Therefore no bias due to missingness is induced when observed data are MCAR. When data are MAR, observed data are a stratified random sample of complete data, with strata defined by fully observed covariates (i.e. being missing is conditional on observed covariates). Whether data is MAR is an untestable assumption in data, and instead relies on knowledge about why data came to be missing.[214] If data can reasonably be assumed to be MAR, then bias due to missingness can be corrected using multiple imputation.[215] However, if missing data is conditional on unobserved covariates (due to being measured but missing or due to not being measured), then data is missing not at random (MNAR) and bias due to missingness cannot be corrected with multiple imputation.[212, 214]

We evaluated whether data was <u>not</u> MCAR in the following way: We then used log binomial regression to see if any observed covariates, including the exposure, were significantly associated with the missing data If any observed covariates were associated with an indicator for missingness (1=missing, 0=observed), then missing data were considered not to be MCAR. In all 4 analyses, several covariates predicted missing data. Therefore the data was considered not to be MCAR.

Hypothesized reasons for missing information: In order to evaluate the reasonableness of the untestable assumption that data were MAR, we assessed reasons data were likely to be missing. We hypothesized that the primary predictors of missing information were likely to be related to health status (being too sick from causes unrelated to pregnancy, such as tuberculosis, to get to a health facility) or access to healthcare due to socioeconomic status or possibly distance from a health facility. Health status was captured by a number of covariates, including BMI, CD4 count, WHO clinical stage, hemoglobin and tuberculosis status. Access to healthcare is also captured by several covariates, including education, number of ANC visits and age. Distance to a health facility is not measured in the ZEPRS database and is unlikely to be a major barrier to accessing healthcare since the study takes place in the urban Lusaka area.

Justification for proceeding with multiple imputation under MAR assumption: In an effort to make the MAR assumption as reasonable as possible, the imputation model for each type of missing data (see analyses 1-4 above) included predictors of missingness, predictors of the outcome and confounders of duration of cART and LBW.[216] Further, after reviewing possible reasons for missing data, we did not hypothesize that data were likely to be missing in a manner associated with unmeasured covariates. Therefore, we felt it is reasonable to assume that data is MAR conditional on observed covariates and proceed with MI.

3. Additional sensitivity analyses: We additionally performed several sensitivity analyses. First, to minimize the impact of length of time in care, we compared women in each category of cART duration to a referent of women who never initiated cART, but were enrolled in ANC for the same duration of time. Second, we stratified the association between cART duration and LBW by baseline CD4 count to see if women with greater baseline immunosuppression were at a higher risk of LBW. Third, we assessed women's duration of cART at 37 weeks

gestation (instead of at delivery) so that treatment duration was completed before women reached term. Fourth, we calculated gestational age using LMP at or before 20 weeks gestation and fundal height only after 20 weeks gestation. Finally, we assessed the association between duration of cART and birthweight using a linear and quadratic term for duration of cART to relax the assumption of linearity.

For Aim 1b, we conducted the following sensitivity analyses:

1. **Amount of variance due to measurement error:** Since the amount of measurement error in gestational age was not known (i.e. based on previous literature) or could not be estimated from our data (i.e. with repeated measures), a range of variances due to measurement error were considered, corresponding to 15%, 30% and 60% of the variance in gestational age. This range allowed us to assess the impact of increasing measurement error in gestational age on associations between duration of cART and preterm birth and SGA.

A5. Limitations and Challenges

For Aim 1a:

1. **Study population restricted to term births:** Our study population did not include pregnant women who miscarried or delivered prior to seeking institutional healthcare, which could limit the external validity of our results.
2. **Measurement error in gestational age**: Despite limiting our analyses to term births, measurement error in gestational age may still be present.

For Aim 1b:

1. **Reference values based on a population of HIV-uninfected women**: The reference curve values used to assess and correct measurement error in gestational age are based on a population of HIV-uninfected women. The distribution of birthweight within each week of

gestational age may vary between HIV-uninfected women and HIV-infected women, limiting our ability to correct the measurement error in gestational age in a population of HIV-infected women.

For Aim 1a and Aim 1b:

1. **Unmeasured confounding**: We did not have access to information on important confounders, including markers of HIV disease (e.g., viral load, WHO clinical stage), antiretroviral regimens, or adherence. While it is difficult to predict the direction of bias due to unmeasured confounding overall, if women with more advanced HIV were more likely to be on cART longer, one would expect to see an increased risk of LBW among women on cART the longest. Such an association was not observed.
2. **Selection bias due to not all women seeking care**: As with all analyses of routinely collected clinical data, selection bias may arise since not all women present for care. However, clinical findings are applicable only in clinical settings, so associations observed among those who present for care may be generalizable to other populations in care.
3. **Effect estimates for are interpretable as associations and not causal effects because not all women are observed from the beginning of pregnancy:** To estimate the causal effect of duration of cART on pregnancy outcomes we might wish to randomize women to a trimester to initiate cART in at the start of pregnancy and assess their pregnancy outcomes. In our observational data, women who initiate treatment earlier may have health-seeking behavior which makes them more likely to initiate cART early during pregnancy and have better birthweight outcomes. Educational attainment and number of ANC visits [203-205] were used as proxies for health-seeking behavior and controlled for in our analysis. More formally, the fact that not all women are observed from the beginning of pregnancy, and that in fact the

majority will not be observed until the second or third trimester, indicates that our study sample was left truncated.[217] For example, women who may have been randomized in a trial to initiate cART during their second trimester, but who miscarry in their first trimester, do not show up in our analysis. Left truncation precludes a causal interpretation of effect estimates from Aim 1a and Aim 1b. However, conditional on having made it to 28 weeks gestation (37 weeks for Aim 1a) and delivering in a health facility, this analysis provides important information to clinicians treating HIV-infected women, policy-makers considering changes to treatment guidelines for pregnant women and guide the way to future causal analyses.

B. Aim 2 – Predicting postpartum LTFU among HIV-infected women initiating cART during pregnancy

B1. Overview and Study Design

We conducted a retrospective cohort analysis of HIV-infected pregnant women attending public antenatal and HIV healthcare facilities in Lusaka, Zambia. Lusaka is an urban setting where antenatal care (ANC) coverage has been consistently reported at >95% [197]. HIV testing and CD4 screening are conducted within public antenatal clinics. Among women attending ANC, HIV testing is near universal and CD4 count screening coverage is approximately 80% [14]. During the study period (2009-2011), women with a CD4 count $\leq$350 were eligible for lifelong cART and services were provided either in integrated ART-ANC clinics or stand-alone HIV treatment departments, typically co-located at the same health facility. Data for the present analysis come from the Zambian Electronic Perinatal Record System (ZEPRS), which has collected comprehensive obstetric information on mothers and infants through delivery since 2007 [14], and SmartCare, an electronic medical record system for HIV clinical information. Prior to 2011, ZEPRS and SmartCare data could be linked using a medical record number across

all 24 public healthcare facilities in Lusaka, thus providing a complete clinical picture from entry into ANC to postpartum HIV care.

B2. Study Population and Eligibility

Women included in our analysis initiated cART during pregnancy, had a CD4 count ≤350 (in accordance with national HIV guidelines at the time) and delivered in a public-sector facility between January 1, 2009 and November 2, 2010. Women who delivered prior to 2009 were not included because the CD4 count initiation guidelines at that time were ≤ 200 for HIV-infected pregnant women in Zambia. Women had to deliver at least 91 days (30 days to have a HIV clinic visit and 61 days to allow the possibility of being LTFU) before the administrative censoring date of February 1, 2011. Women in our cohort were followed for up to 6 months after delivery. During that time they received cART from either HIV clinics or integrated ANC-HIV clinics. We excluded women who died during pregnancy or up to 42 days after delivery in order to exclude possible maternal deaths.[218] Women who are eligible, but did not initiate, cART during pregnancy were not be included in the statistical analysis of LTFU since they did not initiated cART.

Generalizability –Our risk score is intended for use in maternity care settings in SSA where the characteristics of women initiating HIV treatment during pregnancy and the rate of postpartum LTFU are likely to be similar to those in our study population. As such, findings from Aim 2 are generalizable to similar populations of HIV infected pregnant women with a CD4 count ≤350 who initiate cART during pregnancy or at delivery and who deliver in a healthcare facility.

Limitations – Women who do not delivery in a health facility and later returned to HIV care were not included in the risk score analyses, since no data was available for them at the time

of delivery (when the risk score would be assessed). Our analyses were also restricted to women who initiated cART during pregnancy at CD4 count ≤350 in accordance with Zambian policy at the time of data collection. However, the predictors of LTFU among women who initiate cART higher CD4 counts may differ.

B3. Outcome and Covariate Assessment

B3a. Outcome Assessment

Loss to follow-up from HIV care or all-cause mortality by 6 months postpartum was the outcome of interest and was ascertained from the SmartCare HIV database. Loss to follow-up was defined as not presenting to HIV care ≥60 days since the last appointment.[177] Women were marked as LTFU on the 61st day after a missed appointment. Women who never returned to HIV care after delivery were allowed up to 30 days to schedule an appointment and were marked as LTFU 61 days after that time (91 days total of follow-up time). We used a combined outcome of LTFU or death so that death was not a completing risk for LTFU. The dates of each HIV care clinic visit, the next scheduled HIV visit, vital status and date of mortality are recorded in SmartCare and were used to ascertain the outcome up to 6 months postpartum.

B3b. Covariates Assessment

Covariate information was obtained from linked SmartCare and ZEPRS records and covariate values were assessed at the time of cART initiation. Three categories of predictors for LTFU at 6 months postpartum were considered: demographic, obstetric, and HIV characteristics. In identifying candidate predictors, we prioritized information that would be readily available to clinicians at the point of care. We considered the following demographic predictors: age, marital status and employment status. Obstetric predictors considered were: number of ANC visits, parity, and indications of a poor pregnancy outcome, such as low infant birthweight (LBW; <2,500 grams) and preterm birth (<37 weeks gestation). HIV predictors considered were: CD4

count, WHO clinical stage, duration of cART taken during pregnancy, whether or not a woman received a new diagnosis of HIV during pregnancy, BMI, hemoglobin level at entry into ANC and whether or not a woman had active tuberculosis. To allow clinicians to easily calculate the risk score with a pen and paper (and not a computer), candidate predictors were coded as either binary or simple categorical variables. Information on predictors of LTFU such as viral load, disclosure of HIV status to a partner and distance to the clinic were not available.

B4. Statistical Analyses

B4a. Preliminary data exploration

The distribution of the outcome and all covariates were assessed for outliers, implausible values and proportion of missing data before beginning statistical analyses. Covariates for the risk score were selected based on information likely to be available to clinicians at delivery. To allow clinicians to easily calculate the risk score with a pen and paper (and not a computer), candidate predictors were coded as either binary or simple categorical variables.

B4b. Descriptive analyses

In addition to investigating the frequency and proportion of all covariates by LTFU status in tabular format, we performed the following descriptive analyses:

1. **LTFU over time:** We created a bar graph of the frequency and proportion of women LTFU at weeks 11 through 24 postpartum (corresponding to approximately 6 months postpartum). Since women could not be LTFU before not returning to care after a scheduled appointment for 61 days, no women who returned to HIV care after delivery met the definition of LTFU before 11 weeks postpartum. Women who did not return to HIV care after delivery were not included in the bar graph, as they were all marked as LTFU 91 days after delivery (see section B3a).

2. **Mortality:** We assess the frequency and proportion of deaths that occurred (both maternal and non-maternal) within 6 months postpartum.
3. **Sensitivity and specificity:** We graphed the sensitivity and specificity and their respective 95% CIs at each risk score cut-point. Sensitivity, specificity and 95% CIs for each risk score cut-point were also calculated and graphed in the bootstrapped data and compared to the observed data.
4. **Positive predictive value (PPV) and negative predictive value (NPV):** Since PPV and NPV values vary based on the prevalence of the outcome, we graphed PPV and NPV against a range of prevalence values for LTFU and indicated the observed prevalence in our study population with a reference line.

B4c. Risk score development – bivariable and multivariable analyses

The primary goal in developing the risk score was to create an easy to use clinical tool to identify HIV-infected women at delivery, who had initiated cART during pregnancy and may be at high risk of postpartum LTFU. The goal of our analyses was predictive rather than etiologic. Therefore, we will not consider confounders, but rather assess covariates that may predict LTFU at 6 months postpartum.

The analysis occurred in two steps: first we developed a predictive model for LTFU at 6 months postpartum and used the beta coefficients as the basis for the risk score; we then validated the risk score in a bootstrapped dataset.

To develop the risk score, logistic regression was used to estimate unadjusted odds ratios (ORs) and 95% CIs for all candidate predictors. Predictors associated with LTFU at 6 months postpartum with a p-value < 0.25 were retained for the full multivariable model. We used manual backward elimination based on likelihood ratio tests to reduce the full model to a more

parsimonious final model. After the elimination of each variable, the area under the curve (AUC) was compared with the full model to determine whether the two models had comparable predictive ability. Multi-collinearity was assessed using Spearman correlations. For variables that were collinear based on Spearman correlation coefficients, the variable with the strongest predictive power (e.g. largest coefficient) was retained in the final model. Variable elimination stopped when all predictors left in the multivariable model had a p-value ≤ 0.10, in an effort to balance retaining potentially important predictors with parsimony. Model fit was assessed using the Hosmer-Lemeshow test. A priori we specified a risk score cut-point with sensitivity > 80% and specificity >60% as optimal. These sensitivity and specificity values were selected to prioritize identifying the women most likely to be LTFU (higher sensitivity), while maintaining a reasonable ability to identify women most likely not to be LTFU.

To create the final risk score, beta coefficients from the final logistic regression model were multiplied by 10 and rounded to the nearest integer. Values for each person were summed to create an individual risk score. Sensitivity and specificity was assessed at each risk score cut-point.

B4d. Risk score validation

Risk score validation was carried out by bootstrapping the original data set (n=1,000) and comparing the mean, 2.5th and 97.5th percentile values for sensitivity and specificity to their respective values and 95% CIs in the original data.[219] In the bootstrapped data, the mean, 2.5^{th} and 97.5^{th} percentiles of the distribution of sensitivity and specificity correspond to estimated sensitivity or specificity and their 95% CIs in the original data.

B4e. Sensitivity analyses

We conducted a sensitivity analysis where whether or not a women enrolled into ART care prior to initiating cART during pregnancy was additionally considered as a predictor in the development of the risk score.

B5. Limitations and Challenges

1. **Only obstetric and HIV data collected 2007 - 2011 can be linked**: In 2011 the Zambian Ministry of Health stopped providing identifying information, such as date of birth, other than unique medical identifications numbers to CIDRZ for data collected in HIV and obstetric databases. Without additional identifying information, the ability to link the two databases using unique identification numbers alone is limited.
2. **Women with CD4 counts >350 not included in the analysis**: In 2013 Zambia changed their cART guidelines so that all pregnant and HIV-infected women were eligible for lifelong cART. Results from this analysis are not directly generalizable to a setting in which option B+ has been implemented. In settings where Option B+ has been implemented, women not previously eligible for cART (e.g. CD4 count >350 cells/mm3) have had the highest rates of LTFU.[72, 153] As data become available in settings where Option B+ has been implemented, revising the risk score to include women initiating at higher CD4 counts may be important to improve prediction of postpartum LTFU.
3. **Inability to validate deaths**: Zambia does not maintain a national vital registry, therefore we were unable to validate the deaths that were recorded in SmartCare or to ascertain whether some women LTFU later died.
4. **Predictive ability of the risk score may not generalize to other settings**: If women initiating cART in our study population differ from women initiating cART under Option B+

or in other settings, the predictive ability of the risk score may differ. Prior to implementation, the risk score should be validated in the study population in which it will be used.

C. Data Collection, Quality and Management – Aim 1 and 2

Data for all analyses will be abstracted from routinely collected clinical data captured in the ZEPRS and SmartCare electronic medical records systems. Electronic medical records systems have been shown to improve the standardization, quality, and completeness of medical documentation in developing country settings.[220-222] The ZEPRS obstetric database collects extensive obstetric information on each pregnancy, including gestational age, maternal health status and reproductive history, demographic information, HIV testing, status and cART initiation information and delivery characteristics for each pregnancy. The SmartCare HIV database collects information on WHO clinical stage, opportunistic infection, current cART regimen and physical exam information at each clinic visit.

Data for both systems is entered in real-time by nurses, midwives or clerical staff during patient visits. All staff receive a 2-10 day training on using the system, data entry, record retrieval. Data entry systems are pre-programmed to prevent entry of implausible values. The entry of abnormal, but plausible, values is flagged by the system for investigation and correction or intervention by providers, as needed. Data quality is assessed on a monthly basis by a data coordinator. Issues requiring further investigation are reviewed by clerical staff and district nurses at individual clinics. Data entered in each clinic is stored a central server and linked between clinics, making it retrievable from any clinic location within the network.[14] The ZEPRS database covers a network of public clinics providing maternity services in the Lusaka district. The SmartCare database covers a network of public HIV clinics in a broader geographic

area of Zambia, including Lusaka district. Obstetric and HIV records within Lusaka can be linked using patient unique identification numbers.

D. Power and Sample Size

Table 3.2 Statistical power to detect the association between trimester of cART initiation and low birthweight, 2-sided alpha of 5%

	Risk Ratio			
% LBW in unexposed:	1.3	1.5	1.8	2
13%	**0.93**	**>.99**	**>.99**	**>.99**
15%	**0.96**	**>.99**	**>.99**	**>.99**
17%	**0.98**	**>.99**	**>.99**	**>.99**

D1. Aim 1. Given the related outcomes of Aim 1a and Aim1b, we calculated statistical power for an outcome of LBW only. Statistical power was calculated based on estimated available sample size and a binary outcome of LBW, using a 2-sided alpha of 0.05. For the purposes of calculating statistical power, the exposure was timing of cART initiation and was categorized into trimesters (we later moved to an exposure based on duration of cART during pregnancy due to concerns about measurement error in gestational age). Exposure is assessed at the time of delivery and we estimated statistical power for the exposure contrast between initiating cART during the third trimester compared to the second trimester (referent). Second trimester is the referent because the majority of women initiate cART during that time. In the ZEPRS dataset there are 9,419 HIV-infected women with a valid CD4 count ≤350 and delivery information.[14] Published data from Zambia indicates 98% of births are singleton and assuming 3% of women are excluded due to other health condition, the sample size is 8,954.[14, 15] In Zambia, 13% of HIV-infected women present to ANC at ≥28 weeks gestation (3rd trimester) and 33% at ≤20 weeks gestation.[15] To be conservative, we assume only 50% of women who

present at ≤20 weeks do so during their first trimester (<14 weeks gestation). Therefore, we estimate that 17% of women initiate during their first trimester, 71% during their second trimester and 13% during their third trimester. Rescaling the sample size to include only the 84% of the sample who initiated in their second or third trimester, the final sample size is 7,521. We present power estimates varying the percent with the outcome (LBW) in the unexposed (2nd trimester initiators) as 13%, 15% or 17%. We also vary the effect size of the prevalence ratio (PR) at 1.3, 1.5, 1.8 and 2.0. Because our analysis is cross-sectional, we assume no loss to follow-up. Under any of the varied conditions, we have at >90% power to detect a RR of 1.3 or higher for the association between initiating cART during the third trimester compared to the second trimester (Table 3.2).

