MATERNAL RESPONSE TO ANTIRETROVIRAL THERAPY IN JOHANNESBURG, SOUTH AFRICA: ADHERENCE AND DRUG TOXICITIES

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Abstract

CASSIDY E. HENEGAR: Maternal response to antiretroviral therapy in Johannesburg, South Africa: Adherence and drug toxicities
(Under the direction of Daniel Westreich and Annelies Van Rie)

South Africa has one of the highest HIV prevalences in the world, with women of reproductive age disproportionately affected by the epidemic. Access to lifesaving highly active antiretroviral therapy (HAART) is expanding in the region, and many HIV-positive women are experiencing pregnancy after initiating lifelong treatment with HAART. The benefits of continued treatment with HAART during pregnancy include prevention of mother-to-child transmission of HIV, as well as maximization of maternal health. Optimal effectiveness of HAART, however, is dependent on a complex set of factors, most of which have not been adequately described in women established on HAART prior to pregnancy.

Using high quality observational data from a large clinical HIV cohort in Johannesburg, South Africa, and robust epidemiologic methods, including inverse probability weighted marginal structural models, we examined maternal responses to HAART among women established on treatment at the time of pregnancy.

An optimal adherence indicator was derived from routinely collected antiretroviral drug refill data from nearly 9,000 adult HIV-positive men and women, and evaluated based on ability to predict virological failure among the non-adherent. In our cohort of 7,510 HIV-positive women on treatment, pregnancy was common after HAART initiation, with 896 women experiencing at least one pregnancy during follow-up. Risk of non-adherence was similar among non-pregnant and pregnant women (weighted Risk Ratio (RR): 0.95, 95%

confidence interval (CI): 0.78 1.17), while women in the postpartum period, defined as six months after birth, experienced an increased risk of non-adherence compared to non-pregnant women (weighted RR: 1.46, 95% CI: 1.17, 1.82). Among the women in our cohort, we also observed few serious adverse events of renal impairment related to use of tenofovir, a widely used first-line agent in HAART regimens, regardless of pregnancy exposure.

Despite limitations of our pregnancy exposure data, our findings were robust to sensitivity analyses. In general, our results suggest that for women established on treatment prior to conceiving, continuation of HAART through pregnancy, in addition to protective effects against transmission, does not seem to increase maternal risks in respect to adherence or renal toxicity related to tenofovir use.

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ARV	Antiretroviral (drug)
ART	Antiretroviral therapy
BMI	Body mass index (measured kg/m_2)
CD4 (cell)	CD4-positive T-lymphocyte
CI	Confidence Interval
d4T	Stavudine
EFV	Efavirenz
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus type 1
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	Protease inhibitor
PMTCT	Prevention of mother-to-child transmission
sdNVP	Single-dose nevirapine
TDF	Tenofovir
TB	Tuberculosis
TLC	Themba Lethu Clinic
WHO	World Health Organization
ZDV	Zidovudine

Chapter 1

Specific Aims

South Africa has an HIV prevalence among the highest in the world, with women of reproductive age disproportionately affected by the epidemic. Women between the ages of 18 and 45 are up to four times as likely to be infected with HIV compared to men of the same age, and national surveys have estimated an HIV prevalence of 30% among pregnant women. [1,2]

In part due to increased access to antiretroviral therapy, a growing proportion of pregnancies among HIV-infected women are occurring in those already on highly active antiretroviral therapy (HAART) prior to conceiving. [3] The benefits of continued treatment with HAART during pregnancy include prevention of vertical transmission and maximization of maternal health, which is protective for both mother and child.[4] Optimal effectiveness of HAART, however, is dependent on a complex set of factors, most of which have not been adequately described in pregnant women. Existing studies of antiretroviral use during pregnancy, particularly in resource limited settings, have mainly focused on treatment initiated after conception for the primary purpose of preventing mother-to-child transmission. [5,6]

Biological and behavioral changes related to pregnancy and recent delivery could potentially alter tolerability of drug regimens, as well as the ability to maintain the high

degree of adherence required for sustained viral suppression. Increased understanding of the challenges related to treating HIV-infected women initiating HAART for their own health and then experiencing pregnancy could help to optimize care for both mother and child. The analyses in this dissertation aim to address many of the limitations of what is currently known about treating HIV-positive pregnant women.

Specific Aim 1:

Identify an optimal indicator for adherence derived from routinely collected pharmacy refill data.

Rationale: Pharmacy-based adherence measures, such as prescription refill data, are convenient and low resource methods, often utilizing data routinely collected for clinical use. A variety of adherence indicators can be calculated from raw pharmacy refill data, and there is at present no agreed-upon standard of how to calculate or apply these different measures. Given the established relationship between virological response to treatment and degree of adherence, we will assess the association between several calculated indicators and virological failure in order to select the optimal adherence measure to be applied to Specific Aim 2.

Hypothesis for Aim 1: Given the common data source, non-adherence will be associated with virologic failure for each of the derived indicators. Indicators using more extreme definitions of non-adherence will have a stronger association with virologic failure, but will also have a lower sensitivity for identifying true non-adherent behavior.

Specific Aim 2:

Evaluate the effect of pregnancy and the postpartum period on adherence to HAART in HIV-infected women initiated on treatment prior to pregnancy.

Rationale: A high degree of adherence to HAART regimens is required in order to achieve maximal virological suppression, and in the case of pregnant women, to minimize the risk of mother-to-child transmission. Current knowledge about the impact of pregnancy and the postpartum period on maternal adherence to ART is limited in scope. In particular, little is currently known about how pregnancy affects adherence among women that initiate HAART for their own wellbeing and subsequently become pregnant. The longitudinal nature of our data allows comparisons of adherent behavior before, during and after pregnancy, as well as comparisons with women not experiencing pregnancy. We will use marginal structural log-binomial regression models to estimate the effect of pregnancy on adherence.

Hypothesis for Aim 2: Women who are pregnant will be more likely to refill prescriptions on time compared to women who are either not pregnant or postpartum at the time of adherence assessment. The risk of non-adherence will be greatest in the postpartum period.

Specific Aim 3:

Assess the impact of pregnancy and the postpartum period on frequencies of ARV-related drug toxicities, specifically tenofovir-induced renal toxicity

Rationale: One particular challenge to maintaining a high level of adherence is adverse events related to antiretroviral drugs. Treating pregnant women with these potent drugs creates concerns for both maternal and child safety. Most of what is currently known about ARV drug toxicities in pregnant women is limited to new users initiating treatment during a pregnancy.