D2. Aim 2. Aim 2 was originally planned as a time-to-event analysis of the association between timing of cART initiation and LTFU, with death treated as a competing risk. After initial analyses, it was determined that developing a risk score to predict postpartum LTFU would be more clinically useful and that the number of deaths in the cohort were few enough to combine the outcome. Consequently, power analyses presented below are for a time-to-even analysis, accounting for competing risks.

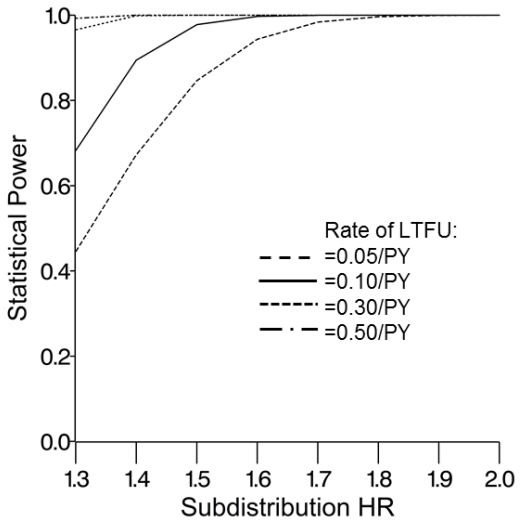


Figure 3.3 Statistical power to detect the association between trimester of cART initiation and LTFU, accounting for competing risks, 2-sided alpha of 5%.

Statistical power was calculated based on estimated available sample size, using a 2-sided alpha of 0.05. For the purposes of calculating power, the exposure, timing of cART initiation, was categorized into trimesters. The primary outcome is

time to LTFU in HIV care, taking into account the competing risk of death, and will be estimated using subdistribution proportional hazard models. As in traditional survival analysis, the subdistribution hazard is directly related to the survival function. Therefore, standard methods to calculate power for survival analyses can be used to calculate statistical power to detect subdistribution hazard ratios.[186, 223, 224] In the ZEPRS dataset there are 4,254 HIV-infected women on cART who delivered a live birth at a health facility between 2007 and 2011.[15] To account for the fact that not all women may have been successfully linked to their HIV records, we estimate a conservative sample size of 3,500. In Zambia, 13% of HIV-infected women present to ANC at ≥28 weeks gestation (3rd trimester) and 33% at ≤20 weeks gestation.[15] To be conservative, we assume only 50% of women who present at ≤20 weeks do so during their first trimester (<14 weeks gestation), and therefore estimate that overall 17% of women present to care and initiative cART during their first trimester. Because the majority of women initiate cART during their second trimester in Zambia, we calculate statistical power for the exposure contrast of third trimester initiation compared to second trimester initiation (referent). Rescaling the sample size to the 83% of the sample estimated to have initiated ART during their second or third trimester, the final sample size is 2,905. We assume participants are followed on average two years and that 84% if the re-scaled population is unexposed (initiated in their second trimester).

Very little published data is available on the rate of loss to follow-up according to trimester of cART initiation. Therefore, we present power curves to detect a subdistribution hazard ratio between 1.3 and 2.0 for LTFU rates among the unexposed ranging from 0.05/person year to 0.50/person year, based on published data from both the Zambian cohort of individuals on cART[63, 180, 225] and HIV-infected pregnant women on cART in SSA.[71, 72]. Even at

the most conservative rate of LTFU (0.05/person year), we have >80% statistical power to detect a subdistribution hazard ratio of ≥1.5 (Figure 3.1). All power calculations were completed using PROC POWER in SAS, version 9.2.

CHAPTER IV. AIM 1A - DURATION OF CART BEFORE DELIVERY AND LOW INFANT BIRTHWEIGHT AMONG HIV-INFECTED WOMEN IN LUSAKA, ZAMBIA

A. Introduction

In 2013, the World Health Organization (WHO) recommended lifetime combination antiretroviral therapy (cART) for all HIV-infected pregnant women in countries with a generalized HIV epidemic. Known as "Option B+," this policy is currently being scaled up in several sub-Saharan African (SSA) countries, including Zambia.[226] Option B+ is expected to streamline treatment initiation for HIV-infected pregnant women by removing the need for CD4 count screening and simplifying treatment recommendations.[227] Improved access to treatment – and the resulting increase in women starting pregnancy already on cART over time – is expected to play a critical role in virtually eliminating new pediatric HIV infections and improving maternal health.[7]

However, cART use during pregnancy could have a negative impact on fetal growth. Adverse associations between cART during pregnancy and birthweight were first reported among HIV-infected pregnant women in Europe.[38, 58] These findings were not confirmed by studies in North and Latin America, where no adverse associations between cART and low birthweight (LBW; <2500 g) were observed.[16, 33, 37, 107] cART with a protease inhibitor (PI) was associated with an increased odds of very low birthweight (VLBW; <1500 g) in a combined cohort study of 7 US sites, but the precision was poor, as very few outcomes were observed (n=16).[39] The relationship between cART and LBW among women in SSA is also unclear. cART has been associated with LBW among women in Cote d'Ivoire and small for

gestational age among women Botswana.[34, 125] As cART access expands throughout SSA, the impact of longer durations of cART during pregnancy on LBW must be understood.

cART use during pregnancy could affect LBW through two different pathways: shortening gestation length (leading to preterm birth (PTB)) or restricting fetal growth. Duration of cART during pregnancy is structurally linked to length of gestation, making it difficult to meaningfully assess the association between duration of treatment and PTB. We, therefore, limited our analysis cohort to those infants delivered at term. This approach allowed us to focus on the potential relationship between duration of cART and LBW due to growth restriction, in a population of HIV-infected women eligible to initiate cART for maternal health in Zambia.

B. Materials and Methods

B1. Study Design and Setting

We conducted a retrospective cohort analysis of HIV-infected pregnant women attending public antenatal healthcare facilities in Lusaka, Zambia. Lusaka is an urban setting where antenatal care (ANC) coverage has been consistently reported at >95%.[197] Over the study period, HIV testing and CD4 screening were conducted within antenatal clinics. Among women attending ANC, HIV testing is near universal and CD4 count screening coverage is approximately 80%.[14] Pregnant women who meet local eligibility criteria for cART receive services either in integrated ART-ANC clinics or stand-alone HIV treatment departments, typically co-located at the same primary health facility. Comprehensive medical information, including HIV treatment information, has been captured by the Zambia Electronic Perinatal Record System (ZEPRS) since 2007 in all 24 public antenatal clinics in Lusaka.[14] Ethical approval was obtained from the University of Zambia Biomedical Research Ethics Committee

(Lusaka, Zambia) and the University of North Carolina, Chapel Hill (Chapel Hill, NC) and informed consent was not obtained for the analysis of routinely collected clinical data.

B2. Study Population

Women were included in the present analysis if they entered ANC after January 1, 2009 and delivered before September 1, 2013, had a CD4 count of ≤350 cells/uL (the threshold for ART eligibility during the study period), were not on cART at the time of presentation and delivered a singleton pregnancy at a public healthcare facility at ≥37 weeks gestation.[228, 229] Since duration of cART is linked to length of gestation and preterm infants are more likely to be LBW, we limited our analysis cohort to those infants delivered at term and focused on an outcome of LBW due to growth restriction. Women with chronic conditions such as known heart disease, hypertension, and diabetes have poorer pregnancy outcomes [230, 231] and may be more likely to seek ANC earlier due to their preexisting conditions. To minimize confounding, this group was also excluded.

B3. Definitions

The exposure of interest was duration of cART before delivery, measured in completed weeks and categorized as never initiated (referent), ≤8 weeks, 9-20 weeks, and 21-36 weeks. Category cut-points were based on the functional form of the relationship between the exposure and outcome, as well as clinical considerations to approximately correspond with cART initiation in early, mid and late pregnancy. We also considered duration of cART before delivery as a continuous measure of weeks on cART. The primary outcome was a binary measure of LBW, defined as <2,500 grams.[199] Mean birthweight as a continuous measure was used as a secondary outcome.

Confounding variables were identified using directed acyclic graphs[201, 202] and included age, baseline body mass index (BMI), baseline CD4 count, baseline hemoglobin, education, parity, previous PTB (<37 weeks gestation), number of ANC visits and gestational age at birth. Number of ANC visits and education were used as proxies for health seeking behavior.[203-205] The functional form of the relationship between continuous confounders and outcome was assessed and confounders were modeled either as linear terms (age, CD4 count and gestational age at birth) or using restricted quadratic splines (BMI and hemoglobin)[206]. All potential confounders were included in multivariable models, unless otherwise noted. Information on viral load, WHO clinical stage, drug regimen and antiretroviral adherence was not available in our electronic medical record.

Gestational age was calculated following standard clinical guidelines by last menstrual period (LMP) for pregnancies <20 weeks at time of enrollment into ANC. For those ≥20 weeks at enrollment, both LMP and symphysis-fundal height were used. If these two methods yielded gestational ages within 3 weeks of each other, the date based on the LMP was used. If not, the fundal height–derived gestational age was used. Gestational age dating based on LMP is known to contain error.[232, 233] To assess the validity of gestational age dating in our data, we compared mean birthweight for each week of gestational age at birth to a reference growth curve adjusted to a Zambian population.[200] Comparisons with a reference growth curve values suggested substantial measurement error in gestational dating for women delivering before 35 weeks. For women delivering at ≥35 weeks gestation, mean birthweights were comparable to reference curve values, indicating relatively unbiased gestational age dating (Figure 4.4).

B4. Statistical analyses

In the primary analysis, we used multivariable Poisson models with robust variance estimators to estimate risk ratios (RRs) and 95% confidence intervals (CIs)[234] for the association between categories of duration of cART before delivery and LBW. In secondary analysis, we used multivariable linear regression to estimate the association between duration of cART before delivery and birthweight, treating duration of cART first as a continuous measure (weeks on cART) and then as a categorical measure.

We additionally performed several sensitivity analyses. First, to minimize the impact of length of time in care, we compared women in each category of cART duration to a referent of women who never initiated cART, but were enrolled in ANC for the same duration of time. Second, we stratified the association between cART duration and LBW by baseline CD4 count to see if women with greater immunosuppression were at a higher risk of LBW. Third, we assessed women's duration of cART at 37 weeks gestation (instead of at delivery) so that treatment duration was completed before women reached term. Finally, we included all women who initiated cART with a CD4 count>350 to make results as generalizable as possible to a setting where Option B+ has been implemented.

As with many sources of routinely collected clinical information, missing data was a concern. To address possible bias from missing data, in a sensitivity analysis we assessed predictors of missing 1) CD4 counts, 2) cART initiation dates 3) confounders and 4) missing data 1-3 combined (all missing data imputed) and performed multiple imputation (n=50 imputations) for the missing data. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc. Cary, NC).

C. Results

Among 50,765 HIV-infected pregnant women enrolled in ANC, 4,474 women met inclusion criteria for our study cohort (Figure 4.1). LBW occurred in 302 of pregnancies (7%). Nulliparity was more common among women who had a LBW infant, than among women with normal birthweight infants (29% versus 18%). Median baseline BMI and CD4 count values were similar between those delivering LBW and normal birthweight infants. Only 10% of women attended ≥4 ANC visits, as recommended by the WHO (Table 4.1).[235]

Of the 4,474 cART-eligible women in our cohort, 2,749 (62%) never initiated treatment, 643 (14%) received ≤8 weeks of cART, 976 (22%) received 9-20 weeks of cART and 103 (2%) received 21-36 weeks of cART (Table 4.2). Of the 2,749 women who never initiated cART, 81% received zidovudine (with or without single-dose nevirapine) to prevent vertical HIV transmission, 13% received only single-dose nevirapine and 6% had no reliable information recorded. Among the 1,722 women who initiated cART, the median time from 1st ANC visit to cART initiation was 36 days (IQR 24, 64 days).

To assess possible selection bias, women included in the study population were compared with eligible women who presented to ANC, but did not deliver in a ZEPRS-supported facility (n=3,921). Women with no delivery information were similar to women included in the study, with a few exceptions. Women without delivery information were more likely to have only 1 ANC visit (75% vs. 47%, p-value <0.01) and less likely to have a cART initiation date recorded (22% vs. 41%, p-value <0.01).

C1. Main analyses

In the primary analysis, there was no evidence to suggest that duration of cART was associated with an increased risk of LBW. Compared to women who never initiated treatment,

there was no evidence that receiving cART for ≤8 weeks (RR 1.22, 95% CI: 0.77, 1.91), 9-20 weeks (RR 1.23, 95% CI: 0.82, 1.83), or 21-36 weeks (RR 0.87, 95% CI: 0.22, 3.46) was associated with an increased risk for LBW after adjustment for multiple confounders (Table 4.2).

Similarly, there was no evidence of an association when birthweight was considered as a continuous outcome, although mean birthweight decreased modestly as duration of cART increased. Compared to women who never initiated cART, the mean birthweight for women receiving ≤8 weeks of cART was lower by 2.35 grams (95 % CI: -53.69, 49.98), lower by 44.19 grams for women receiving 9-20 weeks of cART (95% CI: -89.55, 1.17) and lower by 65.8 grams for women receiving 21-36 weeks of cART (95% CI: -194.92, 63.38). The distribution of birthweights between exposure categories, stratified by gestational age, did not differ meaningfully (Figure 4.2). When weeks on cART before delivery was considered as a continuous measure, a one-week increase in duration on cART before delivery was associated with a decrease in mean birthweight of 2.53 grams (95% CI -5.40, 0.35) (Table 4.3).

C2. Sensitivity analyses and imputation of missing data

Our results were consistent across several sensitivity analyses, although small sample size was limited in some analyses. When women on cART were compared with non-initiators with the same duration of ANC, the RR was 4.20 (95% CI: 0.81, 21.82) for ≤8 weeks of cART, 1.08 (95%CI: 0.71, 1.64) for 9-20 weeks of cART and 0.95 (95% CI: 0.18, 4.92) for 21-36 weeks of cART. Similar findings were observed when we stratified by baseline CD4 count, when cART duration was assessed at 37 weeks gestation and when women who initiated cART with a CD4 >350 were included (Figure 4.3).

A high proportion of CD4 counts, cART initiation dates and confounder data were missing. Among 50,765 HIV-infected pregnant women, 32% (n=16,324) were missing CD4

counts. Of the 4,474 women included in the study, 62% (n=2,773) had ≥1 missing confounder and an additional 18% (n=1,002) were missing a cART initiation date. In general, performing multiple imputation improved precision but resulted in similar point estimates. When all missing data was imputed, point estimates were similar to the primary analysis; receiving cART ≤8 weeks was associated with RR 1.15 (95% CI: 0.87, 1.54), 9-20 weeks RR 1.35 (95% CI: 1.07, 1.70) and 21-36 weeks RR 0.96 (95% CI: 0.52, 1.79).

D. Discussion

D1. Main findings

Among women with CD4 count ≤350 who delivered at ≥37 weeks, longer duration of cART during pregnancy did not result in increased risk of LBW or decreased mean birthweight. These findings did not change meaningfully across numerous sensitivity analyses. Modest decreases in birthweight were observed with increasing cART duration when cART duration was continuously measured. However, this small decrease must be weighed against the substantial benefits of cART in preventing mother-to-child (PMTCT) transmission. Our analysis included only term births, and therefore our findings are focused on LBW due to fetal growth restriction.

D2. Limitations and Strengths

We note several limitations of this work. First, we did not have access to information on important confounders, including markers of HIV disease (e.g., viral load, WHO clinical stage), antiretroviral regimens, or adherence. While it is difficult to predict the direction of bias due to unmeasured confounding overall, if women with more advanced HIV were more likely to be on cART longer, one would expect to see an increased risk of LBW among women on cART the longest. Such an association was not observed. Second, as with all analyses of routinely collected clinical data, selection bias may arise since not all women present for care. However, clinical

findings are applicable only in clinical settings, so associations observed among those who present for care may be generalizable to other populations in care. In addition, our analysis cohort did not include pregnant women who miscarried or delivered prior to seeking institutional healthcare, which could limit the external validity of our results. Finally, despite limiting our analyses to term births, measurement error in gestational age may still be present (Figure 4.4).

Strengths of our study included the use of data from an electronic medical record system covering 24 public health clinics in Lusaka, the use of a study population with uniform eligibility for cART initiation and the consistency of results across multiple sensitivity analyses. In addition, our analysis provides some of the first evidence about the lack of an association between duration of cART and the risk of LBW due to fetal growth restriction among HIV-infected women in SSA.

D3. Interpretation

The aim of this analysis was to determine the association between cART duration during pregnancy and LBW due to growth restriction. PI-based cART has been hypothesized to impact fetal growth by inhibiting progesterone production during pregnancy.[131] Among HIV-uninfected women, low progesterone levels have been associated with lower birthweights.[236, 237] In animal models, PI-based cART regimens have been associated with decreased progesterone levels, which correlated with lower fetal weight.[131] In this respect, our results provide some level of reassurance. Given our focus on LBW due to growth restriction, our results may be helpful to clinicians caring for patients at low-risk for PTB. Additional work is needed to understand how duration of cART affects preterm delivery.

Although the association between PTB and cART duration is of significant interest, we were unable to examine this specific research question in the current context. Studying the

relationship between duration of cART and PTB is difficult because duration of treatment is tied to length of gestation and timing of delivery. Women on cART longer are, by definition, closer to term and therefore less likely to have a PTB. In populations at high risk for PTB, this could result in longer durations of cART looking protective against PTB. Because of these constraints, we focused on LBW due to fetal growth restriction (as opposed to PTB) by restricting our analysis to term births.

Our findings are consistent with other work looking at treatment duration and LBW among term and preterm births. cART initiated either early during pregnancy (≤25 or <28 weeks gestation) or late (≥28 or 32 weeks gestation) was not associated with LBW in studies in South Africa and the US. However, since term and preterm births were combined in these studies – without further stratification as in this report – it is difficult to distinguish whether late initiators had no increased risk of LBW, or were simply less likely to have a PTB by virtue of starting treatment later during pregnancy. Birthweight Z-scores adjusted for gestational age were considered in a French study and no association with duration of cART was found [90]. An analysis from Malawi and Mozambique attempted to stratify gestational age at birth, but was unable to investigate the association between duration of cART and LBW among term infants due to the small sample size (n=496)[113]. Our study, therefore, provides some of the first evidence from SSA on associations between duration of cART and LBW due to fetal growth restriction.

Finally, this analysis cannot establish causality and we caution against overinterpretation in this regard. Prospective randomized studies investigating the effect of timing of cART initiation on LBW could help to establish causality. However, such a randomized study would pose serious ethical challenges, and might itself leave questions unanswered since women

included in randomized controlled trials are often a highly selected group, and may not be generalizable to a real-world clinical population of HIV-infected pregnant women. Analysis of retrospective routinely collected clinical data alone is unlikely to establish causality. However, given the challenges of understanding how timing of treatment may impact LBW, such analyses still provide important information about LBW trends among women initiating cART during pregnancy.

E. Conclusion

There was no evidence that longer duration of cART was associated with poor fetal growth among term pregnancies in our cohort. Despite slight decreases in birthweight with increasing cART duration, the benefits of cART during pregnancy for PMTCT continue to outweigh the risks. Our findings remained consistent across numerous sensitivities analyses, providing some level of reassurance about our primary findings. However, the relationship between cART use and adverse pregnancy outcomes – particularly those associated with PTB – remain complicated and continued work is required to investigate causality. An understanding of the relationship between cART and adverse pregnancy outcomes is of particular importance, as maternal combination regimens become the cornerstone of PMTCT programs globally.