With updated treatment guidelines, tenofovir use is now widespread in first line HAART regimens, yet little is currently known about its safety profile in pregnant women, particularly in resource limited settings. While adverse events are generally lower after the initial phase of treatment, the effect of pregnancy on drug toxicities later in treatment has not been established for many ARVs, including tenofovir.

Hypothesis: Incidence of moderate and severe renal toxicity will be rare, regardless of pregnancy status. Reduced renal function, determined by assessment of creatinine clearance, will occur more frequently in individuals on HAART regimens containing tenofovir than other antiretroviral regimens.

As pregnancy cannot be randomized, our specific aims can only be addressed using observational data. This research will be conducted at Themba Lethu Clinic in Johannesburg, South Africa, one of the largest ARV treatment sites in sub-Saharan Africa, initiating more than 20,000 adults on treatment since opening in 2004. [7] The analysis of high quality patient level observational data from this site allows a unique opportunity to ask new questions and use innovative methods in order to better understand the effects of pregnancy on being treatment with HAART.

Chapter 2

Review of the Literature

A. General Background

Epidemiology of HIV/ AIDS in sub-Saharan Africa

Worldwide, more than 34 million people were living with AIDS in 2011. [1] The global prevalence of HIV remains high, in part because of sustained rates of incident infection, as well as increased life expectancy attributable to effective antiretroviral therapy. Despite improvements to treatment and access to care, HIV/AIDS causes 1.7 million deaths annually, and to date has taken more than 30 million lives. [1]

Sub-Saharan Africa has been disproportionately affected by the epidemic and is home to more than two thirds (22.5 million) of all people living with HIV/AIDS. Sixty-nine percent of incident infections (1.8 million) and seventy percent (1.2 million) of fatalities due to HIV occurred in this region in 2011.[1] HIV has had far-reaching effects in sub-Saharan Africa, impacting economic and social development, and decreasing the life expectancy by as much as 20 years in some countries. [8]

HIV/ AIDS in South Africa

The HIV epidemic has been particularly devastating in South Africa, which continues to have one of the highest rates of HIV-infection in the world, as well as more individuals living with HIV than any other country. With an overall adult prevalence of 17%, an estimated 5.1 million South Africans were HIV-positive in 2011 .[1] Although the country is home to just 0.7% of the world's population, 17% of all global HIV cases are found there. [9] Twenty-three percent of the estimated 1.8 million new adult cases in sub-Saharan Africa in 2009 also occurred in South Africa. [9]

There is evidence to suggest that South Africa's epidemic is stabilizing, but prevalence and incident infection rates remains exceptionally high, particularly in certain segments of the population. [2] These high risk groups for infection include young women, creating unique challenges for treatment and infection control.

Gender, Age, and HIV in South Africa

Women are disproportionately affected by HIV in many parts of the world, particularly in sub-Saharan Africa. This is especially true for women of reproductive age. In this region, young women 15-24 years old are as much as 8 times more likely than men the same age to be HIV positive. [10]

In South Africa, women make up 3.3 million of the country's 5.6 million cases (59%). [2] The situation is even more disparate in young women. The prevalence in 15-24 year olds is estimated at 13.6% for women, compared to 4.5% in men the same age. [1] Prevalence may be as high as 33% in women 25-29 years old, compared to an overall adult prevalence of 17%. [2]

Young women have a greater chance of being infected even when practicing less high risk behaviors than men of the same age. [11,12] Despite interventions to educate youth on safe sex practices, both young women and men commonly participate in high risk behaviors. In a representative survey of South African youths 15-24 years old, the majority of both men and women reported not using condoms consistently, and 25% of women and 15% of men reported never being tested for HIV. [13]

B. HIV and Pregnancy

HIV and pregnancy in South Africa

An estimated 1.4 million HIV-infected women gave birth in low and middle income countries in 2008, with 75% of these births occurring in sub-Saharan Africa. [4,14] In South Africa prevalence of HIV in pregnant women has been consistently high over the last decade, with 30% of pregnant women attending public sector health facilities infected in 2010. [3] (Figure 2.1)

The increased prevalence of HIV among pregnant women compared to the general population is a reflection of both high rates of infection and high incidence of pregnancy among young women in this population. Likelihood of becoming pregnant is closely correlated with age, regardless of HIV status, and the incidence of pregnancy highest for women between the ages of 15 and 24. [15,16] A nationally representative survey of young people in South Africa found that 33% of 15-19 year olds and 59% of 20-24 years olds reported ever being pregnant. [17] HIV prevalence among pregnant women is predicted to remain high as the number of women receiving antiretroviral therapy continues to increase. [18]

Impact of HIV on incidence of pregnancy

Prior to widespread availability of ARVs, HIV-infected women were much less likely to both conceive and experience live births compared to uninfected women. [19-22] Biologically, a woman's degree of immunosuppression is associated with her ability to conceive. Fertility decreases with duration of infection. A woman in the early stages of HIV may experience fertility rates similar to uninfected women. [23,24] A trend has been demonstrated between decreasing CD4 cells/mm^3, also associated with duration of infection in untreated individuals, and reduced fertility. Those most immunosuppressed (CD4 < 100 cells/mm^3) rarely experience pregnancy. [15,25,26] The further along in the disease process she is, the lesser her likelihood of becoming pregnant, particularly if her disease has progressed to AIDS. [25]

Women with greater disease progression are also more likely to be symptomatic, and reduced fertility is more common among women with clinical symptoms related to HIV. Feeling physically ill can create behavioral changes, including declines in sexual activity. [22,27] However, low pregnancy rates have also been observed in HIV-positive women not displaying symptoms. Changes in menstruation, including amenorrhea and anovulation, are common in HIV-infected women, and could be attributed to altered hormone production related to HIV or reduced BMI associated with more severe disease progression. [28]

Reduced female fertility related to HIV is only one explanation for lower incidence of pregnancy among HIV-infected women in the pre-HAART era. If a woman's sexual partner is also infected with HIV, his illness can also contribute to reduced sexual activity and reduced sperm viability.[29] Concerns about mother-to-child transmission or fear of being unable to care for a child while sick could also potentially cause altered behavior in women

and their partners in order to avoid pregnancy, including abstinence from sexual activity and use of contraceptives.

HIV and pregnancy outcomes

Untreated HIV-infected women who become pregnant are less likely to carry a healthy child to term compared to uninfected women who conceive. Maternal HIV has been associated with increased adverse pregnancy outcomes including still birth, infant mortality, intrauterine growth retardation, and low birth weight. [14,30] Conducted by Brocklehurst and French, a meta-analysis of early studies (1983-1996) found an association between HIV infection and adverse pregnancy outcomes across diverse settings, with the exception of infant mortality, which was only associated with HIV in developing countries. [30] HIV has also been associated with increased risk of spontaneous abortion. [31] It has been hypothesized that in addition to reduced fertility, decreased pregnancy rates among HIV-positive women could actually be attributed to early fetal loss due to infection before pregnancy is recognized.[31] Several mechanisms for HIV-related adverse events during pregnancy have been proposed, including the direct effect of the HIV on the placenta, thymic abnormalities, altered cytokine production, and cumulative effects of immunosuppression that may facilitate infection. [32]

Pregnancy among HIV-infected women is also associated with increased risk of maternal death, potentially independent of disease progression. [33] Infected women are more likely to die from both HIV-specific and obstetric causes. [34] This increased risk may extend into the postpartum period as well. While risk of death is closely correlated with CD4 count, an HIV-infected woman is at increased risk of dying during pregnancy even when her

immune function is comparable to that of an uninfected pregnant woman. [33] In 2011, 70% of maternal deaths in South Africa were associated with HIV infection. [35] Globally, HIV is the leading cause of death in women of reproductive age, and contributes more to maternal mortality than any single obstetric cause.

Pregnancy and HIV disease progression

There is some evidence to suggest that pregnancy can biologically affect the progression of maternal HIV disease, although the data have been conflicting and appear to be setting dependent. In the pre-HAART era, studies from high income countries did not show an association between pregnancy and disease progression, either during the period of pregnancy or long-term. [36-38] Studies in low income countries in the same time period, however, suggested a possible association between accelerated HIV progression and pregnancy. [39- 41] These studies were conducted in populations receiving either no ART or a single dose nucleoside reverse transcriptase inhibitor.

More recent studies conducted among women with access to HAART have reported a potential protective effect against disease progression for women on treatment. Two studies from the same cohort in the US examined the effect of pregnancy on disease progression in women on HAART. The initial study by Tai, et al. examined the incidence of AIDS-defining events or death among women, both pregnant and non-pregnant, on lifelong HAART. [42] The authors concluded that pregnancy was consistently associated with lower disease progression, although care should be taken in interpreting the results due to methodological flaws in the analysis. In this analysis, pregnancy was assigned as a baseline exposure, with those experiencing pregnancy at any time during follow-up treated as pregnant the entire

duration. In reality, women who were pregnant during follow-up contributed both exposed and unexposed person-time if they were in care beyond just the duration of their pregnancies.

Conducted within the same clinic population as the Tai, et al. study, Melekhin, et al. looked at the effect of timing of HAART initiation (before, during or after pregnancy) on HIV disease progression and found that women initiating HAART during pregnancy experienced both improved immunologic and virologic response to treatment compared to women initiating treatment after pregnancy.[43] In this study, the authors appropriately dealt with the exposure of pregnancy by ending individual follow-up at the end of pregnancy. All covariates, however, were fixed at baseline, a potential limitation for this longitudinal study.

Immunologic and virologic protective effects of pregnancy, however, have not been seen in all settings. In a study using data from the TLC cohort (the same population studied in this dissertation) conducted by Westreich, et al., women experiencing pregnancy after HAART treatment initiation had a modest increased risk of virologic failure. [44] The study design allowed women to contribute both exposed and unexposed time on study, and appropriate methods were used to control for time-varying confounders, namely marginal structural models.

C. HIV, Pregnancy and Antiretroviral Therapy

Antiretroviral therapy in South Africa

Even with effective antiretroviral regimens developed and distributed in many parts of the world, access to the drugs in South Africa remained extremely limited until late in the history of the country's epidemic due to governmental resistance and other political challenges. [45]

The first national PMTCT program in South Africa put in place in 2002. Early PMTCT policy called for provision of sdNVP for all HIV-infected pregnant women and their infants, as well as expansion of related health services and HIV counseling and testing. Ongoing maternal HIV treatment after delivery was not addressed in this early program, as HAART was not widely available to anyone until 2004. [46,47]

In 2004 the South African government introduced programs for comprehensive care and management of HIV, which included the provision of antiretroviral drugs free of charge to eligible individuals. National standards for eligibility were created using internationally recognized clinical guidelines for the initiation of HAART. This marked the start of the national HAART rollout program. Under these new treatment guidelines, pregnant women with CD4 cell counts < 200 cells/mm^3 were eligible to begin lifelong treatment with HAART. [3,18]

In part due to its more established infrastructures and greater financial resources than many other countries with large HIV burdens, dissemination and uptake of HIV services were relatively rapid once programs were put in place. The number of HIV-infected individuals on HAART rose from less than 2,000 in 2003 to more than 200,000 in 2005. [48] Access to HAART continues to expand, although there are still substantial gaps in coverage for eligible (according to clinical guidelines) individuals. In 2009, it was estimated that 42% of South Africa's 2.3 million eligible adults were receiving HAART, rising to 66% of 2.4 million eligible adults in 2011. [1]

Uptake of PMTCT services in South Africa was even more dramatic. In 2005, under 50% of all pregnant women were tested for HIV prior to delivery. [49] Today maternal testing is essentially universal, and more than 95% of HIV-infected women are treated with

appropriate ART for PMTCT. [1] The number of infants born to HIV-infected mothers that are tested using PCR within the first two months of life has also increased, from 36% in 2008 to 70% in 2011. [1] The measure of success in any PMTCT program is the transmission rate between mother and child. The proportion of infected infants born to HIV-positive mothers continues to decline in South Africa. The first national population-based surveys on early HIV transmission in infants took place in 2010 and 2011, reporting transmission rates of 3.5% and 2.7%, respectively. [8]

Guidelines for treatment with HAART

When government provided HAART was first introduced in 2004, HIV-infected adults, including pregnant women, were considered eligible to initiate lifelong treatment with ART if they had a CD4 cell count of less than 200 cells/mm^3, or had experienced a WHO stage IV AIDS-defining illness, irrespective of CD4 count. Unless contraindicated, all treatment naïve patients were initiated on one of two regimens consisting of two nucleoside transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitors: stavudine (d4T) and lamivudine (3TC), plus either efavirenz (EFV) or nevirapine (NVP). The standard second line regimen was zidovudine (ZDV), didanosine (ddI) and lopinavir/ritonavir (LPV/r, Kaletra ®). [50]