F. Tables and Figures

Table 4.1. Sociodemographic and obstetric characteristics of 4,474 HIV-infected women eligible for cART initiation during pregnancy in Lusaka, Zambia 2009-2013.

Characteristic	Low Birthweight (<2,500g) N(%) or Median (IQR) N=302 (6.9)	Normal Birthweight (≥2,500g) N(%) or Median (IQR) N=4,070 (93.1)
Age	27 (23, 32)	28 (24, 32)
Education		
Primary or None	117 (45.5)	1,599 (44.7)
Secondary or Tertiary	140 (54.5)	1,979 (55.3)
Parity		
0	79 (29.4)	669 (18.2)
1	68 (25.3)	930 (25.2)
2	50 (18.6)	919 (24.9)
2+	72 (26.8)	1167 (31.7)
Body mass index (kg/m2)	22.3 (20.5, 24.7)	23.7 (21.8, 26.2)
CD4 count (cells/uL)	237 (171, 286)	243 (174, 300)
Hemoglobin (g/L)	10.6 (9.7, 11.8)	10.8 (9.9, 11.7)
Number of ANC visits		
1	137 (45.4)	1920 (47.2)
2	81 (26.8)	1038 (25.5)
3	59 (19.5)	715 (17.6)
≥4	25 (8.3)	397 (9.8)
Previous preterm birth [a]		
No/unknown	292 (96.7)	3949 (97.0)
Yes	10 (3.3)	12 (3.0)
Gestational age at birth	39 (37, 40)	39 (38, 40)

[a] Previous preterm birth: delivery <37 weeks gestation. Missing data: LBW (2.3%), age (0.1%), education (12.6%), parity (9.8%), BMI (32.2%), CD4 (0%), hemoglobin (8.4%), number of ANC visits (0%), previous preterm birth (0%), gestational age at birth (0%).

Table 4.2. Results for the association between duration of cART before delivery and low birthweight.

Weeks on cART before delivery	Low Birthweight N(%) N=302(6.9)	Normal Birthweight N(%) N=4069 (93.1)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)[a]
21-36	4 (1.3)	95 (2.3)	0.64 (0.24, 1.69)	0.87 (0.22, 3.46)
9-20	83 (27.5)	872 (21.4)	1.38 (1.07, 1.77)	1.23 (0.82, 1.83)
≤8	46 (15.2)	590 (14.5)	1.15 (0.84, 1.57)	1.22 (0.77, 1.91)
Never initiated	169 (56.0)	2,512 (61.7)	1.00	1.00

[a] Adjusted for: number of ANC visits, age, BMI, CD4 count, education, hemoglobin, parity, previous preterm birth and gestational age at birth.

Table 4.3. Multivariable results for the association between duration of cART and before delivery and birthweight.

Exposure Specification	Mean Birthweight (SD)	Unadjusted Mean Change in Birthweight (95% CI)	Adjusted Mean Change in Birthweight [a] (95% CI)
Weeks on cART before delivery, continuous	3040.00 (428.19)	-3.23 (-5.20, -1.26)	-2.53 (-5.40, 0.35)
Weeks on cART before delivery			
21-36	3059.90 (421.46)	1.89 (-83.85, 87.62)	-65.77 (-194.92, 63.38)
9-20	2995.79 (418.38)	-62.22 (-93.79, -30.65)	-44.19 (-89.55, 1.17)
≤8	3027.44 (439.57)	-30.57 (-67.52, 6.37)	-2.35 (-54.69, 49.98)
Never initiated	3058.01 (428.22)	0.00	0.00

[a] Adjusted for: number of ANC visits, age, BMI, CD4 count, education, hemoglobin, parity, previous preterm birth and gestational age at birth.

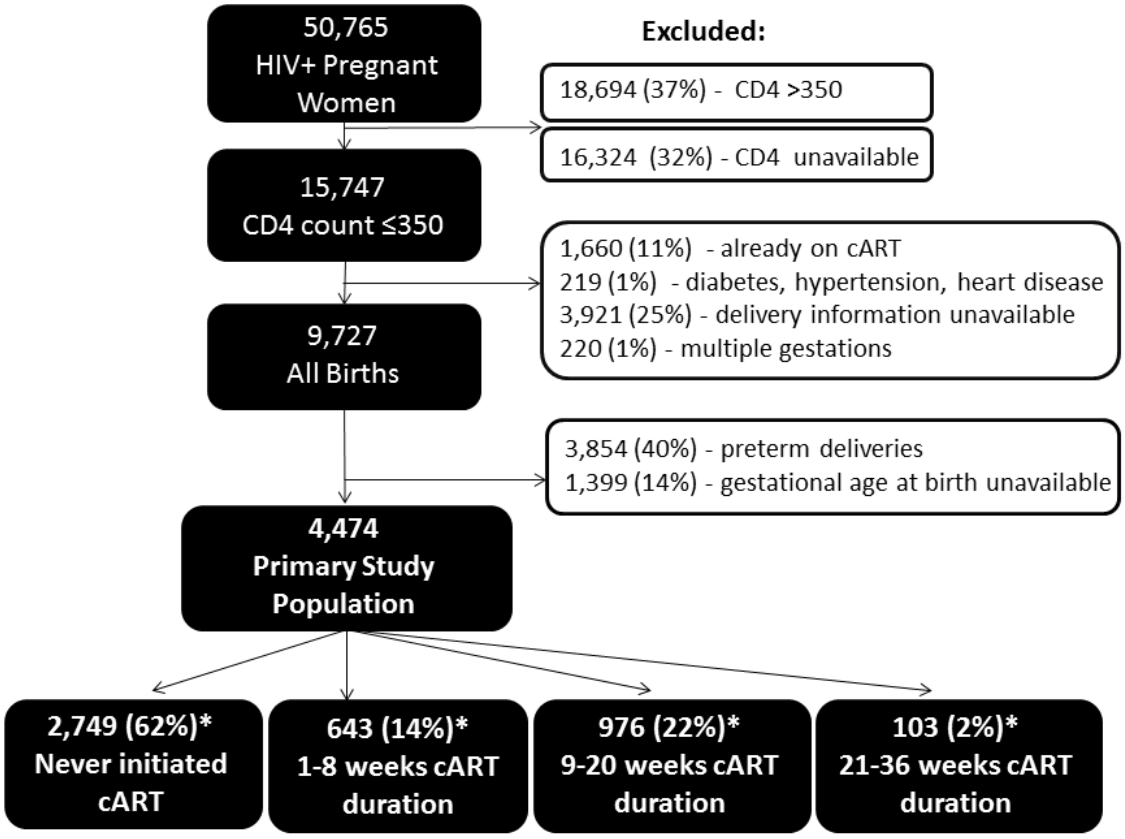


Figure 4.1. Inclusion criteria for 4,747 term births among HIV-infected pregnant women included in the study population. *Duration of cART could not be calculated for 3 women who had no delivery date available.

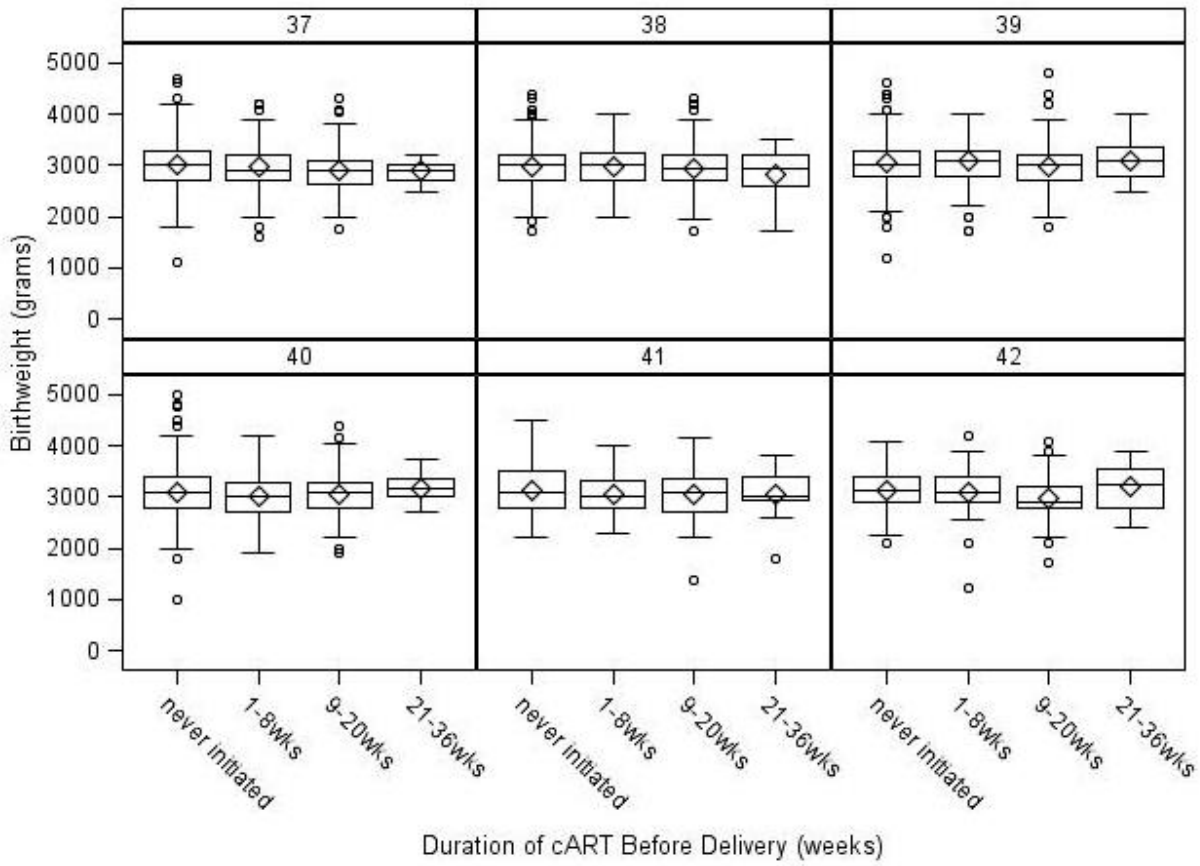


Figure 4.2. Boxplots of infant birthweight by duration of cART, for each week of gestational age at birth (weeks 37 through 42).

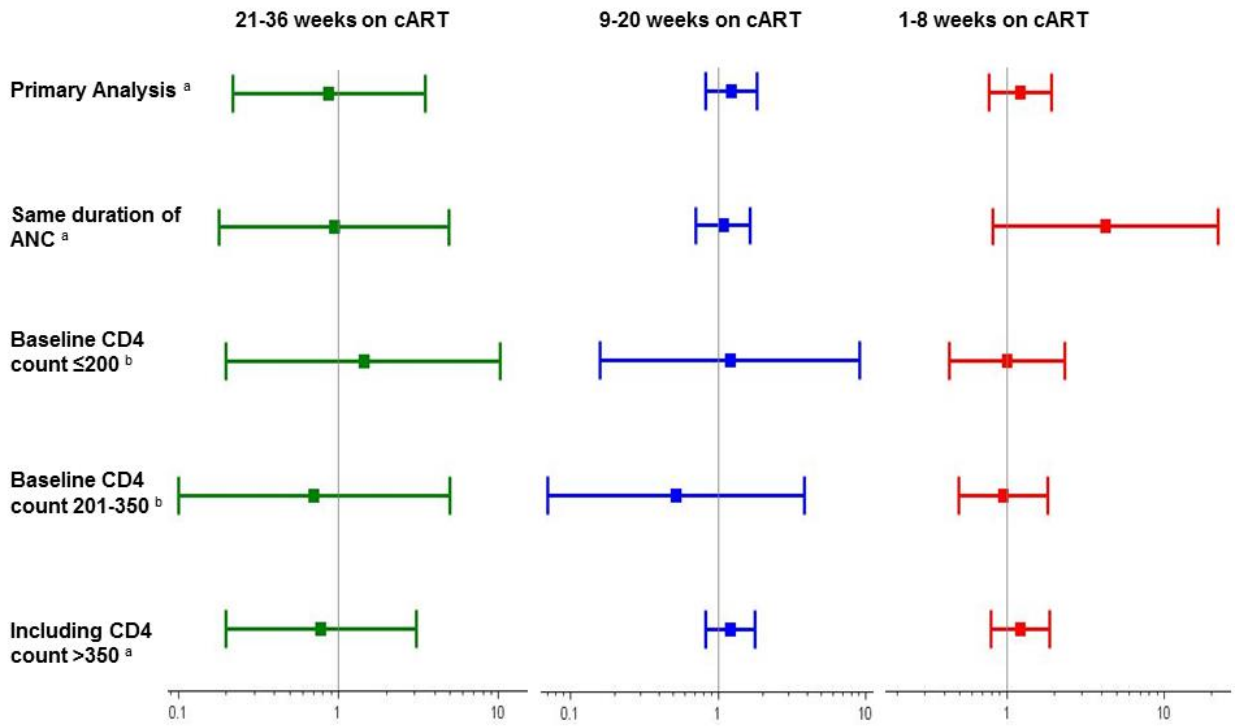


Figure 4.3. Risk ratios and 95% confidence intervals for the association between duration of cART and LBW, among term infants. [a] Adjusted for: number of ANC visits, age, BMI, CD4 count, education, hemoglobin, parity, previous preterm birth and gestational age at birth. [b] Adjusted for: number of ANC visits, age, BMI, education, hemoglobin, parity, previous preterm birth and gestational age at birth.

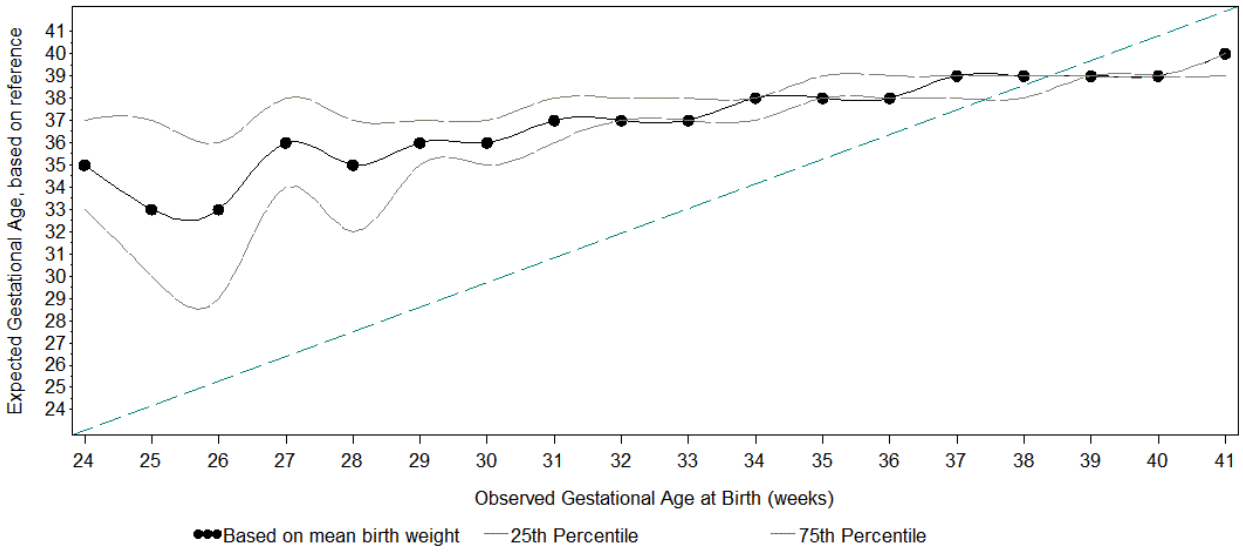


Figure 4.4. Comparison of gestational age data with reference curve values, adjusted to a Zambian population. The figure shows observed versus expected gestational age at birth, based on mean birthweight. The dashed diagonal line indicates perfect agreement between observed and expected values.

CHAPTER V. AIM 1B - THE RELATIONSHIP BETWEEN DURATION OF cART BEFORE DELIVERY AND PREGNANCY OUTCOMES: MULTIPLE OVERIMPUTATION AS A METHOD TO ADDRESS MISSING DATA AND MEASUREMENT ERROR

A. Introduction

In 2013 the WHO recommended lifetime combination antiretroviral therapy (cART) for all HIV-infected pregnant women in countries with a generalized HIV epidemic.[226] When taken during pregnancy and breastfeeding, cART reduces the risk of mother-to-child HIV transmission in resource-limited settings to <5%.[238] However, the impact of cART on fetal growth and length of gestation remains controversial. cART use during pregnancy has been associated with preterm delivery (delivery < 37 weeks gestation)[35, 36] and low infant birthweight(LBW; <2,500 grams)[58] in Europe and the United States.[33] In resource-limited settings, cART use during pregnancy has also been associated with an increased risk of preterm birth [239], LBW [34]and small for gestational age (SGA; birthweight < the 10^{th} percentile for gestational age).[32]

The impact of cART on pregnancy outcomes is a great concern in sub-Saharan Africa (SSA), where HIV prevalence remains high among women of reproductive age and lifetime cART initiation during pregnancy is rapidly being scaled up.[240] With nearly 13 million people on cART globally, the need to understand cART impact on pregnancy outcomes is critical.[241] In resource-limited settings, investigations of cART's association with pregnancy outcomes often rely on routinely collected clinical data.[32] Clinical data provides an important source of

information for monitoring pregnancy outcomes of HIV-infected women; but is typically not collected for research purposes. Routinely collected clinical data is consequently often plagued by missing data and measurement error.[242]

Missing data and measurement error in gestational age may introduce bias when evaluating the relationship between cART and gestational-age based outcomes like preterm birth and SGA. Gestational age dating based on last menstrual period (LMP) may include error due to natural variation in when women ovulate, errors in recall or missing LMP dates.[232] Inaccurate LMP dates may be more common among women in the developing world, where malnutrition, high fertility rates and longer breastfeeding duration may mean that many women do not resume regular menstrual cycles before becoming pregnant again.[243] Menstrual abnormalities are common in HIV-infected women, which may further limit the reliability of LMP dating.[244] Missing or mismeasured gestational age values in routinely collected clinical data may have implications for our understanding of cART's association with preterm birth and SGA.

To assess the impact of bias from missing data and measurement error in gestational age on the associations of duration of cART with SGA and preterm birth, we used multiple overimputation (MO). MO, a recently developed method from the social sciences, is a convenient approach to address both missing data and measurement error. The purpose of the current analysis is to illustrate the use of multiple overimputation, using routinely collected clinical data from public health facilities in Lusaka, Zambia.