In 2010, the national eligibility criteria and standardized regimens were updated to reflect current WHO recommendations. Pregnant women and patients with TB are now eligible to initiate lifelong treatment with HAART with a CD4 count of 350 cells/mm^3 or less. Individuals with stage IV HIV disease or drug resistant TB are still encouraged to start ART immediately, independent of CD4 count. A CD4 cell count of 200 cells/mm^3 remains

the recommended cutoff for starting HAART in adult patients not meeting these special criteria. [51]

For treatment naïve patients initiating HAART, the current recommended first line regimen now consists of tenofovir (TDF), either lamivudine (3TC) or emtricitabine (FTC), and either EFV or NVP. Patients on d4T- based regimens prescribed under prior guidelines and who are tolerating treatment well are encouraged to maintain these regimens. Standard second line regimen options have been updated as well. For those failing on a d4T or AZT based regimen, the recommended replacement is TDF, 3TC/FTC, and LPV/r. For individuals failing on a TDF-based first line regimen, the recommended second line regimen is AZT, 3TC, and LPV/r. [51]

For women who become pregnant after initiating ART, continuation of the current treatment regimen is encouraged, given that there are no other indicators for drug substitution. If a pregnancy is recognized before the 12^{th} week of gestation and EFV is part of the current HAART regimen, NVP should be substituted due to concerns about potential birth defects (discussed in greater detail in section D). [51] Until this year, women who were eligible to initiate treatment for their own health according to the most current CD4 count thresholds were initiated on standard first line regimens as appropriate, and women that were not eligible for HAART were started on a PMTCT regimen. The most recent recommendations for these regimens were daily AZT from 14 weeks gestation, sdNVP plus AZT every three hours during delivery, and single dose TDF and FTC post-delivery.

Regardless of whether the mother is on lifelong HAART during pregnancy, infants should be given NVP at birth and daily for 6 weeks after birth. If the mother is not on HAART and breastfeeding, daily NVP should continue for the duration of breastfeeding.

Maternal treatment with HAART should continue through pregnancy, delivery and breastfeeding, with a few exceptions. For women eligible to initiate HAART for their own health during pregnancy, treatment is recommended regardless of gestational age.

The recommendations for PMTCT in South Africa are currently being updated with the goal of further reducing mother-to-child transmission rates and improving maternal health outcomes. The newest guidelines recommend starting all pregnant women not currently on HAART on a standard triple drug regimen regardless of CD4 count. Treatment would continue through pregnancy and for the duration of breastfeeding. If the woman is eligible to initiate lifelong treatment, the triple drug regimen taken during pregnancy should be continued after breastfeeding has stopped. This treatment strategy is known as Option B.[52]

Pregnancy among HIV-infected women in the HAART era

With increased access to HAART in South Africa, women are initiating treatment more frequently and earlier in their disease processes. A corresponding increase in the incidence of pregnancy among women on lifelong HAART has been observed.

Because widespread access to long-term treatment with ART is relatively new in sub-Saharan Africa, there are limited reports of the effect of treatment on incident pregnancy. Myer et al. compared the incidence of pregnancy among women participating in the MTCT-Plus Initiative in six African countries: Cote d'Ivoire, Kenya, Rwanda, South Africa, Uganda, and Zambia. Pregnant and recently postpartum women receiving PMTCT services were enrolled in the study, regardless of disease stage. [15] After completing treatment with PMTCT regimens, women who were eligible according to WHO guidelines were initiated on

lifelong HAART. Both women who were on treatment and those that ceased ART after delivery or breastfeeding were then followed until they experienced another pregnancy or were administratively censored at the end of the study period. Women who became eligible and initiated HAART during the course of follow-up contributed both pre-ART and on-ART time on study. The rate of incident pregnancies was higher among women on ART (9.0 pregnancies/ 100 person-years) than women not on ART (6.5 pregnancies/ 100 person-years), with an adjusted hazard ratio of 1.74 (95% CI: 1.19, 2.54). Among women on ART, the likelihood of pregnancy increased the longer they were followed up on treatment.

The women contributing time on treatment in the Myers, et al. study were a combination of those initiating HAART during pregnancy and those initiating at some point after the index pregnancy. These women experienced one of two general scenarios: a) they were healthy enough to become pregnant but still immunosuppressed enough to initiate HAART during pregnancy, and spent the entire duration of follow-up being treated, or b) they were healthy enough to conceive and healthy enough to avoid starting HAART during pregnancy, but then experienced disease progression severe enough to make them eligible for treatment at a later point in follow-up. It is reasonable to think that person-time contributed on treatment between these two groups of women may not be comparable in regard to factors associated with conceiving another child. Further, although there was variability in the degree of immunosuppression at the time of pregnancy, all of these women were at least healthy enough to conceive and carry a child, potentially indicating that they were healthier at baseline than the general population of HIV-infected women.

A second study conducted by Westreich, et al., and using data from the TLC clinical cohort, also examined the incidence of pregnancy among women on HAART. Women who

were both pregnant and not pregnant at the time of treatment initiation were included in this analysis. Women pregnant when starting HAART were younger and healthier than those not pregnant at initiation. In this cohort, pregnancy after HAART initiation was common, particularly among younger women, with an overall cumulative incidence in six years of follow-up of 22.9% (95% CI: 20.6%, 25.4%), and a cumulative incidence of 52.2% (95% CI: 35.0%, 71.8%) among 18-25 year olds. Women pregnant when starting HAART conceived more frequently (6.2 pregnancies/ 100 person-years, (95% CI: 5.1, 7.7%)) than women not pregnant when starting treatment (5.0 pregnancies/ 100 person-years, (95% CI: 4.7, 5.5%)). [53]

Treatment with effective ART may result in a variety of biological and behavioral changes that contribute to increased incident pregnancy in HIV-infected women. A trend of increasing rates of pregnancy with increasing CD4 counts has been observed in various settings. [53] As previously discussed, women who are highly immunosuppressed are more likely to have symptoms, as well as HIV-related opportunistic infections. After initiating HAART, improvements to general physical wellbeing may make women more likely to engage in sexual activity. As treatment continues, other conditions associated with HIV disease progression may resolve, including low BMI and anemia, making a woman's body more capable of supporting a pregnancy, with a higher likelihood of conception and lower risk of early fetal loss. [54,55]

Additionally, access to HAART and the associated improvements to health and wellbeing could increase the likelihood that HIV-positive women will actively attempt to become pregnant. Studies in various settings, including sub-Saharan Africa, have indicated that improved health after initiating ART is associated with an increased desire to have

children among both HIV-infected women and their partners. [56- 58] Driving this increased desire to have children may be an improved perception of the risks involved with pregnancy among HIV-infected women, driven by the positive improvements to pregnancy outcomes associated with access to appropriate ART during pregnancy.