B. Methods

B1. Multiple overimputation

MO is a convenient approach to address missing data and measurement error simultaneously. Although often viewed as separate forms of bias, missing data and measurement

error can be viewed as linked, where each one representing a degree of the other one.[245, 246] To illustrate this, consider the classical measurement error model, where for normal random measurement error a participant's observed value of x (x_i) is equal to the true value of x (x^*_i) plus measurement error (u_i) (equation 1a).[247] In the case of normal non-random measurement error, an off-set value (a_i) is added to the equation (equation 1b). With this measurement error model in mind, missing data can be conceptualized as a situation where we have no information about x^*_i.[210] With no available information on x^*_i for missing data, we may therefore wish to impute values based on observed data. For mismeasured values, we may also chose to impute the missing information on x^*_i, however the observed value x_i offers a considerable amount of prior information about the value of x^*_i.[210]

$$observed\ x_i\ = true\ x_i^* + u_i, \qquad u_i|x_i^* \sim N(0, \sigma_{ui}^2) \tag{1a}$$

$$observed\ x_i\ = true\ x_i^* + u_i + a_i\ , \qquad u_i|x_i^* \sim N(0, \sigma_{ui}^2) \tag{1b}$$

MO has been described in the political science literature,[208-210] but has not been widely used in epidemiology. Briefly, with MO missing data are addressed in the same way as multiple imputation. Missing values are multiply imputed based on observed covariates. Observed, but mismeasured, values are handled slightly differently. Mismeasured values are overimputed (replaced) with multiply imputed values based on observed covariates, with the additional step of observed mismeasured value serving as observation-level Bayesian priors in the imputation model.[210] In this way, the available prior information (in the form of the observed value x_i) about the true value x^*_I is incorporated into the imputation model. Whereas multiple imputation rests on the assumption that that data are missing at random, conditional on observed covariates, MO rests on a weaker assumption that measurement error for an observed value is random, conditional on the observation-level prior and observed covariates.[210]

Observation-level Priors

The goal of specifying observation-level Bayesian priors is to incorporate prior knowledge, as well as appropriate uncertainty, about the true value of x (x^*_i) into the imputation model.[210] In the case of MO, the observed value of x_i provides considerable prior knowledge about the value of x^*_i, however other sources of information can be incorporated to provide additional information about x^*_i, such as information from a validation study or reference standard.

Specifying observation-level Bayesian priors involves two parts: specifying the mean of prior distribution and the variance due to measurement error. The mean of the prior distribution is set to a proxy value (w_i) most likely to reflect x^*_i, since x^*_i is unknown.[210]. In considering what value to set w_i to, it is useful to again consider the classical measurement error model (equation 1a and 1b). If x_i can reasonably considered to be equal to x^*_i, plus random noise (the measurement error), then the observed x_i can be considered a proxy for the true value and w_i is equal to x_i (equation 2a).[210] That is, in the case of normal random measurement error, the observed value x_i serves as the mean of the prior distribution (w_i). In the case of normal non-random measurement, an offset (a_i), which is either known or estimated from the data, is added to x_i when setting w_i (equation 2b).[210]

$$w_i = observed\ x_i, \qquad w_i \sim N(x_i^*, \sigma_{ui}^2) \tag{2a}$$

$$w_i = observed\ x_i + a_i, \qquad w_i \sim N(x_i^*, \sigma_{ui}^2) \tag{2a}$$

For example if investigations into measurement error in gestational age using other sources of information (i.e. reference curve values) suggested that observed gestational age values of 35 were closer 38 weeks based on mean birthweight, then a_i would equal -3 for individuals with an observed value of 35 weeks gestational age.

After the mean of the prior distribution has been specified, to appropriately account for uncertainty about $x^*_{i,}$ we must specify a variance around w_i. The variance around w_i can be conceptualized as the variance due to measurement error, and is chosen or estimated in one of three ways. The value of the variance due to measurement error may be chosen based on published estimates.[211] If repeated measures of the mismeasured covariate are available, the variance due to measurement error can be directly estimated from the data.[210] Finally, if repeated measures or published information is not available, the variance due to measurement error can be conceptualized as the proportion of the observed covariate's (x) variance due to measurement error and estimated from the data. The proportion of variance due to measurement error is an arbitrary value assigned by the investigator and therefore is best considered as a range of values (e.g. 30%-70%). However, even with very little information on the proportion of variance due to measurement error, MO can provide more information than assuming no measurement error. For example, in simulations where the true proportion of variance due to measurement error was 50%, assuming any proportion of variance due to measurement error <80% resulted in lower mean squared error , compared to assuming no measurement error.[210]

Within the multiple overimputation framework, only mismeasured values are given observation-level priors; missing values are not. The reason for this is that within the MO framework, missing data represent an severe case of measurement error, where no prior information about x^*_i exists on which to base w_i and the variance due to measurement error is infinity.[210]

B2. Duration of cART and SGA – An example of multiple overimputation

Data for the present retrospective cohort analysis come from the Zambia Electronic Perinatal Record System (ZEPRS), which has collected comprehensive maternity and HIV care

information on mothers and infants in 24 public facilities in Lusaka, Zambia since 2007. A related analysis looking at LBW as an outcome used a similar study population and setting and has been described elsewhere.[248] Briefly, women who presented for antenatal care (ANC) and delivered between January 1, 2009 and September 2, 2013 were included in the present analysis if they had a CD4 count of ≤350 cells/uL at entry into ANC, were not on cART at entry into ANC and delivered a singleton pregnancy at a public healthcare facility at ≥28 weeks gestation (fetal viability cut-off in Zambia). Women with chronic conditions such as known heart disease, hypertension, and diabetes were excluded since they have poorer pregnancy outcomes [230, 231] and may be more likely to seek ANC earlier due to their preexisting conditions. Ethical approval for the analysis of routinely collected clinical data was obtained from the University of Zambia Biomedical Research Ethics Committee (Lusaka, Zambia) and the University of North Carolina, Chapel Hill (Chapel Hill, NC).

B2a. Definitions

For the present analysis, SGA was considered the primary outcome and defined as birthweight below the 10^{th} percentile for each week of gestational age at birth (weeks 28-41). The reference curve used to define SGA was based on fetal weight and adjusted to account for lower overall birthweights in a Zambian population.[200] Preterm birth (delivery at <37 weeks gestation) was considered as a secondary outcome. The exposure of interest was duration of cART before delivery, measured in completed weeks and assessed at 32 weeks gestation. Duration of cART during pregnancy is intrinsically linked to length of gestation. Consequently, longer duration of treatment may appear protective against preterm birth or SGA simply by being a marker of longer gestation. To mitigate the connection between duration of treatment and duration of gestation and allow the majority of women to complete their exposure duration

before delivery, we assessed cART duration at 32 weeks. Duration of cART was categorized into never initiated or did not initiate by 32 weeks gestation (referent), 1-8 weeks and 9-32 weeks. Category cut-points were based on the functional form of the relationship between the exposure and outcome, as well as clinical considerations to approximately correspond with early and mid-to-late pregnancy.

Likely confounders were identified using directed acyclic graphs [201, 202] and included age, baseline body mass index (BMI), baseline CD4 count, baseline hemoglobin, education, intermittent presumptive therapy (IPT) for malaria, parity, previous preterm birth (<37 weeks gestation), syphilis screening/treatment, self-reported tuberculosis status and number of ANC visits. Number of ANC visits and education were used as proxies for health seeking behavior.[203] The functional form of the relationship between continuous confounders and outcome was assessed and confounders were modeled using restricted quadratic splines.[206] All confounders were included in multivariable models. Information on viral load, WHO clinical stage, antiretroviral adherence and drug regimen was not available.

B2b. Assessment of Measurement Error in Gestational Age

Following standard clinical practices in resource-limited settings,[198] gestational age was calculated by last menstrual period (LMP) for pregnancies less than 20 weeks at time of enrollment into ANC. For those ≥20 weeks at enrollment, both LMP and symphysis-fundal height were used. If these two methods yielded gestational ages within 3 weeks of each other, the date based on the LMP was used. If not, the fundal height–derived gestational age was used.

We assessed the presence and direction of measurement error in observed gestational age values by comparing the mean birthweight for each week of gestational age at birth to a reference curve adjusted to a Zambian population. For each observed week of gestational age at

birth, we determined the expected gestational age value by finding the smallest absolute difference in mean birthweight among reference curve values. For example, if the mean birthweight for infants born at observed gestational age 35 weeks was 2,900 grams, based on reference value mean birthweights, the gestational age is more likely to be 38 weeks. We repeated the same process for the 25^{th} and 75^{th} percentiles of weight to assess whether the pattern in observed versus expected values was consistent across the birthweight distribution (Figure 5.1).

To investigate the possibility of digit preference in measured birthweight values (which could affect expected gestational age values), we inspected histograms of birthweight for each week of gestational age (Figure 5.5). We attempted to account for digit preference in gestational age values by choosing off-set values (a_i) between observed and expected gestational ages. Offset values from 4 to 0 weeks were used to account for both random and non-random measurement error across the distribution of gestational age (Table 5.2; Figure 5.2).

B2c. Statistical Analyses

We used Poisson models with robust variance estimators to estimate risk ratios (RR) and 95% confidence intervals (CIs),[207] due to lack of convergence of log-binomial models, for the associations of duration of cART with SGA and preterm birth in three separate analyses. First, we conducted a complete-case analysis using only the observed data (naïve-analysis). Second, we used multiple imputation (MI) to impute missing data for all confounders, the exposure and the gestational age as a continuous measure (used to define both outcomes). The imputation model included predictors of any missing data (not only missing gestational age), all confounders, the exposure and the outcome.[209] Third, we used MO to impute missing confounder and exposure information and to overimpute gestational age. Due to the fact that the

data arise from routine clinical care and not all women are under observation from a uniform time point in pregnancy, we note that reported effect estimates are interpretable as associations and not causal effects.

Observation-level priors were specified for all measured values of gestational age, with a mean of w_i and a variance due to the measurement error in gestational age. The mean of the prior distribution (w_i) was calculated by taking the observed value of gestational age (x_i) and adding an off-set (a_i), equal to the difference between the observed and expected values of gestational age. A range of variances due to measurement error were considered, corresponding to 15%, 30% and 60% of the variance in gestational age. MO was performed using the Amelia II package in R; all other statistical analyses were performed using SAS version 9.2 (R Development Core Team Vienna, Austria; SAS Institute Inc. Cary, NC).[208]

C. Results

Among 50,765 HIV-infected pregnant women, 9,529 women met inclusion criteria for our study population. Of the 9,529 women included, 583 (8%) delivered SGA infants, 3,656 (45%) delivered preterm infants and 108 (1%) delivered infants that were both preterm and SGA. Most women did not initiate cART by 32 weeks gestation (n=6,925, 77%; of these, 778 initiated cART at or after 32 weeks), 1415 (16%) women had between 1 and 8 weeks of cART by 32 weeks gestation and 611 (8%) had between 9 and 32 weeks of cART by 32 weeks gestation.

Missing data were common. Of the 9,529 women included, 1,399 (15%) were missing gestational age at birth. A SGA value could not be calculated for additional 733 women who had recorded gestational ages >41 weeks (SGA defined for weeks 24-41 only) or who were missing birthweight information. These women were, therefore, marked as missing for SGA (total missing SGA: n=2,132 (22.4%)). Only 5% (n=542) of women were missing exposure

information, due to not having a gestational age at first ANC value recorded. Missing confounder data was also common, with body mass index (33%), tuberculosis status (27%) and education (13%) having the highest degrees of missing data. Overall, 6,625 (70%) of women were missing exposure, outcome or confounder information (Table 5.1).

Comparisons of birthweight within weeks of gestational age with reference curve values suggested significant measurement error in gestational age. Particularly for preterm births (gestational age <37 weeks), observed gestational age was much lower than expected, based on comparisons of mean birthweight with the reference curve values. This suggests that the measurement error in gestational age <37 weeks is not random, but rather biased (non-random) in the direction of gestational age being incorrectly specified as too early. At gestational ages ≥37 weeks, measurement error appeared to be more randomly distributed (i.e. expected values randomly distributed around observed values) (Figure 5.1). Information on expected gestational age was used to specify observation-level priors for each observed value of gestational age. Inspection of histograms of birthweight overall and by gestational age week revealed apparent digit preference at birthweight 3,000 grams (Figure 5.5).

Small for gestational age. In the naïve analysis, receiving 9-32 weeks of cART was associated with RR 1.14 (95% CI 0.70, 1.85) and ≤8 weeks of cART was associated with RR 0.94 (95% CI 0.64, 1.37), compared to not initiating cART by 32 weeks gestation (Figure 5.3). When MI and MO were performed, point estimates 9-32 and ≤8 weeks of cART were attenuated and moved to the other side of the null. In the MO analysis, when 60% of variance due to measurement error was assumed, receiving 9-32 weeks of cART was associated with RR 0.92 (95% CI 0.70, 1.21) and ≤8 weeks of cART was associated with RR 1.01 (95% CI 0.84, 1.21). As the amount of variance in gestational age due to measurement error increased, the joint

distribution of gestational age and mean birthweight approached that of the reference curve values, suggesting that values for gestational age from the MO analysis were closer to what we might have expected them to be, based on reference curve values (Figure 5.4).

Preterm birth. Similarly, duration of cART and preterm birth were not associated in the naïve-analysis. Receiving 9-32 weeks of cART was associated with RR 0.98 (95% CI 0.83, 1.16) and ≤8 weeks of cART was associated with RR 1.02 (95% CI 0.94, 1.13). When MO was performed, point estimates for 9-32 weeks of cART moved away from the null as the variance due to measurement error increased. When 60% of variance due to measurement error was assumed, receiving 9-32 weeks of cART was associated with RR 1.22 (95% CI 1.02, 1.47) and ≤8 weeks of cART was associated with RR 0.95 (95% CI 0.83, 1.09), suggesting the risk of preterm birth may increase slightly with longer durations of cART(Figure 5.3). The proportion of preterm births decreased from 45% in the naïve-analysis to 30%, when 60% of the variance in gestational age was considered due to measurement error. For both SGA and preterm birth outcomes, performing MI alone dramatically improved precision, compared with the naïve-analysis. Additionally performing MO appropriately propagated uncertainty about the true value of gestational age through to final confidence intervals, and therefore slightly decreased precision from the MI analysis.

D. Discussion

In our analysis of duration of cART before delivery and its associations with SGA and preterm birth, MO was a convenient method to simultaneously address missing data and measurement error in gestational age. Here, application of MO showed that duration of cART before delivery was not associated with SGA, even after accounting for missing data and measurement error. Duration of cART before delivery was also not associated with an increased

risk of preterm birth in the naïve-analysis or when only missing data was imputed. However, when measurement error in gestational age was additionally considered in the MO analysis, point estimates for 9-32 weeks of cART moved away from the null, suggesting a possible increased risk of preterm birth with longer duration of cART.

Our findings largely align with previous studies examining the relationships between duration of cART with SGA.[18, 90] An association between cART and SGA was noted in a study in Botswana, however the analysis did not account for duration of treatment.[32] Early cART initiation (<14 or <28 weeks gestation)[18, 40] and late ($\geq$ 20 weeks gestation) during pregnancy has been associated with preterm birth.[130] Regimens containing protease inhibitors (PIs) have been of greatest concern.[18] Regimen information was not available in our dataset. However, PI-based cART is typically reserved for second line treatment in Zambia. This may be one reason why we did not observe a strong association between longer duration (which corresponds to earlier initiation) of cART and preterm birth. In our analysis, point estimates for the association between 9-32 weeks of cART and preterm birth did move away from the null as increasing amounts of measurement error were assumed, suggesting that measurement error in gestational age may have biased results towards the null.

Multiple overimputation is likely to be most useful in settings where a considerable amount of both measurement error and missing data exist and therefore it is advantageous to handle both issues simultaneously. To expand on this idea: one advantage to MO is simply the convenience of handling missing data and measurement error in one unified step. A second possible advantage is that MO allows information about the "true" values of mismeasured variables (in the form of observation-level priors) to be incorporated into the imputation model. This additional information, theoretically, improves the imputation model in MO, compared to if

one imputed missing data first and then subsequently correcting measurement error.[209] Addressing missing data and measurement error simultaneously also allows the appropriate uncertainty about missing and mismeasured values to be propagated through to the final point estimate's confidence interval. Thus, confidence intervals appropriately reflect increased precision (from imputing missing data), as well as increased uncertainty (from measurement error in gestational age).

MO also offers a flexible approach to handling measurement error. In contrast to other methods to address measurement error, such as regression calibration,[249] MO can be easily adapted to handle non-random (e.g. differential) forms of measurement error. And unlike regression calibration, moment reconstruction[250] or multiple imputation[246] methods to correct for measurement error, MO also does not require validation data. In fact, MO may be particularly useful in settings where validation data is not available, but other external sources of information (such as a reference curve) are available to help assess and correct measurement error.[210] Finally, unlike maximum likelihood estimation to address measurement error,[251] MO is easily performed in R using the Amelia II package.[208] Data can then be combined and analyzed in any statistical package.

Measurement error is rarely addressed in epidemiologic studies, yet the ability to measure variables correctly is fundamental to making inference.[252] For example, even a randomized controlled-trial with perfect follow-up could have biased results if the exposure is mismeasured. MO offers a convenient approach to address measurement error and missing data. However, correct application requires an understanding of MO's underlying assumptions. For MO to produce unbiased overimputed values, the measurement error model must be correctly specified.[210] This means that all predictors of measurement error, within levels of observed

mismeasured values, must be included in the imputation model and that the specified prior and measurement error variance are approximately correct. Typically, this untestable assumption can be made more reasonable by including a number of covariates in the imputation model and considering a range of measurement error variances. Varying the amount of measurement error assumed also allows the analyst to assess the sensitivity of point estimates to a range of assumptions about measurement error. Additionally, as with MI, MO rests on the assumption that data have a multivariate-normal distribution; an assumption violated by categorical or binary variables. Simulations by Blackwell et al. suggest that MO is robust to violations of normality and works. well for categorical variables when the amount of measurement error specified is approximately correct [210] Additional work is needed to evaluate the use of MO for mismeasured categorical variables and to compare MO with other methods to address measurement error. Bearing these caveats in mind, epidemiologists should consider using MO to address missing data and measurement error moving forward.

E. Tables and Figures

Table 5.1. Sociodemographic and obstetric characteristics of 9,529 HIV-infected women eligible for cART initiation during pregnancy in Lusaka, Zambia 2009-2013.

Characteristic	Small for gestational age [a] N(%) or Median (IQR) N=583 (7.9)	Normal for gestational age [a] N(%) or Median (IQR) N=6,814 (92.1)	Missing Data N (%) N=9,529
Age	27 (23, 31)	27 (23, 31)	17 (0.2)
Education			1,201 (12.6)
Primary or None	252 (49.7)	2,720 (45.8)	
Secondary or Tertiary	255 (50.3)	3,223 (54.2)	
Parity			903 (9.5)
0	145 (26.9)	1,150 (18.7)	
1	140 (26.0)	1,571 (23.5)	
2	108 (20.0)	1,517 (24.7)	
2+	146 (27.1)	1,912 (31.1)	
BMI	22.6 (20.8, 24.7)	23.4 (21.5, 25.8)	3,130(32.9)
CD4 count	230 (156, 286)	239 (168, 297)	0 (0.0)
Hemoglobin	10.6 (9.6, 11.6)	10.8 (9.8, 11.6)	759 (8.0)
Syphilis screening			0 (0.0)
Non-reactive	372 (63.8)	4,246 (62.3)	
Reactive	28 (4.8)	331 (4.9)	
Not tested	183 (31.4)	2,237 (32.8)	
Tuberculosis			2,590 (27.2)
No	410 (96.9)	4,380 (97.2)	
Yes	14 (3.1)	137 (2.8)	
IPT [b] for Malaria			0 (0.0)
None	154 (26.4)	1,701 (25.1)	
SP1	301 (51.6)	3,543 (52.0)	
SP2	82 (14.1)	1,086 (15.9)	
SP3	46 (7.9)	478 (7.0)	
Number of ANC visits			0 (0.0)
1	298 (51.1)	3,607 (53.9)	
2	141 (24.2)	1,734 (25.5)	
3	97 (16.6)	978 (14.4)	
≥4	47 (8.1)	432 (6.3)	
Previous Preterm birth			0 (0.0)
No	564 (96.7)	6,602 (96.9)	
Yes	19 (3.3)	212 (3.1)	

[a] Missing outcomes: SGA 2,132 (22.4%), preterm birth 1,399 (14.7%). [b] IPT: intermittent preventative therapy.

Table 5.2. Assigned proxy and offset values for each gestational age week. Proxy and offset values were assigned in between observed and expected gestational age values, no based on expected gestational age only, due to concerns over digit preference in birthweight (which could inflate the expected gestational age).

Observed GA* (x_i)	N	Observed mean birthweight	Reference curve mean birthweight	Expected GA*, based on reference curve	Assigned proxy value (w_i) of GA*	Offset (a_i), based on assigned proxy (w_i)
24	16	2343.13	620.2	35	27	3
25	22	1954.21	724.2	33	28	3
26	48	2009.33	839.6	33	29	3
27	56	2507.09	966.6	36	30	3
28	93	2287.27	1104.9	35	31	3
29	117	2455.73	1254.2	36	32	3
30	151	2475.5	1413.5	36	33	3
31	224	2630.47	1581.9	37	34	3
32	338	2651.87	1757.8	37	35	3
33	424	2726.22	1939.4	37	36	3
34	632	2782.36	2124.8	38	37	3
35	751	2893.9	2311.5	38	37	2
36	814	2918.75	2496.8	38	38	2
37	840	2979.31	2677.9	39	38	1
38	879	2975.1	2851.9	39	39	1
39	964	3038.74	3015.8	39	39	0
40	709	3081.8	3166.7	39	40	0
41	432	3098.28	3301.6	40	41	0

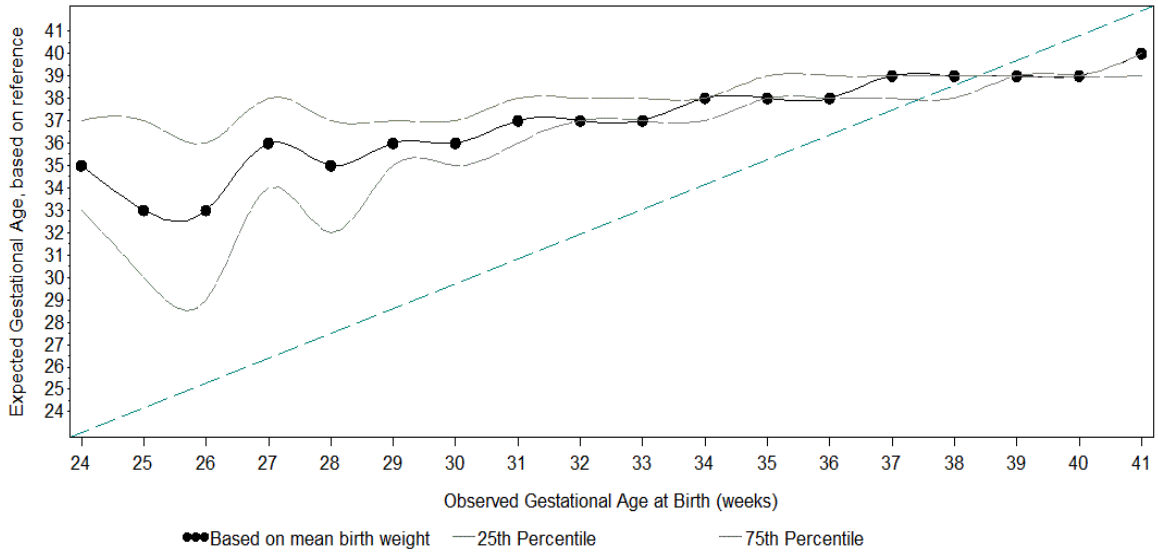


Figure 5.1. Observed versus expected gestational age of 9,529 HIV-infected women in Lusaka, Zambia 2009-2013. The X-axis indicates the value of gestational age observed in the data. To calculated expected values of gestational age (Y-axis), we compared each week of gestational age at birth to a reference curve adjusted to a Zambian population and found the smallest absolute difference in birthweight among reference curve values. The dotted line indicates expected gestational age based on mean birthweight and the dashed lines indicate expected values based on the 25th and 75th percentiles of birthweight. The diagonal line indicates perfected agreement between observed and expected values. The divergence of the dotted and dashed lines from the line of agreement at gestational ages <37 weeks) suggests that the measurement error in at these gestational ages is non-random in the direction of gestational age being incorrectly specified as too early. At gestational ages ≥37 weeks, measurement error appeared to be more randomly distributed around the line of agreement.

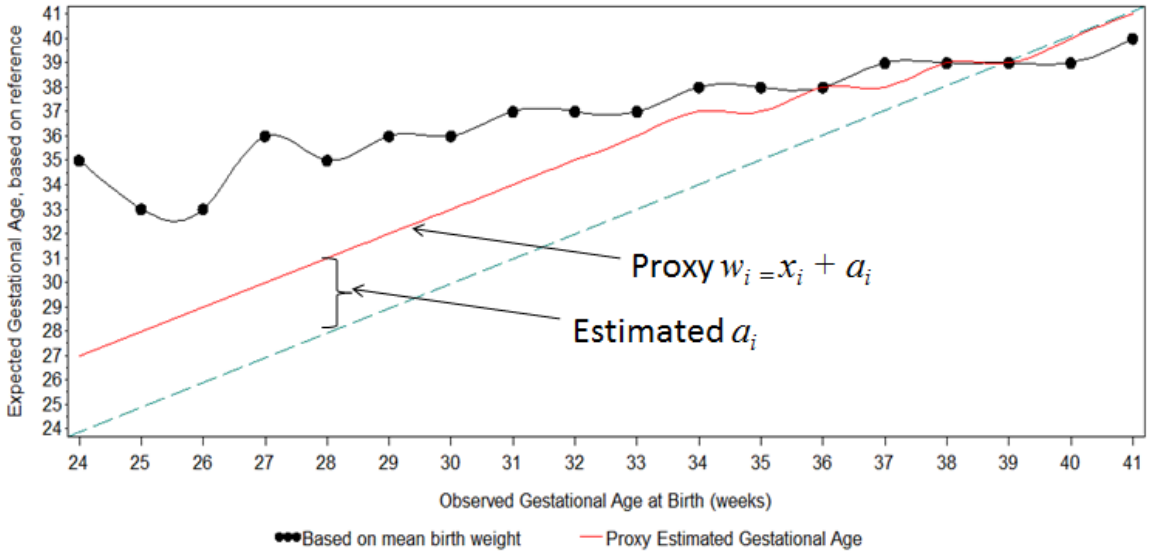


Figure 5.2. Estimated offset (ai) and proxy gestational age values (wi) for 9,529 HIV-infected women in Lusaka, Zambia 2009-2013. The dotted line indicates expected gestational age values, based on reference curve mean birthweights. The dashed line indicates perfect agreement between observed and expected gestational age values. The divergence of expected values from the line of agreement indicates non-random measurement error. To correct this non-random measurement error, an offset was calculated (a_i) and added to each observed value of gestational age to create a proxy value (w_i; solid line) for gestational age more likely to reflect the 'true' value of gestational age. Due to concerns over digit preference at earlier gestational ages, we selected proxy values (w_i) that were between expected values and observed values. To accommodate the apparent shift from non-random and to random measurement error across the distribution of gestational ages, we selected offset values ranging from 4 to 0 weeks.

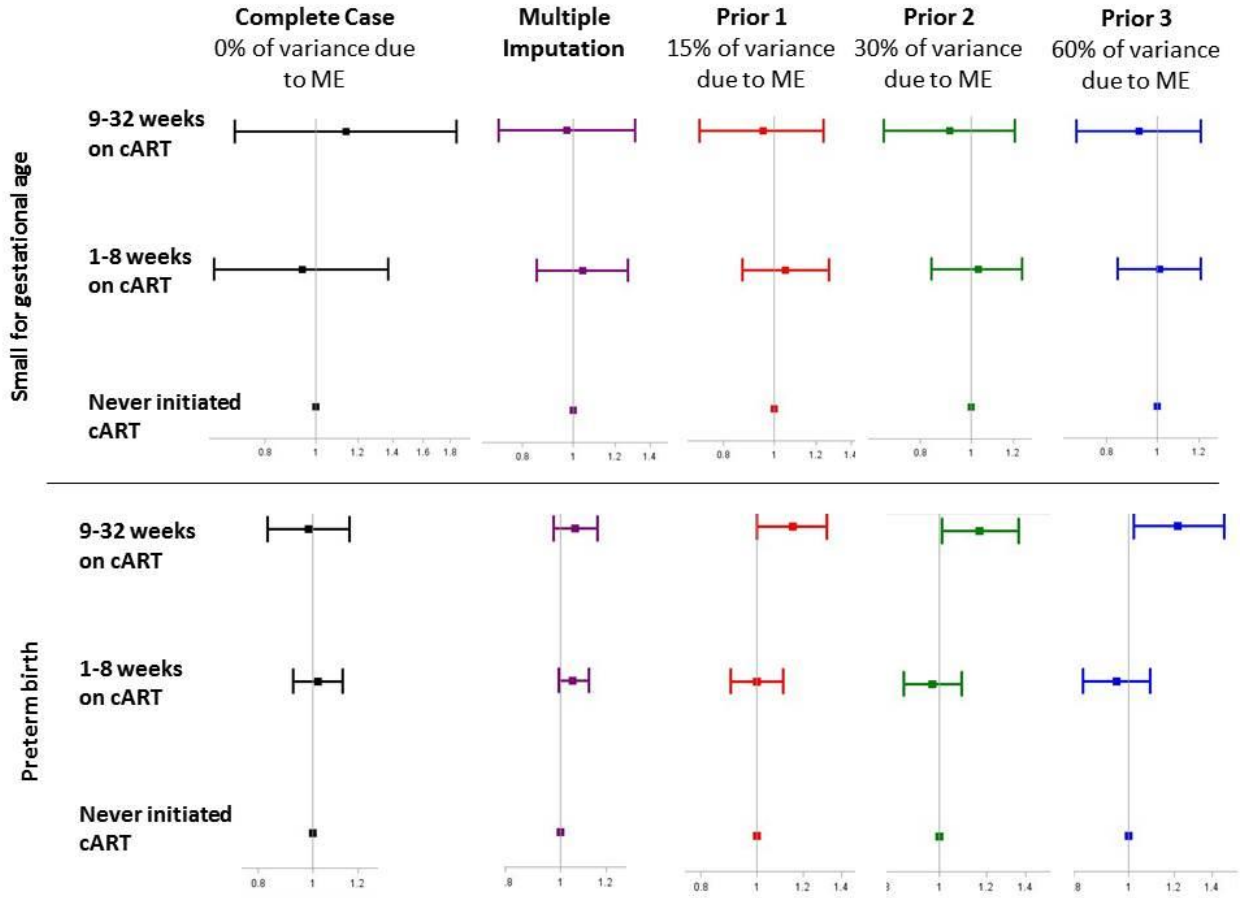


Figure 5.3. Associations between duration of cART before delivery with SGA and preterm birth (RRs and 95% CIs). All models adjusted for: number of ANC visits, age, BMI, CD4 count, education, hemoglobin, intermittent presumptive therapy, parity, syphilis screening/treatment, tuberculosis status and prior preterm birth. All models estimated with Poisson models and robust variance estimators.

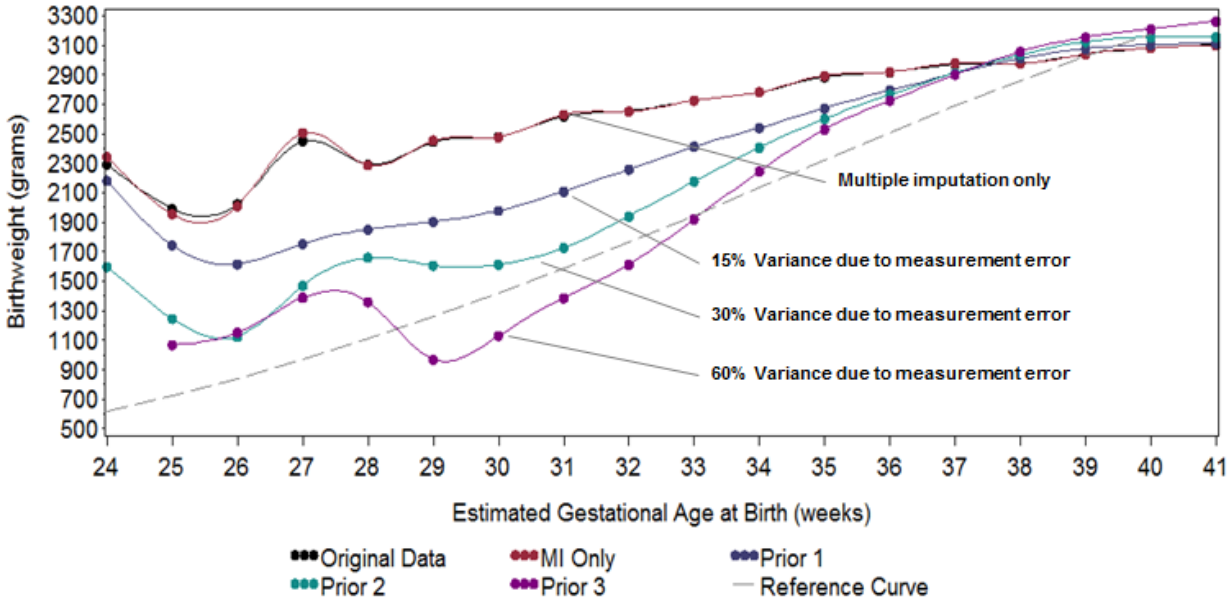


Figure 5.4. Gestational age at birth by mean birthweight for 9,529 HIV-infected women in Lusaka, Zambia 2009-2013 in the observed, multiply imputed and multiply overimputed data. The dashed line indicates reference curve values for gestational age by mean birthweight.

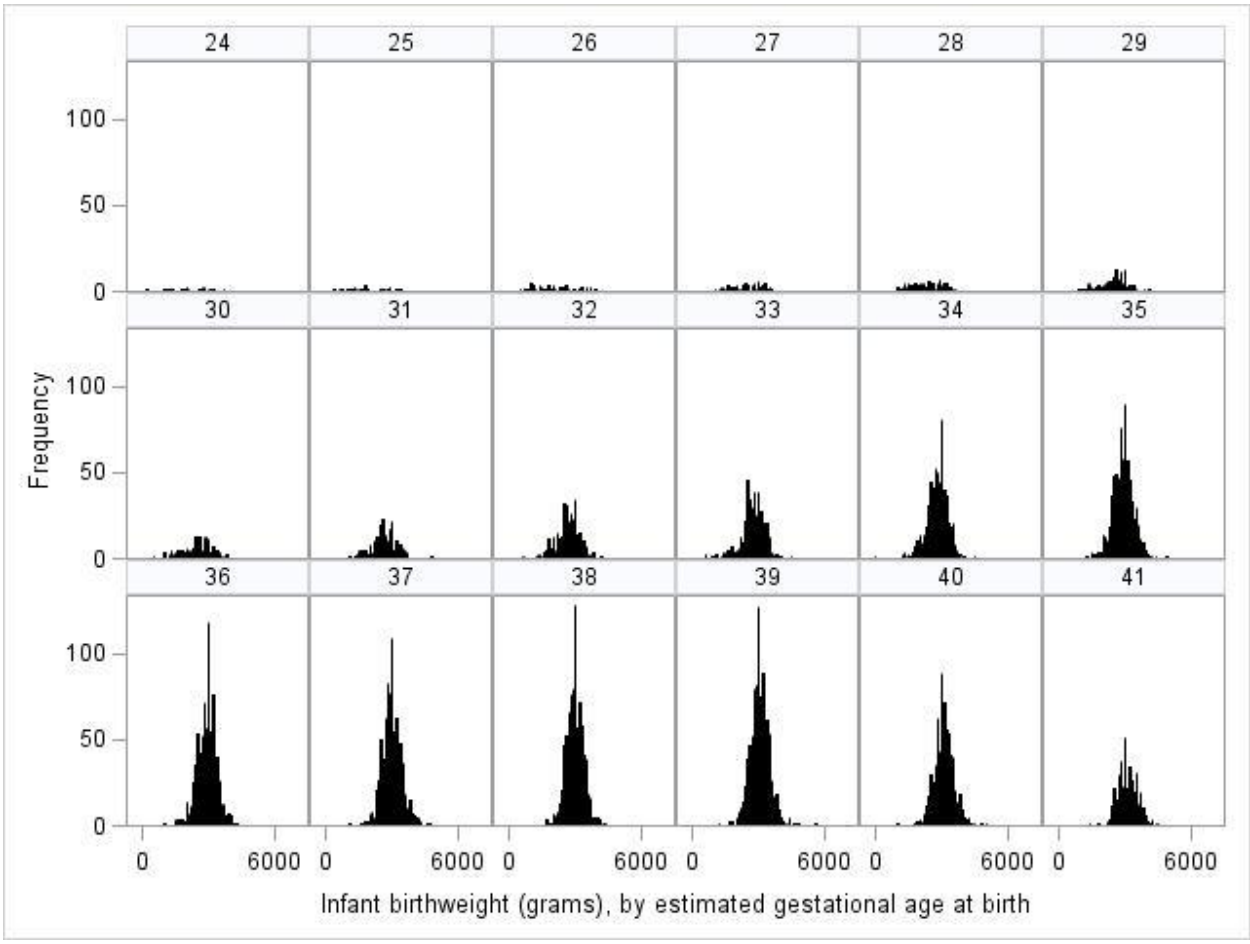


Figure 5.5. Histograms of infant birthweight (in grams) for each observed week of gestational age at birth.

CHAPTER VI. AIM 2 - IDENTIFYING HIV-INFECTED WOMEN AT HIGHEST RISK OF POSTPARTUM LOSS TO FOLLOW UP: DEVELOPMENT OF A CLINICAL RISK SCORE

A. Introduction

Access to lifelong combination antiretroviral therapy (cART) is rapidly being scaled-up to HIV-infected pregnant and breastfeeding women throughout sub-Saharan Africa (SSA). In 2013, an estimated 68% of HIV-infected pregnant women received some type of antiretroviral drug during pregnancy and delivery, doubled from 33% in 2009 [241]. In countries with a generalized HIV epidemic, the World Health Organization (WHO) now recommends lifelong cART for all pregnant and breastfeeding women [154, 226]. This strategy, known as Option B+[12], has become the standard of care in many SSA countries. It is recognized as a critical component for preventing mother-to-child transmission of HIV (PMTCT), improving maternal health, and reducing the risk of sexual transmission among serodiscordant couples [227].

Of course for Option B+ to improve maternal HIV outcomes, women must remain in HIV care and maintain viral suppression [63, 64, 69]. Unfortunately, loss to follow-up (LTFU) during and after pregnancy is common [152]. In Malawi, 17% of women initiating lifelong cART during pregnancy were LTFU by 6 months after treatment initiation. Retention at 12 months was lower among pregnant women than other adults initiating cART [153, 154]. In South Africa, 32% of women initiating cART during pregnancy were lost by 6 months postpartum [147]. Several factors have been associated with LTFU among pregnant women, including

younger age [71], initiating treatment at higher CD4 counts [153], timing of presentation to antenatal care (ANC) [74, 253] and receiving a new HIV diagnosis during pregnancy [70]. To date, strategies to identify women at time of delivery who are at the highest risk of subsequent LTFU are lacking.