Access to lifelong HAART has dramatically altered survival time and quality of life for HIV-infected women. Before effective antiretroviral regimens were available, long-term survival for women, as well as for children conceived after maternal infection, was low. Without access to appropriate treatment, HIV greatly reduces life expectancy, and dramatic declines in health and quality of life typically occur within a few years of primary infection. In some settings, HIV-positive individuals treated with HAART have life expectancies comparable to uninfected individuals. [1]

Without the intervention of antiretroviral therapy during pregnancy and delivery, the risk of transmission from mother to child is as high as 15-30%. Breastfeeding adds an additional 5-20% risk of transmission. [4] Most vertical transmission occurs in the intrapartum period, but can also occur during delivery or breastfeeding. [4] Risk of transmission is affected by the mother's disease progression, duration of ruptured membranes, premature birth and exposure to genital secretions related to STI co-infections common in HIV-infected women.[14]

The use of ART during pregnancy, particularly combination therapy, is associated with greatly reduced risk of vertical transmission. [59] In South Africa, universal HIV testing in pregnant women and nearly 100% coverage with some form of ART during pregnancy and delivery have resulted in low transmission rates (< 3.0%) comparable to those seen in high income countries (<2.0%). [60] As part of more comprehensive HIV care, women are

also exposed to more extensive counseling on issues relating to pregnancy, including appropriate treatment to prevent mother-to-child transmission, appropriateness of breastfeeding, and the importance of early infant HIV testing.[3,61]

Access to knowledge on how to have a healthy pregnancy and delivery, as well as reduced fears about mother-to-child transmission, poor birth outcomes, or early maternal mortality, may encourage more women to actively try to conceive, or to be less likely to terminate an unplanned pregnancy. [56] With improved access to both ART and HIV testing, more women are being assessed for treatment and initiating lifelong HAART when appropriate. The result is a growing number of HIV-infected women, living longer and healthier lives, creating increased opportunities for incident pregnancies among women on HAART.

D. Pregnancy and Drug Toxicities Related to Antiretroviral Therapy

Adverse events related to treatment in pregnant women

While HAART provides many benefits for eligible HIV-infected individuals, there are also challenges to optimizing therapy for maximal response; primary among these concerns are drug toxicities. Adverse events related to therapy range from mild reactions that can be a nuisance to the patient, generally lowering quality of life and potentially leading to poor adherence or treatment interruption, to more severe reactions, which can be life threatening or have long-term physical effects.

Treatment with ART during pregnancy creates additional concerns about drug toxicities, as both the safety of the mother and infant must be balanced with maintaining optimal viral load suppression. Potential changes to pharmacokinetics in the body of a

pregnant woman could also alter the risk profile for certain toxicities. Adding to the challenges of treating pregnant women in resource-limited settings is the reduced selection of available drugs, limiting options for regimen changes when faced with intolerable side effects.

There is evidence to suggest that pregnancy may affect the incidence of treatment-related adverse events. Women in general differ from men in their likelihood of experiencing certain drug toxicities, regardless of pregnancy. Observational studies have reported a higher risk of adverse events due to ARVs in women compared to men. [62] Pharmacokinetics, the process by which drugs are absorbed, metabolized and eliminated from the body, differ by gender, with women tending to have higher concentrations or lower clearance of several drugs, including indinavir, EFV, LPV and NVP. [62] Women are also at higher risk for toxicities related to these drugs, including lactic acidosis and NVP-associated rashes and hepatotoxicity. [63-65]

Looking again within the setting of TLC, Sanne, et al. [66] observed that during the four year period of follow-up, women were more than twice as likely to experience at least one drug substitution, an indicator of drug intolerance, than men (HR: 2.19, 95% CI: 2.00-2.39). Looking at specific toxicities, women were significantly more likely to experience lipodystrophy, lactic acidosis, and symptomatic hyperlactemia compared to men, although peripheral neuropathy did occur more commonly in men.

Higher incidences of both rash and hepatotoxicity associated with NVP use have also been reported in women. In a multicenter cohort study from 7 clinics in the US, Bersoff-Matcha, et al., observed that women were seven times more likely than men to develop a rash, and 3-5 times more likely to discontinue NVP use due to the rash. [67] Changes to HIV

treatment since the time of this study (1993-1998), however, may limit the generalizability of the findings. Results of a randomized trial in South Africa to examine the safety and efficacy of 3TC compared to FTC, given in combination with d4T or NVP were reported by Sanne, et al.[68] Hepatotoxicity early in treatment occurred in 17% of the NVP group, but in none of the d4T group. Hepatotoxicity occurred in 12.8% (N=20) of men taking NVP and 20.1% of women (N=46) (aRR 3.9, 95% CI 1.9-8.0).

Differences in size and body volume between genders may partially explain the increased drug concentrations found in women. Women tend to have greater intolerance for drugs that require dosage adjustment for weight. [62] Under the same mechanism, increases in body size and blood volume associated with pregnancy may lead to reduced drug concentrations and fewer drug toxicities. A few small studies have indicated that pregnant women have lower concentrations of several antiretroviral drugs compared to non-pregnant women. [69,70]

Fluctuation in drug concentrations of antiretrovirals in pregnant women have also been attributed to differences in enzymatic activity.[71] Cytochrome P450 is the primary hepatic enzyme responsible for metabolizing PIs and NNRTIs. The production of this enzyme differs by sex, but is also altered by hormones present in pregnancy. [72,73] Induction of P450 enzymes may increase the likelihood of hepatotoxicity. [74,75] Pregnancy has been shown to be a risk factor for hepatotoxicity in other conditions, including hepatitis E. [74,76]

Changes in maternal blood volume during pregnancy, in combination with drug activity, have been associated with the increased risk of anemia in women taking AZT.

Anemia in pregnancy is common due to the 50% increase in plasma volume balanced with only a 30% increase in red blood cell mass. [59] When taking antiretroviral agents that alter the production of red blood cells, such as AZT, the risk of anemia is even greater. [77,78]

Changes to hormone production in pregnancy have also been linked to antiretroviral-related adverse events. Pregnancy hormones are known to have an anti-insulin effect.[79] Treatment with protease inhibitors has been shown to have an effect on glucose metabolism in non-pregnant HIV-positive adults. [80] The combination of the two effects may explain the increased risk of gestational diabetes seen in HIV-positive women taking PIs during pregnancy. [79] Pregnancy has also been associated with low levels of riboflavin, which potentially increases the risk of mitochondrial toxicity, including severe lactic acidosis. [81,82]

It is also plausible that the incidence of ARV-related adverse events is associated with adherence, both generally and in pregnant women. If pregnancy has an impact on compliance with taking pills as prescribed, independent of experiencing side effects, the degree of adherence could affect drug concentrations circulating in the body. This potential relationship will be discussed in more detail in the review of literature examining adherence to ART during pregnancy.

General safety profiles and guidelines for use in pregnancy of ARVs by drug class

While toxicities in pregnant women have not been adequately evaluated for all antiretroviral drugs, certain drugs and drug combinations are not recommended during pregnancy due to observed or suspected toxic effects. The following is a summary of

guidelines and potential toxicities for the antiretroviral drugs most commonly available to pregnant women in South Africa:

Nucleoside Reverse-Transcriptase Inhibitors (NRTIs):

Zidovudine (AZT) and Lamivudine (3TC)

AZT and 3TC are the most common NRTIs used in South Africa, particularly prior to updates to treatment guidelines in 2010. These drugs are used during pregnancy, both in HAART regimens for women initiating or continuing lifelong ART, as well as in combination PMTCT regimens. In general, the drugs appears to be well-tolerated.[4,83] Hematological toxicities have been associated with AZT, including anemia and neutropenia. For women presenting with severe anemia at initiation, alternative NRTIs (TDF) can be prescribed. [84]

Stavudine (d4T)

The use of d4T in pregnant women is no longer recommended as a preferred option for ART. Its use is associated with increased risk of mitochondrial toxicity, which can result in lipoatrophy, peripheral neuropathy, lactic acidosis and pancreatitis. [85] Case reports and cohort studies in developing countries have indicated that d4T during pregnancy, particularly when taken with didanosine (ddl), can lead to increased rates of life-threatening lactic acidosis.[59,81] For this reason, the combination of these two drugs should be avoided during pregnancy.

Tenofovir (TDF)

TDF is currently recommended for first line ART regimens, including regimens for pregnant women.[4] Updated recommendations to initiate adults on TDF-based HAART were based on clinical trials and observational studies indicating that TDF has comparable or better efficacy than other first line drugs , including d4T, but has a better safety profile. [86,87,88,89] A multi-site trial in Africa found associations between TDF and increased risk of moderate or severe nephrotoxicity, but still an infrequent occurence (1.3%).[90] Few studies have looked at TDF use specifically among pregnant women in resource limited settings, but existing literature suggests that the drug is well tolerated among pregnant women and an effective means of PMTCT. [91]

Non-nucleoside Reverse-Transcriptase Inhibitors (NNRTIs)

Nevirapine (NVP)

Historically NVP has been the most widely used NNRTI in the developing world, regardless of pregnancy status. The most common toxicities associated with NVP are cutaneous rash and hepatotoxicity.[59] The frequency of NVP-induced adverse events reported in the literature varies widely depending on study population and design, as well as definitions of adverse events. NVP related rash and hepatotoxicity can be life-threatening, especially in women with CD4 counts above 250 cells/mm^3. [92,93]

Studies on the effect of pregnancy on NVP toxicity have produced conflicting results as to whether or not pregnant women are at increased risk of hepatotoxicity.

Efavirenz (EFV)

In addition to NVP, EFV is the other recommended first line NNRTI. The most significant toxicities associated with its use are neuropsychiatric disorders. Development of rash is also common. The primary concern with EFV exposure during pregnancy is the potential association with neural tube defects when taken in the first trimester of pregnancy.[94] Research to date has not established a definitive link between EFV and impairment of fetal neural tube development in humans, but avoidance of the drug early in pregnancy is recommended if possible. [4]

Protease inhibitors (PIs):

Lopinavir/ritonavir (LPV/r)

LPV/r has been associated with weakness, headaches, digestive disorders and metabolic complications. [59,90] There have also been reports of increased risk of low birth weight infants women taking LPV/r. [4] There have been conflicting findings from observational studies on the effect of PIs on duration of pregnancy. A joint analysis of two large European cohorts including over 4,000 mother-child pairs found that antenatal ART including PIs were associated with 2.6 greater risk of premature birth compared to women taking no treatment.[95] Data from the Women and Infants Transmission Study in the US, however, found no differences in the rates of premature births between those taking combination therapy including PI's and AZT monotherapy. [96]

Toxicities related to tenofovir among pregnant women

Most of what is currently known about drug toxicities related to ARV use during pregnancy is derived from studies conducted in high resource settings. Research to date has also disproportionately focused pregnancy outcomes and long term effects for the infant, rather than toxicities to the mother. Among studies looking at maternal toxicities conducted in high income countries, most examined the incidence of adverse events in pregnant women, without a comparative group of non-pregnant women. [65,70,71,75,77,79,93,97- 101]

Further, while a limited number of studies included women initiating HAART prior to becoming pregnant, none specifically focused on this group of HAART users. In general, antiretroviral use during pregnancy appears relatively well tolerated among women in high and low income settings.

Due to widespread use in both long-term HAART and PMTCT regimens, the safety TDF during pregnancy is of particular interest. Limitations of the study populations and designs used to date have left unresolved questions, especially concerning incidence of drug toxicities related to TDF among pregnant women established on ART prior to conception.

In the general adult population on ART, TDF has an excellent safety profile, with low incidence of nephrotoxicity, proteinuria, and renal tubular dysfunction with Fanconi Syndrome reported. [102- 104] The standard use of TDF in first line combination ART regimens for pregnant women is a relatively new and not universal, and as such there are limited studies on the safety of its use during pregnancy. TDF remains a category B drug for pregnancy according to WHO classifications, indicating that more data on safety in mothers and infants is needed. Of particular concern are potential impact on bone mineralization (seen in animal studies) and renal impairment. [15,105]

Using data from the Development of AntiRetroviral Therapy in Africa (DART) trial, Gibb, et al. examined the effect of TDF exposure during pregnancy on birth outcomes.[106] In this study, the frequency of birth defects was similar in TDF exposed and unexposed infants, and *in utero* use did not appear to increased the risk of renal impairment or hypophosphataemia. This study had the advantage of data from a large trial of women initiating ART for their own health during pregnancy in Africa, where data is still scarce, but maternal adverse events related to the drug were not evaluated.