To address this gap, we developed and evaluated a risk score to identify women LTFU from HIV care by 6 months postpartum using data from public health facilities in Lusaka, Zambia. Demographic, obstetric, HIV predictors were used to develop a simple, user-friendly scoring system that could be implemented by frontline clinicians at the time of delivery, to identify women at highest risk of LTFU for targeted retention interventions. Since Zambia only recently implemented Option B+ nationwide, our analysis focused on treatment-eligible women who initiated cART during pregnancy in the pre-Option B+ era.

B. Methods

B1. Study design and population

We conducted a retrospective cohort analysis of HIV-infected pregnant women attending public antenatal and HIV healthcare facilities in Lusaka, Zambia. Lusaka is an urban setting where the proportion of pregnant women attending at least 1 antenatal care (ANC) visit consistently exceeds 95%[197]. HIV testing and CD4 screening are conducted within public antenatal clinics. Among women attending ANC, HIV testing is near universal and CD4 count screening coverage is approximately 80% [14]. During the study period (2009-2011), women with a CD4 count $\leq$350 cells/uL were eligible for lifelong cART and services were provided either in integrated ART-ANC clinics or stand-alone HIV treatment departments, typically co-located at the same health facility. Data for the present analysis were derived from two sources: (1) the Zambian Electronic Perinatal Record System (ZEPRS), which collects comprehensive

obstetric information on mothers and infants through delivery [14], and (2) SmartCare, an electronic medical record system for HIV clinical information.

Women included in our analysis initiated cART during pregnancy, had a CD4 count ≤350 cells/uL (in accordance with national HIV guidelines at the time) and delivered in a public-sector facility between January 1, 2009 and November 2, 2010. Women were followed for up to 6 months after delivery. We excluded women who died during pregnancy or up to 42 days after delivery in order to exclude possible pregnancy-related deaths [218]. Ethical approval for the analysis of routinely collected clinical data was obtained from the University of Zambia Biomedical Research Ethics Committee (Lusaka, Zambia) and the University of North Carolina, Chapel Hill (Chapel Hill, NC).

B2. Outcome and predictor definitions

The primary endpoint was LTFU at 6 months postpartum. LTFU was defined as the first time a woman did not present to HIV care within 60 days of the last scheduled appointment. Women were categorized as LTFU on the 61st day after a missed appointment, based on previous work by our group [177]. We considered both clinic visits and pharmacy refill appointments. Women who never returned to HIV care after delivery were allowed up to 30 days to schedule an appointment and were classified as LTFU 61 days after that time (91 days total of follow-up time).

Three categories of predictors for LTFU at 6 months postpartum were considered: demographic, obstetric, and HIV characteristics. We prioritized information that would be readily available to clinicians at the point of care in settings like Zambia. We considered the following demographic predictors: age, level of educational, marital status and employment

status. Obstetric predictors considered were: number of ANC visits, parity and a poor pregnancy outcome, such as low infant birthweight (LBW; <2,500 grams) and preterm birth (<37 weeks gestation). HIV predictors considered were: CD4 count, WHO clinical stage, duration of cART taken during pregnancy, whether or not a woman received a new diagnosis of HIV during pregnancy (defined as a positive HIV test within 7 days of entry into ANC), body mass index (BMI), hemoglobin level at entry into ANC and self-report of whether or not a woman had active tuberculosis during pregnancy. Our overarching aim was to develop a risk score that would be easy to calculate by frontline providers; as such, candidate predictors were coded as binary or categorical. Information on predictors of LTFU such as viral load, disclosure of HIV status to a partner and distance to the clinic were not available and thus were not included. We conducted a sensitivity analysis where whether or not a women enrolled into ART care (but did not start treatment) prior to initiating cART during pregnancy was additionally assessed as a candidate predictor.

B3. Statistical analysis

The primary goal in developing the risk score was to create an easy to use clinical tool to identify HIV-infected women at delivery, who had initiated cART during pregnancy and may be at high risk of postpartum LTFU. The analysis comprised two steps. First, we developed a predictive model for LTFU at 6 months postpartum and used the beta coefficients as the basis for the risk score. We then validated the risk score by bootstrapping [219].

To develop the risk score, we used logistic regression to estimate unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) for all candidate predictors. Predictors associated with LTFU at 6 months postpartum with a p-value $\leq$ 0.25 were identified and included in the full multivariable model. We used manual backward elimination based on likelihood ratio tests to

reduce the full model to a more parsimonious final model. After the elimination of each variable, the area under the curve (AUC) was compared with the full model to determine whether the two models had comparable predictive ability. Multi-collinearity was assessed using Spearman correlations. For variables that were collinear based on Spearman correlation coefficients, the variable with the strongest predictive power (e.g. largest coefficient) was retained in the final model. Variable elimination stopped when all predictors left in the multivariable model had a p-value ≤ 0.10, in an effort to balance retaining potentially important predictors with parsimony. Model fit was assessed using the Hosmer-Lemeshow test. *A priori* we specified a risk score cut-point with sensitivity > 80% and specificity >60% as optimal. These sensitivity and specificity values were selected to prioritize identifying the women most likely to be LTFU (higher sensitivity), while maintaining a reasonable ability to identify women most likely not to be LTFU.

To calculate the final risk score, beta coefficients from the final logistic regression model were multiplied by 10 and rounded to the nearest integer. Values for each person were summed to create an individual risk score. Sensitivity and specificity was assessed at each risk score cut-point. Risk score validation was carried out by bootstrapping the original data set (n=1,000) and comparing the mean, 2.5^{th} and 97.5^{th} percentile values for sensitivity and specificity to their respective values and 95% CIs in the original data[219]. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, North Carolina).

C. Results

Of 4,305 identified in ZEPRS who started cART during pregnancy, 2,982 (69%) could be linked to their SmartCare records. Demographic and obstetric characteristics were similar between women who could be linked, and those who could not. Of the 2,982 women with linked

records, 925 (31%) were excluded due to having the date of first cART dispensation outside the window of the index pregnancy (n=876) or having previously been on cART (n=49) and 551 (18%) were excluded who had delivery dates outside the study period. An additional 759 women were identified in SmartCare who initiated cART during pregnancy. Out of a total of 2,265 women who initiated cART for the first time during pregnancy, 2,108 (93%) had a CD4 count ≤350 and 2,034 (96%) had delivery information. Five women (0.2%) died within 42 days of delivery and were not included, for a final sample size of 2,029 women.

A total of 507 (25%) women were lost to follow-up (LTFU) by 6 months postpartum; 10 (0.5%) of whom died from non-pregnancy related causes. Of the 507 women who were LTFU, 285 (56%) never returned to care after delivery. Among those who initially returned to care but were later LTFU, over half were lost by 18 weeks postpartum (Figure 1).

Overall, most women were >25 years of age (66%), married or cohabitating (93%), either unemployed or a housewife (74%) and attended only 1 or 2 ANC visits (65%) (Table 1). Most women received a new diagnosis of HIV (87%) during the current pregnancy. Women were overwhelmingly in WHO clinical stage 1 or 2 at cART initiation (93%) and received ≥4 weeks of cART during pregnancy (80%).

C1. Model development

In bivariable analyses, age, level of education, employment status, WHO clinical state, duration of cART during pregnancy, hemoglobin, number of ANC visits, parity and preterm delivery predicted LTFU at 6 months postpartum (all, $P < 0.25$; Table 2). In the sensitivity analysis, having enrolled in pre-ART care prior to initiating treatment during pregnancy was a

strong predictor of LTFU at 6 months postpartum (unadjusted OR 2.08 95% CI (1.11, 3.88)), but very few women (2%) were enrolled in pre-ART care.

In the multivariable analysis, parity, level of education, employment status, WHO clinical stage, duration of cART during pregnancy, and number of ANC visits predicted LTFU at 6 months postpartum (all $P < 0.10$; Table 2). The strongest predictors of LTFU were primiparity, WHO clinical stage 1 or 2, and receiving only 1 to 4 weeks of cART before delivery (Table 2). Overall, discrimination in both the full (included all predictors identified in the bivariable analysis) and final (excluded age, hemoglobin, and preterm delivery) models was marginal (full model area under the curve (AUC) 0.64; final model AUC 0.63).

C2. Risk score performance

Risk score values associated with individual predictors ranged from 3 to 6 (Table 2). When values were summed to create individual risk scores, the risk scores ranged from 3 to a maximum score of 26. We include an example score sheet (Figure 6.4). In the bootstrapped data, mean sensitivity and specificity values were consistent with sensitivity and specificity values in the observed data, as was 95% CI coverage, indicating good internal validity of the risk score.

No risk score cut-point met our *a priori* targets for acceptable test performance (sensitivity >80%, specificity >60%). Overall, sensitivity was approximately ≥80% for any risk score cut-point below 12, indicating a reasonably good ability at that range of risk score cut-points to accurately identify women who became LTFU. Specificity for the same range of risk scores was low (0-22%). For example, selecting a risk score cut-point of 11 resulted in 85% sensitivity (95% CI 0.82, 0.88) and 22% specificity (95% CI 0.20, 0.24) to detect women LTFU by 6 months postpartum (Figure 2). With a 25% prevalence of LTFU in our population, the positive predictive value (PPV) for a cut-point of 11 was 0.27 (95% CI 0.24, 0.29) and the

negative predictive value (NPV) was 0.81 (95% CI 0.78, 0.85; Figure 3). Using a risk score cut-point of 11 would identify a large group of women for intervention and exclude only 411 (20%) of the 2,029 women in our study population from an intervention to support retention.

Alternatively, a risk score cut-point with lower sensitivity and higher specificity could be used to target a smaller group of women with high likelihood of LTFU for a more intense retention intervention. For example a risk score cut-off of 18 identified the 23% of women (n=460) with the highest probability of LTFU and had sensitivity 0.32 (95% CI 0.28, 0.36) and specificity 0.80 (95% CI 0.78, 0.82). PPV improved slightly to 0.35 (95% CI 0.31, 0.40) and NPV) was 0.78 (95% CI 0.76, 0.80 at a cut-point of 18. Estimates of sensitivity and specificity for each risk score cut-point changed minimally when enrollment in pre-ART care was included as a predictor into the risk score.

D. Discussion

We developed a simple and easy to use clinical risk score to identify women initiating cART during pregnancy with a high likelihood of LTFU by 6 months postpartum. No risk score cut-point met our *a priori* criteria for a satisfactory combination of sensitivity and specificity (sensitivity >80% and specificity >60%). PPV was also low, but will vary with the underlying prevalence of LTFU in the population (Figure 2). However, the risk score might still be useful to target a subgroup of women at delivery for retention in care interventions. In our study population, a risk score cut-off of 11 identified 80% of our study population for intervention and may be useful cut-point for identifying a larger group of women at moderate risk of LTFU for a low-intensity intervention. Alternatively, a cut-point of 18 identified the 23% of our study population with the highest likelihood of LTFU and may be more appropriate for targeting a

smaller group of women for a high-intensity intervention to prevent LTFU. Examining these tradeoffs in more detail will be a subject for future investigations.

Our risk score is intended for use in maternity care settings in SSA, where the characteristics of women initiating HIV treatment during pregnancy and the rate of postpartum LTFU are likely to be similar to those in our study population. Prior to discharging women after delivery, clinicians could complete a simple checklist to assess likelihood of being LTFU (Figure 6.4). The risk score could then be used to either identify those women less likely to become LTFU, so that resources are appropriately directed to those at greater risk. Alternatively, the tool could be used to identify those women at highest risk of LTFU, an approach that – in our opinion – may be more practical in settings where limited funds are available for retention interventions. If adequate funds are available, it may be preferable to target all women initiating treatment for retention in care interventions. Prior to implementation, however, our risk score should be validated in the field setting. If women initiating cART in our study population differ from women initiating cART under Option B+ or in other settings, the predictive ability of the risk score may differ.

Predicting postpartum LTFU is difficult. In our analysis, we used data likely to be available to clinicians at the time of delivery. However, none of the characteristics strongly predicted LTFU at 6 months postpartum. To improve the predictive ability of the risk score, collecting information on additional predictors may be necessary. Clinicians could consider asking women at delivery about whether they have disclosed their HIV status to their partner [84, 141], face structural barriers to seeking care such as transportation or financial burdens [77, 79] or have concerns about stigma associated with seeking HIV care [77, 139]. All of which have been shown to predict LTFU, but such information is rarely collected as part of routine care.

Further research is needed to determine whether information on social and structural barriers to care is sufficient to produce a risk score with satisfactory sensitivity and specificity.

Many of the predictors identified in our analysis have previously been associated with LTFU. We found that a lower level of education and being a housewife or unemployed predicted LTFU. Higher education, which may influence employment status, has been shown to improve cART adherence, as well as retention in care [74, 254, 255]. We also found that fewer ANC visits and shorter duration of cART during pregnancy predicted LTFU, which aligns with previous findings that late presentation to ANC is associated with postpartum LTFU [74]. We were unable to assess whether initiating treatment with a CD4 count >350 cells/uL predicted LTFU, because of existent thresholds for CD4 eligibility during the study period. However, as Option B+ is scaled up, the need for CD4 count monitoring may become obsolete, since women will initiate treatment regardless of CD4 count. In our analysis, WHO stage 1 or 2 was associated with LTFU, suggesting that women who are otherwise asymptomatic and healthy (ie., CD4 >350 cells/uL) may be at an increased risk of LTFU [72, 153]. As seen in other studies [71], enrollment in pre-ART care prior to pregnancy strongly predicted LTFU; however very few women met this definition and thus this finding may well be the result of selection into pre-ART care by factors which predict LTFU.

LTFU in our cohort was high. Of the 2,029 women included in the study population, 25% (n=507) were LTFU by 6 months postpartum. In countries that have implemented Option B+ similar trends have been seen, where 12-24% of women have been reported LTFU or to have no follow-up by 6 months after cART initiation [153, 154]. Among women LTFU in our study, 56% (n=285) did not return to care after delivery. The high proportion of LTFU immediately following delivery has also been reported in South Africa, where among women who initiated

cART during pregnancy, 64% of women LTFU never returned to care after delivery[72]. In Malawi, women who started cART while pregnant were five times as likely to never return to care, compared to non-pregnant patients initiating cART for their own health (OR 5.0, 95% CI 4.2–6.1)[153].

Improving retention in care is critical to the success of scaling up lifetime cART to pregnant and breastfeeding women [12, 227]. Women initiating treatment during pregnancy face the dual stresses of maintaining engagement in HIV care and having a new infant to care for, making them particularly vulnerable to LTFU [70, 146]. In our population, over half of the women who were LTFU never returned to HIV care after delivery. In our study, it is possible that some of these women may have migrated from Lusaka after the birth of their child and later returned to care. However the high proportion of women lost immediately after delivery suggests that interventions to improve postpartum retention in care may need to engage with women before delivery and focus on keeping women in care immediately following birth.

A number of interventions have improved retention in care. These include, improving patient preparedness [71, 164], using community-based care [78, 165, 256] or mobile phone strategies [167] to trace those with a missed visit, and reducing structural barriers such as financial constraints and transportation to clinics [150, 170, 257]. Many of these interventions have been effective among HIV-infected adults, but have not been tested among pregnant and postpartum women. Clinical risk scores could be used to select women with a high likelihood of LTFU for pilot interventions. If found to be effective, such interventions could then be scaled up to all women initiating treatment during pregnancy. More research is needed to assess whether such a targeted approach translates into meaningful reductions in LTFU at a population level.

E. Conclusions

Maintaining engagement in care for HIV-infected women initiating cART is essential for PMTCT and improving the health of HIV-infected mothers. As Option B+ is scaled up throughout SSA, healthcare systems must balance the additional costs of providing lifelong treatment to pregnant and breastfeeding women with prevention efforts to reduce LTFU [258, 259]. Risk scores offer a simple and easy clinical tool that can be employed at delivery, when women are still engaged in care, to identify women most likely to benefit from an intervention to reduce LTFU. Our clinical risk score may be useful to identify a subset of women most likely to be LTFU or those most likely to be retained in care without intervention. However, accurately predicting LTFU among postpartum women is difficult and supplementary information may be needed to maximize the effectiveness of a risk score. Additional work is needed to update it as data become available for women initiating treatment under Option B+.

F. Tables and Figures

Table 6.1. Clinical characteristics of 2,029 HIV-infected women who initiated cART during pregnancy in Lusaka, Zambia 2009-2010.

Characteristic	Lost to Follow-up at 6 months N=507 (25.0) N(%)	Remained in HIV Care at 6 months N=1522 (75.0) N(%)	Total N=2,029 N (%)
Age			
≤ 25	201 (39.6)	483 (31.8)	684 (33.7)
>25	306 (60.4)	1,037 (68.2)	1,343 (66.3)
Level of education			
None or primary	211 (48.0)	540 (40.4)	751 (42.2)
Secondary or tertiary	229 (52.1)	798 (59.6)	1,027 (57.8)
Marital status			
Married or cohabitating	450 (91.7)	1,353 (91.6)	1,804 (92.6)
Singled, divorced or widowed	41 (8.4)	125 (8.5)	166 (8.4)
Employment status			
Employed or student	85 (19.1)	380 (28.1)	465 (25.8)
Housewife or unemployed	361 (80.9)	974 (71.9)	1,335 (74.2)
CD4 count (cell/uL)			
≤150	118 (23.5)	377 (25.1)	495 (24.7)
151-251	202 (40.2)	557 (37.1)	759 (37.9)
251-350	118 (36.3)	566 (37.7)	748 (37.4)
WHO stage			
1 or 2	458 (92.9)	1,360 (91.1)	1,818 (92.5)
3 or 4	35 (7.1)	133 (8.9)	168 (8.5)
Duration of antenatal cART			
1-4 weeks	18 (27.2)	274 (18.0)	412 (20.3)
> 4 weeks	369 (72.8)	1,248 (82.0)	1,617 (79.7)
New HIV diagnosis during pregnancy			
Yes	439 (86.6)	1,328 (87.3)	1,767 (87.1)
No	68 (13.4)	194 (12.8)	262 (12.9)
Body mass index (kg/m^2)			
<18.5	15 (3.2)	46 (3.2)	61 (3.2)
18.5 - <25	306 (64.7)	923 (63.4)	1,229 (63.7)
25 - <30	125 (26.4)	392 (26.9)	517 (26.8)
≥30	27 (5.7)	96 (6.6)	123 (6.4)
Hemoglobin (g/dL)			
<8	17 (3.7)	45 (3.2)	62 (3.4)

8-<10	105	(22.9)	357	(25.7)	462	(25.0)
≥ 10	337	(73.4)	990	(71.1)	1,327	(71.7)
Active tuberculosis						
Yes	10	(2.0)	23	(1.5)	33	(1.6)
No	497	(98.0)	1,499	(98.5)	1,996	(98.4)
Number of ANC visit						
1 or 2	358	(70.6)	966	(63.5)	1,324	(65.3)
≥3	149	(29.4)	556	(36.5)	704	(34.8)
Parity						
0	108	(22.0)	252	(17.2)	360	(18.4)
1	126	(25.7)	358	(24.4)	484	(24.7)
2	112	(5.7)	343	(23.4)	455	(23.3)
>2	144	(29.4)	515	(35.1)	659	(33.7)
Low birthweight (<2,500 grams)						
Yes	82	(16.5)	204	(13.7)	286	(14.4)
No	414	(83.5)	1287	(86.3)	1,701	(85.6)
Preterm delivery (<37 weeks gestation)						
Yes	179	(41.2)	461	(35.6)	640	(37.0)
No	256	(58.9)	835	(64.4)	1,091	(63.0)

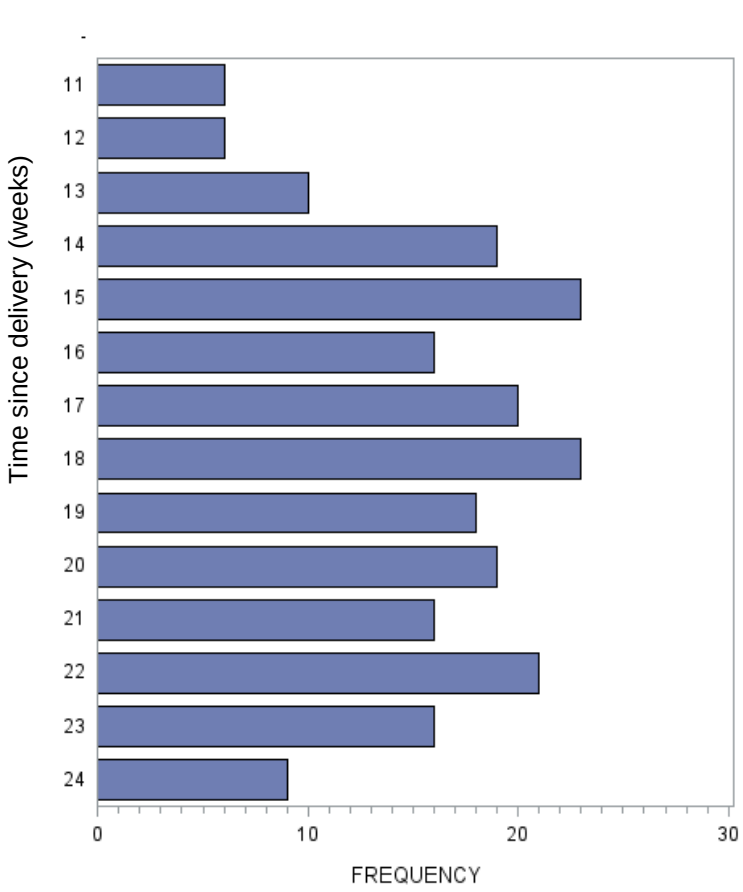
Table 6.2. Predictors of loss to follow-up from HIV care at 6 months postpartum among HIV-infected Zambian women initiating cART during pregnancy.