To date, there is only one large scale cohort study in sub-Saharan Africa examining the effect of pregnancy on TDF-related adverse events. Johnson, et al. assessed predictors of renal impairment, including pregnancy, in adults initiating ART in Malawi.[107] Renal impairment was evaluated using laboratory results for creatinine clearance (CrCl). While pregnancy itself did not appear associated with renal impairment, other predictors of reduced CrCl varied by pregnancy status. Among pregnant women, only increases in age were associated with increased risk of reduced CrCl, while low BMI and hemoglobin were risk factors for non-pregnant adults. This study, however, did not address the long-term risks of TDF-related adverse events in adults, pregnant and non-pregnant, among individuals established on treatment.

Maskew, et al. assessed the impact of TDF exposure among women receiving HAART on incident pregnancy in the TLC clinical cohort. [108] Results of the analysis suggest that women on ART regimens containing TDF compared to those on d4T-based regimens may be slightly less likely to become pregnant while on treatment. The effect estimates, however, were modest and imprecise. Further, incident pregnancy while on HAART is common in this population, suggesting that with most women now initiating on

TDF-based regimens, there will still be a large number of women conceiving while on the drug regardless of a slightly reduced risk of incident pregnancy. [53]

E. Pregnancy and Adherence to Antiretroviral Therapy

Maintaining a high degree of adherence to HAART regimens is challenging, yet proper compliance is vital to achieving viral suppression, avoiding viral resistance, and prolonging life. Adherence to ART is one of the strongest predictors of progression to AIDS and death. [109] Adherence during pregnancy is of particular importance, as it protects both maternal health and lowers the risk of mother-to-child transmission of HIV. [109- 111]

The exact degree of adherence required to achieve and maintain viral suppression is drug and regimen dependent. Earlier HAART regimens demonstrated a threshold of 95% adherence for reduced risk of virological failure and poor clinical outcomes. [110] The higher potency and longer half-lives of current HAART regimens, however, appear to require a more moderate degree of adherence to maintain viral suppression. Recent studies have suggested that regimens containing ritonavir-boosted PIs or NNRTIs suggest that viral suppression can be achieved with 70-80% adherence. [112,113] Given the observed linear relationship between degree of adherence and virological success, however, the goal for individuals patients should still be complete adherence. [113] Further, a high but imperfect level of adherence (80-90% of doses taken on time) has been associated with a greater risk of developing mutations for drug resistance than moderate to low levels of adherence. [114]

Review of studies of ART adherence during pregnancy

An individual's adherence is determined by a complex set of mental, biological and social factors. The experience of pregnancy while on HAART adds complexity to understanding and measuring adherence during this time period. Studies examining adherence to ART during pregnancy have been limited in scope, revealing currently unanswered questions.

Most studies examining adherence to ART among pregnant women have indicated a high degree of compliance during pregnancy, particularly when compared to the postpartum period. During pregnancy, women may have motivating factors beyond their own wellbeing to comply with treatment guidelines. The same patterns can be seen for other conditions, such as cessation of smoking during pregnancy, or better dietary control among diabetic pregnant women. [115] Pregnant women also experience more frequent interactions with care providers, which may introduce additional opportunities for adherence counseling or simply encouragement to maintain healthful practices. [116,117]

High pill burden has been associated with reduced adherence, due to complexity of the regimen or an individual simply feeling overwhelmed. In some settings, pregnant women may actually have a lower pill burden compared to non-pregnant women, a factor which could contribute to maintaining high levels of adherence. [117]

It is also possible that challenges specific to pregnancy could interfere with achieving maximal adherence. [118,119] While estimates of adherence during pregnancy are typically high, some studies have reported low rates of compliance, and nearly all indicate room for improvement. There are many potential barriers to maximal adherence unique to pregnancy and the postpartum period. Women who experience side effects related to pregnancy, such as

nausea or headaches, may avoid taking medication which could exacerbate these symptoms. [120] Treatment fatigue from dealing with both pregnancy and HIV-related issues, as well as depression, both during pregnancy and during the postpartum, could impact personal motivation to adhere to medical routines during pregnancy. [121,122] After delivery, the demands of caring for an infant, in addition to no longer being concerned about transmitting HIV to the baby, may partially explain the lower proportion of adherent women typically observed in the postpartum period.

Nachega, et al. published the only current review of the existing literature on adherence to ART during pregnancy and the postpartum. [6] The authors evaluated adherence estimates from 72 studies, and using a threshold of 80% adherence or higher, reported pooled estimates of 76% (95% CI: 72%, 80%) of women being adherent during pregnancy and 53% (95% CI: 33%, 73%) during the postpartum period. The variation in study designs and populations, however, makes the value of a pooled estimate questionable.

The threshold for identifying adherent and non-adherent behavior varied between values of 80%, 90%, 95%, and 100%. Studies with thresholds below 80% (n=2) were excluded from the analysis. The virological and clinical outcomes of someone maintaining 100% adherence compared to someone with 80% adherence, particularly when drug regimen and year of study are taken into account, may vary substantially, making it inappropriate to label the two types of behavior as the same. This is especially true when considering that pooled estimates included adherence measures for sdNVP given at the onset of labor, AZT taken for PMTCT during pregnancy, and combination ART taken for both the purposes of PMTCT and lifelong maternal treatment. Both the behavior required to be fully adherent, as well as the motivation and challenges associated with these different regimens, suggests

these forms of ART should be considered separately. Nachega, et al. did estimate separate pooled adherence based on type of ART, but estimates were not stratified by any other variable, including whether data was collected from the pregnancy or postpartum periods. Not surprisingly, adherent behavior for combined pregnancy and postpartum periods was reported more frequently in sdNVP (79% of women adherent (95% CI:70%, 87%) and AZT (79% (95% CI: 74%, 83%) regimens compared to combination ART regimens (64% (95% CI: 56%, 71%). In the analysis of cART regimens, no distinction was made between women taking combination therapy for the duration of pregnancy for PMTCT purposes, women initiating lifelong combination therapy for their own health during pregnancy, and women becoming pregnancy while on cART. A closer look at the individual studies examining adherence to combination therapy reveals a dearth of information on women initiating HAART for their own health prior to pregnancy, particularly in low and middle income countries.

Of the twenty four studies that evaluated adherence to cART during pregnancy, twelve included women initiating or continuing treatment for their own health, while the remainder focused on combination therapy for PMTCT purposes alone. Studies of cART for PMTCT purposes alone tended to be older, using data collected prior to changes in treatment guidelines which encouraged initiation of lifelong ART among eligible pregnant women. Among studies of pregnant women taking HAART for their own health, four were restricted to women initiating treatment during pregnancy (Ciabrone (2006), Kierten (2011), Mirkenzie (2011), Shapiro (2010)), and three did not differentiate between women starting treatment before or during pregnancy in their analyses (Caswell (2011), Louis (2005), Zorilla (2003)). Mellins, et al. assessed timing of ART initiation, but based assessment of ART experienced

vs. naïve on exposure before enrollment, which occurred during the third trimester. [129] Women starting HAART in the early phases of pregnancy would be classified as ART experienced, along with women starting treatment prior to pregnancy.