	Unadjusted	Full Model	Final Model	Risk Score
Covariate	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	
Age				
>25	1.00	1.00		
≤ 25	1.41 (1.15, 1.74)[a]	1.10 (0.77, 1.56)		
Education				
Secondary or tertiary	1.00	1.00	1.00	--
Primary or none	1.36 (1.10, 1.69)[a]	1.33 (1.01, 1.76)	1.41 (1.10, 1.80)[b]	3
Marital status				
Married or cohabitating	1.00	--		
Singled, divorced or widowed	1.18 (0.86, 1.62)	--		
Employment status				
Employed or student	1.00	1.00	1.00	--
Housewife or unemployed	1.66 (1.27, 2.16)[a]	1.47 (1.06, 2.03)	1.56 (1.17, 2.07)[b]	4
CD4 count (cell/uL)				
251-350	1.00	--		
151-250	1.13 (0.89, 1.42)	--		
≤150	0.97 (0.75, 1.27)	--		
WHO Clinical Stage				
3 or 4	1.00	1.00	1.00	--
1 or 2	1.28 (0.87, 1.89)[a]	2.18 (1.26, 3.75)	1.73 (1.10, 2.73)[b]	5
Duration of cART before delivery				
> 4	1.00	1.00	1.00	--
1-4	1.70 (1.35, 2.16)[a]	1.69 (1.23, 2.32)	1.73 (1.32, 2.27)[b]	5
HIV diagnosis during pregnancy				
No	1.00	--		
Yes	0.94 (0.70, 1.27)	--		
Body mass index (kg/m^2)				

18.5 - <25	1.00	--		
<18.5	0.98 (0.54, 1.79)	--		
25 - <30	0.96 (0.76, 1.22)	--		
≥30	0.85 (0.54, 1.33)	--		
Hemoglobin (g/dL)				
≥ 10	1.00	1.00		
8-<10	0.86 (0.67, 1.11)[a]	0.86 (0.63, 1.18)		
<8	1.11 (0.63, 1.97)	1.83 (0.87, 3.83)		
Active tuberculosis				
No	1.00	--		
Yes	1.31 (0.62, 2.77)	--		
Number of ANC visits				
≥ 3	1.00	1.00	1.00	--
1 or 2	1.38 (1.11, 1.72)[a]	1.31 (0.97, 1.76)	1.31 (1.02, 1.68)[b]	3
Parity				
>2	1.00	1.00		--
2	1.17 (0.88, 1.55)	1.09 (0.76, 1.57)	1.31 (0.96, 1.81)[b]	3
1	1.26 (0.96, 1.66)[a]	1.19 (0.80, 1.76)	1.42 (1.04, 1.94)[b]	3
0	1.53 (1.15, 2.05)[a]	1.30 (0.80, 2.10)	1.83 (1.29, 2.59)[b]	6
Low birthweight (<2,500 grams)				
No	1.00	--		
Yes	1.25 (0.95, 1.65)	--		
Preterm delivery (<37 weeks gestation)				
No	1.00	1.00		
Yes	1.27 (1.01, 1.58)[a]	1.09 (0.82, 1.44)		

[a] $P \leq 0.25$, [b] $P \leq 0.10$.

Figure 6.1. Timing of loss to follow-up among 222 women returned to HIV care after delivery and who were subsequently lost to follow-up. An additional 285 women (507 women LTFU in total) never returned to HIV care after delivery and are not included.



	FREQ.	CUM. FREQ.	PCT.	CUM. PCT.
11	6	6	2.70	2.70
12	6	12	2.70	5.41
13	10	22	4.50	9.91
14	19	41	8.56	18.47
15	23	64	10.36	28.83
16	16	80	7.21	36.04
17	20	100	9.01	45.05
18	23	123	10.36	55.41
19	18	141	8.11	63.51
20	19	160	8.56	72.07
21	16	176	7.21	79.28
22	21	197	9.46	88.74
23	16	213	7.21	95.95
24	9	222	4.05	100.00

Figure 6.2. Sensitivity (solid line) and specificity (dotted line) at each risk score cut-off (range 3-26) to predict LTFU at 6-months postpartum among 2,029 HIV-infected Zambian women who initiated cART during pregnancy.

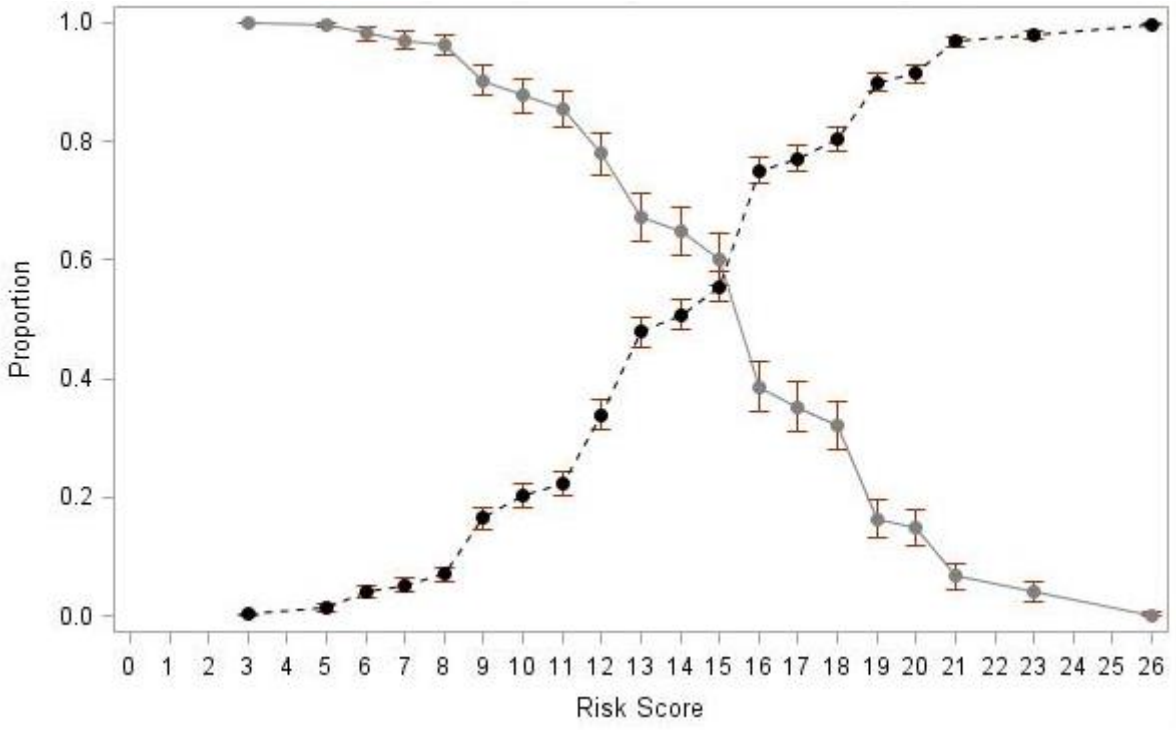


Figure 6.3. Positive predictive value (PPV; dashed line) and negative predictive value (NPV; solid line) for risk score cut-point 11, as the prevalence of LTFU varies.

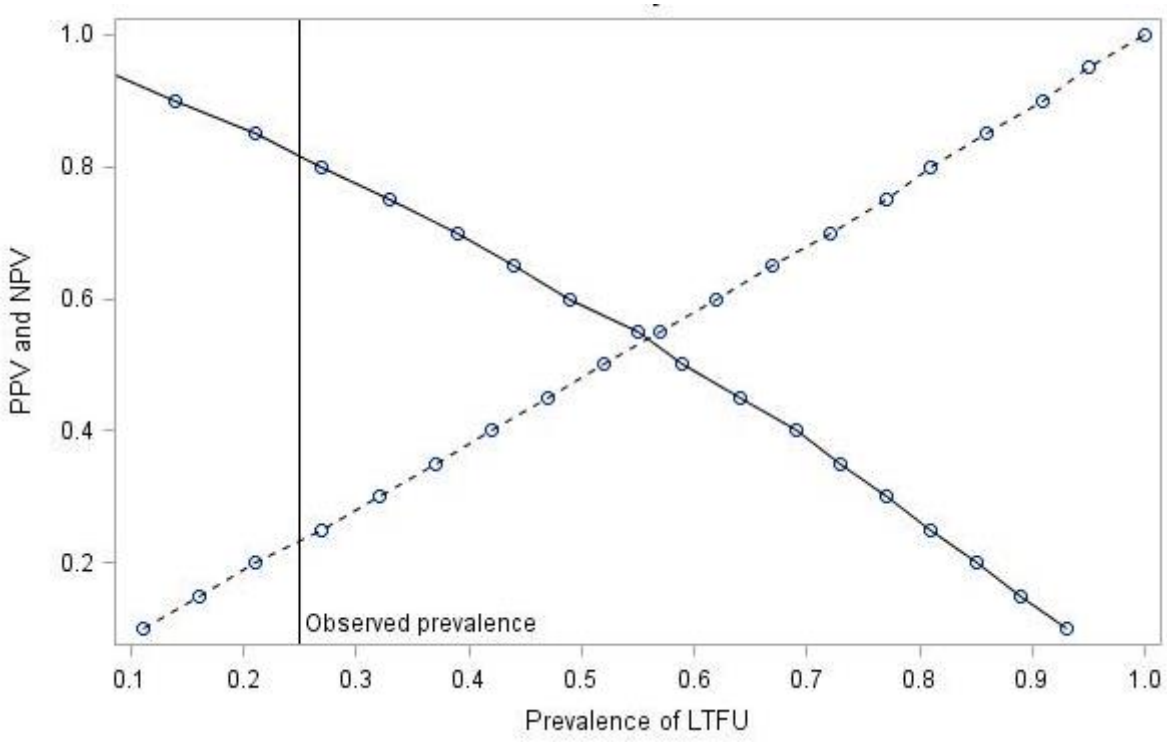


Figure 6.4. Risk Score for Loss to Follow-up by 6 months Postpartum.

Directions:

- All women start with a score of 0.
- Check off box correct category for each risk factor.
- Sum the points for all risk factors and record the final risk score at the bottom.

Risk Factor	Check correct box	Points
Education level		
Secondary or tertiary	☐	**0**
Primary or none	☐	**3**
Employment status		
Employed or student	☐	**0**
Housewife or unemployed	☐	**4**
WHO clinical stage (at entry into ANC)		
3 or 4	☐	**0**
1 or 2	☐	**5**
Duration of cART before delivery (current pregnancy)		
<4 weeks	☐	**0**
1-4 weeks	☐	**5**
Number of ANC visits (current pregnancy)		
>3	☐	**0**
1 or 2	☐	**3**
Parity (number of previous deliveries)		
>2	☐	**0**
2	☐	**3**
1	☐	**3**
0	☐	**6**

Sum points for all checked boxes

RISK SCORE ☐

CHAPTER VII. CONCLUSIONS

A. Summary of main findings

Aim 1a – Duration of cART and infant birthweight

The Aim 1a analysis focused on duration of cART during pregnancy and infant birthweight, assessed as both a binary measure of LBW and a continuous measure of birthweight. Among women with CD4 count ≤350 who delivered at term (≥37 weeks gestation), longer duration of cART during pregnancy did not result in increased risk of LBW or decreased mean birthweight. These findings did not change meaningfully across numerous sensitivity analyses. Modest decreases in birthweight were observed with increasing cART duration when cART duration was assessed as a continuous variable. However, this small decrease must be weighed against the substantial benefits of cART for PMTCT. Our analysis included only term births, and therefore our findings focus on LBW due to fetal growth restriction.

Among our study population of women delivering at term, LBW occurred in 302 of pregnancies (7%). Women included in our analysis had a CD4 count $\leq$ 350 at entry into ANC and therefore were eligible to initiate cART during pregnancy under the Zambian national guidelines at the time. However, the majority of eligible women (62%) never initiated combination treatment, but nearly all (94%) received some form of antiretroviral prophylaxis for PMTCT.

Aim 1b – Multiple overimputation to address missing data and measurement error in gestational age, with an application to duration of cART and preterm birth and SGA

The goal of Aim 1b was to demonstrate the use of multiple overimputation as a method to address missing data and measurement error, with an application to investigating the association between duration of cART during pregnancy with preterm birth and SGA. In our analysis of duration of cART before delivery and its associations with SGA and preterm birth, MO was a convenient method to simultaneously address missing data and measurement error in gestational age. Application of MO showed that duration of cART before delivery was not associated with SGA, even after accounting for missing data and measurement error. Duration of cART before delivery was also not associated with an increased risk of preterm birth in the naïve-analysis or when only missing data was imputed. However, when measurement error in gestational age was additionally considered in the MO analysis, point estimates for 9-32 weeks of cART moved away from the null, suggesting a possible increased risk of preterm birth with longer duration of cART.

Among the HIV-infected women with a CD4 count $\leq$ 350 included in our study population, the proportion of preterm births was very high (45%). Conversely, the proportion of SGA infants was lower than expected (8%) and there were very few infants that were both preterm and SGA (1%). The observation that the majority of preterm infants were not SGA aligns with findings from our assessment of measurement error in gestational age, which suggested that overall gestational age values were recorded as earlier than birthweights would suggest.

Aim 1 – Duration of cART and pregnancy outcomes

Taken together, the combined results from Aim 1 suggest that longer duration of cART during pregnancy is not associated with adverse pregnancy outcomes. We saw no evidence that compared to never initiating cART, any duration of cART during pregnancy increased the risk of LBW due to growth restriction. Similarly, there was no evidence of an association between duration of cART and SGA. The lack of any associations between duration of cART and LBW or SGA suggests that in our study population, duration of cART did not have a strong impact on fetal growth. In the MO analysis, we saw some suggestion that duration of cART may impact length of gestation. As increasing amounts of measurement error in gestational age were assumed, the association between longer durations of cART and preterm birth moved away from the null. However, associations were imprecise and confidence intervals included the null.

Aim 2. Predicting postpartum LTFU among HIV-infected women initiating cART during pregnancy

In Aim 2, we developed a simple and easy to use clinical risk score to identify women who initiated cART during pregnancy with a high likelihood of LTFU by 6 months postpartum. HIV and obstetric predictors likely to be available to clinicians at delivery were used to develop the risk score. Unfortunately, no risk score cut-point met our a priori criteria for a satisfactory combination of sensitivity and specificity (sensitivity >80% and specificity >60%). PPV was also low, given the underlying prevalence of LTFU in our study population. However, the risk score approach may still be useful to target a subgroup of women at delivery for retention in care interventions. In our study population, a risk score cut-off of 11 identified 80% of our study population for intervention and may be useful cut-point for identifying a larger group of women at moderate risk of LTFU for a low-level intervention. Alternatively, a cut-point of 18 identified

the 23% of our study population with the highest likelihood of LTFU and may be more appropriate for targeting a smaller group of women for more intense intervention to prevent LTFU.

Within our study population of HIV-infected women who initiated cART, there were very few deaths between delivery and 6 months postpartum. A total of 15 women died; 5 (0.3%) that were considered maternal deaths (and excluded from analyses) and 10 (0.5%) who died subsequent to being LTFU. There is no death registry or other means of validating deaths in Zambia, so it is likely that deaths were underestimated in our study.

Over half of women who were LTFU in our study, were lost immediately following delivery. Given this high proportion of LTFU, it is possible that some of these women may have died, or have migrated out of Lusaka following the birth of their baby. However, it is not possible to verify migration or death in the available data. Among women who returned to HIV care after delivery and were later LTFU, the rate of LTFU was fairly constant between 14 and 24 weeks postpartum (recall that women had to miss a scheduled HIV appointment by 60 days to be defined as LTFU, so LTFU did not start occurring until week 10). However, among women who did return to HIV care after delivery, over half were lost by 18 weeks postpartum.

B. Public health importance

Aim 1 – Duration of cART and pregnancy outcomes

Low birthweight and small for gestational age

The relationship between cART and adverse pregnancy outcomes remains controversial. Numerous studies have evaluated the associations between cART and LBW, preterm birth and SGA. The balance of evidence from the developed world suggests that cART likely is not

associated with LBW SGA, [33, 38, 58] but there is less certainty about cART impact on LBW and SGA in the developing world.[32, 34, 125]

The associations of cART duration with LBW and SGA have been evaluated, although most studies have combined term and preterm births. cART initiated either early during pregnancy (≤25 or <28 weeks gestation) or late (≥28 or 32 weeks gestation) was not associated with LBW in studies in South Africa and the US. However, since term and preterm births were combined in these studies it is difficult to distinguish whether late initiators had no increased risk of LBW, or were simply more likely to make it to term by virtue of starting treatment later during pregnancy. Birthweight Z-scores adjusted for gestational age were considered in a French study and no association with duration of cART was found [90]. An analysis from Malawi and Mozambique attempted to stratify gestational age at birth, but was unable to investigate the association between duration of cART and LBW among term infants due to the small sample size (n=496)[113].

Despite the fact that cART use during pregnancy has not led to an increase in LBW or SGA in most analyses, there is evidence to suggest that PI-based cART may directly impact fetal growth. cART has been hypothesized to impact fetal growth by inhibiting progesterone production during pregnancy.[131] Among HIV-uninfected women, low progesterone levels have been associated with lower birthweights.[236, 237] In animal models, PI-based cART regimens have been associated with decreased progesterone levels, which correlate with lower fetal weight.[131]

Our Aim 1a and Aim 1b analysis provides some of the first evidence from SSA on associations between duration of cART and LBW due to fetal growth restriction of SGA. Given our focus on LBW due to growth restriction in Aim 1a, these results may be helpful to clinicians

caring for patients at low-risk for preterm birth. However, our analysis of duration of cART and SGA among all infants (term and preterm) in Aim 1b align with these findings, lending further support that duration of cART is not associated with fetal growth restriction. Our results provide early and preliminary evidence that as Option B+ is scaled up throughout SSA and women are on cART for longer periods of time during pregnancy, there is unlikely to be a large increase in LBW due to growth restriction or SGA. Additional research is needed to assess whether women who initiate cART pre-conception are at an increased risk of fetal growth restriction.

Preterm birth

The greatest controversy over the use of cART during pregnancy has been surrounding its possible association with preterm birth. Several studies, both in the developed world [33, 35, 36, 38] and developing world [32, 239] have observed associations between cART and preterm birth. Most analyses have been observational, however one randomized trial reported an increased risk in preterm birth among women randomized women that PI-based, versus or NRTI-based cART.[239] Early cART initiation (<14 or <28 weeks gestation)[18, 40] and late (≥ 20 weeks gestation) during pregnancy has been associated with preterm birth.[130] PI-based cART has caused the greatest concern about increasing the risk of preterm birth.[18] The mechanism(s) by which PI-based cART could impact length of gestation is not well understood. Several mechanisms have been proposed, including mitochondrial toxicity, [126-129] placental insufficiency, [118, 130] inhibited progesterone production [131] and a TH2 to TH1 cytokine shift.[41, 42]

One of the reasons for the ongoing controversy is the difficulty in establishing causality in the relationship between cART and preterm birth. In particular, understanding whether cART initiation during specific times during gestation increases the risk of preterm birth is challenging

because duration of treatment is tied to length of gestation and timing of delivery. Women on cART for longer durations are, by definition, closer to term and therefore less likely to have a preterm birth. In populations at high risk for preterm birth, this could result in longer durations of cART looking protective against preterm birth. Because of these constraints, we focused on LBW due to fetal growth restriction by restricting our analysis to term births in our Aim 1a analysis and assessed duration of cART at 32 weeks gestation in our Aim 1b analysis.

Prospective randomized studies investigating the effect of timing of cART initiation on preterm birth could help to establish causality. Such prospective trials should seek to enroll women at a uniform time-point close to conception, randomize them to gestational age at cART initiation and confirm gestational age using ultrasound-based measurements. However, such a randomized study would pose serious ethical challenges, and might itself leave questions unanswered since women included in randomized controlled trials are often a highly selected group, and may not be generalizable to a real-world clinical population of HIV-infected pregnant women. Further, such a study can assess one specific causal contrast about when to initiate cART during pregnancy (such as in the 1st trimester versus in the 2nd), but it cannot shed light on the mechanisms by which cART may impact preterm birth – a central and unresolved question.

Analysis of retrospective routinely collected clinical data alone is unlikely to establish causality on its own. However, given the challenges of understanding how timing of treatment may impact preterm birth, such analyses provide important information about preterm birth trends among women initiating cART during pregnancy.

Measurement error in gestational age

In the absence of randomized controlled trials, much of the inference about cART's impact on LBW, preterm birth and SGA has been drawn from observational data. In resource-

limited settings in particular, observational studies of cART's impact on pregnancy outcomes often rely on routinely collected clinical data.[32] Clinical data provides an important source of information for monitoring pregnancy outcomes of HIV-infected women; but is typically not collected for research purposes. Routinely collected clinical data is consequently often plagued by missing data and measurement error.[242]

Missing data and measurement error in gestational age may introduce bias when evaluating the relationship between cART and gestational-age based outcomes like preterm birth and SGA. Gestational age dating based on last menstrual period (LMP) may include error due to natural variation in when women ovulate, errors in recall or missing LMP dates.[232] Inaccurate LMP dates may be more common among women in the developing world, where malnutrition, high fertility rates and longer breastfeeding duration may mean that many women do not resume regular menstrual cycles before becoming pregnant again.[243] Menstrual abnormalities are common in HIV-infected women, which may further limit the reliability of LMP dating.[244] Missing or mismeasured gestational age values in routinely collected clinical data may have implications for our understanding of cART's association with preterm birth and SGA.

The possible impact of measurement error in gestational age on the associations of duration of cART with preterm birth and SGA has not been evaluated. Our Aim 1b analysis results provide the first quantitative assessment of how point estimates for associations between duration of cART with preterm birth and SGA might change under a range of assumptions about measurement error in gestational age. In our results we saw no evidence of an increased risk of SGA. As we increased assumptions about the amount of measurement error in gestational age, the point estimates for the association between longer duration of cART and preterm birth

reversed directions and moved away from the null, highlighting the possible sensitivity of point estimates to measurement error in gestational age.

In addition to assessing how measurement error may impact associations between duration of cART and gestational age-based outcomes, our Aim 1b analysis provides an overview of a general purpose method to simultaneously address measurement error and missing data. Multiple overimputation is a method that was developed by political scientists, [208, 210] but has not been widely used in epidemiology despite the presence of measurement error and missing data in many epidemiologic analyses. Multiple overimputation offers a convenient and straightforward way to handle these issues together, by multiply imputing missing data and using a proxy for observed data as an informative prior when multiply imputing (e.g. overimputing) mismeasured data.[210] The resulting manuscript from our Aim 1b analysis introduces and describes multiple overimputation to an epidemiologic audience, and provides an application of the method to the question of whether duration of cART is associated with preterm birth or SGA.

Aim 2. Predicting postpartum LTFU among HIV-infected women initiating cART during pregnancy

As Option B+ is scaled up throughout SSA, retention in care during and after pregnancy has emerged as an important concern.[152-154] In Malawi, the first country to implement Option B+, 17% of women initiating lifelong cART during pregnancy were LTFU by 6 months after treatment initiation and retention at 12 months was lower among pregnant women than other adults initiating cART.[153, 154] In South Africa, 32% of women initiating cART during pregnancy were lost by 6 months postpartum. [147] In order for increased access to cART to translate into improved PMTCT outcomes, it is essential that women remain engaged in care and adherent to cART during pregnancy and breastfeeding.[173]

However, successfully retaining women in care throughout pregnancy and breastfeeding is challenging. A number of intervention studies [158-161, 173] are underway to investigate strategies to improve retention in care. Yet even with effective strategies, resource limited settings may have to balance the additional costs of providing lifetime treatment to pregnant and breastfeeding women with prevention efforts to reduce LTFU.

Risk scores offer one option for prioritizing groups at highest risk of LTFU for retention interventions. Using a simple pen and paper, clinicians can assess women's risk factors for postpartum LTFU at delivery and use a predefined risk score cut-point to identify a proportion of the population for further intervention. Risk scores have been developed to identify persons with acute HIV-infection [187-190], partners unlikely to seek HIV-testing after being notified of their partner's status [191] and to develop selective screening guidelines for sexually transmitted infections [192, 193]. To our knowledge, this is the first development of a risk score to identify HIV-infected women initiating cART during pregnancy most likely to be LTFU postpartum.

However, predicting postpartum LTFU is difficult. In our analysis, we used data likely to be available to clinicians at the time of delivery and identified several variables that predicted LTFU at 6 months. None of the variables strongly predicted LTFU at 6 months postpartum and no risk score cut-point met our *a priori* criteria for sensitivity and specificity. To improve the predictive ability of the risk score, collecting information on additional predictors may be necessary. Clinicians could consider asking women at delivery about whether they've disclosed their HIV status to their partner [84, 141], face structural barriers to seeking care such as transportation or financial burdens [77, 79] or have concerns about stigma associated with seeking HIV care [77, 139], all of which have been shown to predict LTFU. Further research is

needed to determine if information on social and structural barriers to care is sufficient to produce a risk score with satisfactory sensitivity and specificity.

Improving retention in care is critical to the success of scaling up lifetime cART to pregnant and breastfeeding women [12, 227]. Women initiating treatment during pregnancy face the dual stresses of maintaining engagement in HIV care and having a new infant to care for, making them particularly vulnerable to LTFU [70, 146]. In our population, over half of the women who were LTFU never returned to HIV care after delivery. It is possible that some of these women may have migrated from Lusaka after the birth of their child and later returned to care. However, the high proportion of women lost immediately after delivery suggests that interventions to improve postpartum retention in care may need to engage with women before delivery and focus on keeping women in care immediately following birth.

In sum, while our risk score did not strongly predict LTFU at 6 months postpartum, it may still be useful to identify women at highest risk of LTFU or to identify a larger group of women for a lower level intervention. It also provides a starting point, that may be updated or amended as additional data is available, for strategies that can be used while women are engaged in care (e.g. at delivery) to identify women most likely to be LTFU. It is important to note that in the absence of resource constraints, enrolling all pregnant and breastfeeding women initiating cART into retention interventions may be the most effective strategy to reducing LTFU and improving PMTCT and maternal HIV outcomes.

C. Limitations and Strengths

Aim 1a.

We note several limitations of Aim 1a. First, we did not have access to information on important confounders, including markers of HIV disease (e.g., viral load, WHO clinical stage),

antiretroviral regimens, or adherence. While it is difficult to predict the direction of bias due to unmeasured confounding overall, if women with more advanced HIV were more likely to be on cART longer, one would expect to see an increased risk of LBW among women on cART the longest. Such an association was not observed. Second, as with all analyses of routinely collected clinical data, selection bias may arise since not all women present for care. However, clinical findings are applicable only in clinical settings, so associations observed among those who present for care may be generalizable to other populations in care. In addition, our analysis cohort did not include pregnant women who miscarried or delivered prior to seeking institutional healthcare, which could limit the external validity of our results. Finally, despite limiting our analyses to term births, measurement error in gestational age may still be present.

Strengths of our study included the use of data from an electronic medical record system covering 24 public health clinics in Lusaka, the use of a study population with uniform eligibility for cART initiation and the consistency of results across multiple sensitivity analyses. In addition, our analysis provides some of the first evidence about the lack of an association between duration of cART and the risk of LBW due to fetal growth restriction among HIV-infected women in SSA.

Aim 1b.

We note many of the same limitations in Aim 1b, as in Aim 1a; namely, the presence of unmeasured confounding by HIV disease, antiretroviral regimen and adherence and possible selection bias due to not all women presenting for clinical care. As in Aim 1a, our findings on women in care are generalizable to other clinical population in care. However, they are not causal effects. The fact that not all women present to care, and that those presenting enter care at different times, indicates left truncation in the data. Left truncation in the data precludes the

estimates of causal effects, since women who might have initiated cART during pregnancy, but miscarry early in their pregnancy never show up in the analysis. Specific to Aim 1b, we also note that the reference curve used to correct measurement error in gestational age was based on a population of HIV-uninfected women. Infants born to HIV-infected women may be smaller overall than infants born to HIV-uninfected women. However, a reference curve of gestational age and birthweight is not available for HIV-infected women. Finally, we assessed duration of cART at 32 weeks in order to establish a clear temporal sequence where the exposure was assessed prior to the outcome of preterm birth or SGA, at delivery. There were a small number of women [n=617; 6%], who delivered before 32 weeks gestation and therefore, before exposure assessment.

Strengths of the Aim 1b analysis include the introduction and demonstration of a novel method to simultaneously address measurement error and missing data to an epidemiologic audience. We also offer the first assessment of how point estimates for the associations between duration of cART with SGA and preterm birth may change under a range of assumptions about measurement error in gestational age, while also accounting for missing data.

Aim 2.

We note several limitations to Aim 2. First, we were unable to identify any strong predictors of LTFU at 6 months postpartum. Consequently, our risk score did not met our *a priori* specifications for satisfactory sensitivity and specificity. Second, very few deaths were recorded in our study population between delivery and 6 months postpartum. However, Zambia does not have a death registry or other means of validating deaths. Therefore, we may be underestimating the number of deaths. Third, data used for the Aim 2 analysis comes from 24 public health facilities in Lusaka, Zambia. These facilities have the ability to link data within

their network of clinics, but not to link to data in facilities outside of Lusaka. Over half of the women LTFU in our analysis were lost immediately after delivery. It is possible that some of these women migrated and entered care at a facility outside of Lusaka, but we are unable to measure this within the current data collection structure. Finally, we analyzed retrospective data from pre-Option B+ implementation in Zambia. Women initiating cART under Option B+ may have different risk factors for postpartum LTFU and our risk score may not be generalizable to this population.

Strengths of Aim 2 include the first attempt to develop a risk score to predict postpartum LTFU among HIV-infected women initiating cART during pregnancy and the fact that the risk score can easily be implemented in a clinical setting with only pen and paper. Additionally, our data come from 24 public health facilities in an area with high ANC coverage and HIV testing in Lusaka, Zambia and therefore are likely to be representative of HIV-infected women who initiate cART during pregnancy in similar settings.

D. Future Directions

Aim 1. Duration of cART and pregnancy outcomes

Controversy about cART's impact on pregnancy outcomes is likely to remain until the mechanisms by which cART might impact fetal growth or length of gestation are understood. Several mechanisms have been proposed that could impact pregnancy outcomes, including mitochondrial toxicity,[126-129] which may lead to placental or vascular damage that increases the risk of preterm birth.[118, 130]. PI-based cART has also cART has been hypothesized to impact fetal growth by inhibiting progesterone production during pregnancy.[131] Among HIV-uninfected women, low progesterone levels have been associated with lower birthweights.[236, 237] In animal models, PI-based cART regimens have been associated with decreased

progesterone levels, which correlated with lower fetal weight.[131] cART, particularly if initiated early during pregnancy, has been hypothesized to increase the risk of preterm birth by counteracting the increase in TH2 cytokines that occurs with a healthy pregnancy and inducing a Th2 to Th1 cytokine shift. [41, 42]

The mechanisms by which cART may increase the risk of adverse pregnancy outcomes are likely to be complex and multi-factorial. However, without a better understanding of how cART impacts pregnancy outcomes, it remains difficult to develop clinically meaningful hypotheses to test in future studies. For example, a randomized controlled trial of early versus delayed cART initiation during pregnancy could help to answer the question of when to start cART during pregnancy. However, such a trial will be less informative if timing of cART initiation does not impacts pregnancy outcomes, but rather the choice of regimen does. Moving forward, a greater focus on the mechanisms by which cART may impact pregnancy outcomes is warranted, in order to develop studies that more effectively evaluate whether any biological changes induced by cART during pregnancy translate into clinically meaningful outcomes.

In addition to a better understanding of the mechanisms by which cART impacts pregnancy outcomes, future studies should evaluate what the long-term implications of such outcomes are for infants. For example, do infants who are born preterm to mothers who initiate cART during pregnancy eventually catch up to other infants in terms of growth and development? Or does cART have a longer-term impact on the growth trajectory of an infant? The impact of exposure to HIV (whether or not an infant is infected) on growth and development outcomes has been evaluated in a number of studies,[260-263] and the results have largely been reassuring. However, whether HIV-exposed infants who experience an adverse pregnancy

outcome (preterm birth, LBW or SGA) have different growth and development patterns than infants who do not has received less attention.

Aim 2. Predicting postpartum LTFU among HIV-infected women initiating cART during pregnancy

As lifelong cART is scaled up to all pregnant and breastfeeding women throughout SSA, strategies to target women most likely to fall out of HIV care may be increasingly useful. However, such targeted approaches will only be successful if they to translate into improved PMTCT and maternal HIV outcomes at the population level. Comparisons of strategies that enroll a subset of women, versus all pregnant and breastfeeding women, into retentions interventions should be the focus of future evaluations. In addition, investigating what type of targeted strategy (e.g. enrolling a small group of women at highest risk of LTFU for a high-level retention intervention or enrolling a larger group of women for a lower-level intervention) results in the most gains in retention should also be evaluated.

In order for targeted approaches to be successful, the ability to predict postpartum LTFU also needs to be improved. Our risk score did not identify any strong predictors of LTFU at 6 months postpartum. It is possible that additional information on disclosure, [84, 141] barriers to staying in care,[77, 79] and social support [77, 139] could be asked by clinicians at delivery to improve the prediction of postpartum LTFU. Women with higher CD4 counts at cART initiation are also more likely to be LTFU.[153] However, as Option B+ is scaled up, the need for CD4 count monitoring may become obsolete, since women will initiate treatment regardless of CD4 count. Investigating whether other indicators of HIV disease status, such as suppressed viral load or WHO clinical stage, help to predict LTFU should be the subject of future investigations. As a

complimentary strategy, the development of a tool to assess cART-initiation readiness could be developed and integrated into a risk score to predict postpartum LTFU.

The ability to predict postpartum LTFU will only be useful if there are effective interventions to improve retention in care. A number of combination interventions are currently being tested among pregnant and breastfeeding women.[158-161, 173] Evidence from Malawi and our own study population show that a large proportion of postpartum LTFU is among women who never return to care after delivery or treatment initiation.[153] The high rate of LTFU after delivery and treatment initiation suggests that focusing on the prenatal period, when women typically have some engagement in care through ANC, may be important for retention interventions. After delivery, women may travel to their home village or switch their care to another clinic, making it difficult to track retention in care. Strategies to maintain contact with women, such as mobile phone interventions or integrated data systems across clinics, may help to have an accurate picture of postpartum LTFU and improve retention in care.

Along with effective intervention, meaningful measures of retention in care are also needed. Many studies (including our own) assess LTFU or retention at a uniform time point after delivery or treatment initiation. While this is analytically convenient, it does not given an indication of engagement in care throughout the study period. Engagement in care, as measured by adherence to antiretroviral therapy and viral suppression, are the goals of HIV treatment programs but are often measured by a simple indicator of whether a woman was present at one time point.[173] Developing indicators to more effectively capture women's engagement in care, such as the proportion of visits they attend over a study period, may give a more complete picture of patters of engagement in care and how that impacts PMTCT and HIV outcomes.[173]

E. Summary and Conclusions

Treatment guidelines for HIV-infected pregnant and breastfeeding women have shifted quickly in the last several years from an emphasis on antiretroviral prophylaxis for PMTCT towards lifelong treatment. These changes have led to important reductions in MTCT and are expected to improve maternal HIV outcomes. However, the increasing use of cART during pregnancy and breastfeeding has also lead to challenges. cART's role in causing adverse pregnancy outcomes has been of concern, as has improving postpartum retention in care. In Aim 1, we evaluated associations of duration of cART during pregnancy with LBW (Aim 1a), preterm birth and SGA (Aim 1b). Overall, we did not observe an increased risk of an adverse pregnancy outcome with longer duration of cART. However, the associations between duration of cART and preterm birth and SGA may be susceptible to measurement error in gestational age. We also observed that postpartum LTFU was high, especially immediately after delivery, among HIV-infected women initiating cART during pregnancy. However, predicting postpartum LTFU is difficult. Additional information may be needed to more accurately identify women at the highest risk of LTFU. Further work is also needed to assess how risk scores can most effectively be used to translate into improved HIV outcomes at the population level. As lifelong cART increasingly becomes the standard of care for pregnant and breastfeeding women, efforts to improve retention in care and better understand the mechanisms by which cART may impact pregnancy outcomes are essential if improvements in PMTCT and maternal HIV outcomes are to be realized and sustained.

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