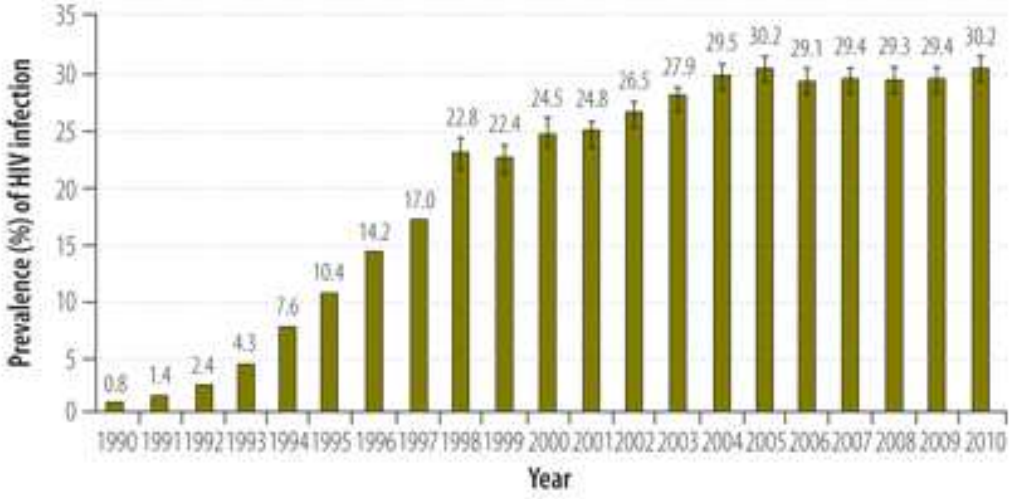
Bardeguez, et al. enrolled HIV-infected women during pregnancy or soon after giving birth, with follow-up continuing through 48 weeks postpartum. [122] Follow-up visits were conducted during each trimester of pregnancy, at delivery, and every 12 weeks postpartum, with adherence at each visit assessed by self-report A total of 519 women were enrolled in the study, 90% (468) before delivery. Three quarters of women reported perfect adherence during pregnancy. Those that self-reported perfect adherence also had lower viral loads than those reporting imperfect adherence. Self-reported adherence fell to 65%, 64%, and 66% at the 6, 24, and 48 week postpartum visits. This study reported higher adherence in women starting ART during pregnancy than in those that were on ART before becoming pregnant (OR for perfect adherence 1.46, 95% CI 1.05-2.02).

These results contradict those of Vaz, et al. in their analysis of a Brazilian cohort of pregnant and non-pregnant HIV-infected women attending outpatient clinics for HIV care.[117] Adherence was ascertained by both pill count and self-report, and was defined as taking at least 95% of prescribed doses. Seventy-two pregnant women, of whom 34 were also assessed in the postpartum period, and 79 non-pregnant women were enrolled in the study. Pill count indicated that pregnant women were more likely to be adherent (p=0.001), with 43% of pregnant women and 18% of non-pregnant taking 95% or more of their pills. Adherence in the post-partum period was significantly lower (20.6%, p=0.0002). Self-reported values for adherence were much higher for both pregnant (83%) and non-pregnant women (72%). This study found that there were no differences in level of adherence

between women who started ART prior to pregnancy and those that initiated once they became pregnant (p=0.49).

F. Figures

Figure 2.1: Prevalence of HIV Infection in pregnant women in South Africa, 1990-2010
(Barron, et al.,2013) [3]



Chapter 3

Research Study Design and Methods

A. **Study Setting and Population**

Study Setting

Prior to 2004, access to life-saving antiretroviral treatment in South Africa was extremely limited. With the launch of the government sponsored national HAART rollout that year, access to antiretroviral drugs and related HIV care began to expand rapidly. By 2010, South Africa had the largest HIV treatment program in the world, with over one million people accessing treatment through public sector services. [1]

Coinciding with the start of the national HAART rollout, the Themba Lethu Clinic (TLC) in Johannesburg opened in April of 2004 as a government run treatment center. The clinic is located in a large, public sector teaching facility, Helen Joseph Hospital, and receives patients referred primarily from within the Gauteng Province. Individuals testing positive for HIV are referred to TLC to be assessed for ART eligibility. Most patients referred to TLC received testing and preliminary counseling from other clinical sites; TLC does, however, conduct about 12,000 HIV tests annually, with immediate referral of HIV-positive adults into care at the facility in most cases. Individuals testing positive but not eligible to initiate HAART can enter into pre-ART care. Eligible patients are started on lifelong treatment with appropriate antiretroviral regimens. Since opening in 2004, the clinic

has initiated over 22,000 adults on ART, with approximately 40% of these individuals still in care at TLC. [7]

In addition to funding and management from the South African Department of Health, TLC is also supported by the NGO Right to Care, which receives partial funding from the United States Agency for International Development (USAID) through the President's Emergency Plan for AIDS Relief (PEPFAR). [7]

Study Population

The base cohort for our analyses included patients initiated on HAART at TLC between April 1, 2004 and September 30, 2011. Patients became eligible for inclusion at the time they initiated treatment with ART, and follow-up time spent in pre-ART care was excluded from all analyses. Patients receiving treatment with ARVs for any reason prior to initiating at TLC were excluded, as well as those with missing data on treatment start date or initial HAART regimen. In Johannesburg, pediatric HIV services, including testing and treatment, are conducted at separate specialized facilities. Therefore, our base cohort was restricted to individuals 18 years of age and older with no upper age limit. Once enrolled, patients were followed until they died, transferred care to another facility, or were lost to follow-up. Patients still in care at the end of the analysis period (September 30, 2011) were administratively censored.

Patient Follow-up and Clinic Procedures

Eligibility for ART initiation was determined using standardized criteria endorsed by the South African Department of Health. [50,51] (Table 3.1) Between April 1, 2004 and March 30, 2010, patients were eligible to initiate ART with a CD4 cell count of less than 200

cells/mm^3, or if determined to have stage 4 HIV disease according to WHO classifications, regardless of CD4. Pregnant women could be initiated at higher CD4 counts based on clinician discretion.

In April of 2010, eligibility requirements were changed to recommend pregnant women and those with tuberculosis start HAART with a CD4 count of less than 350 cells/ mm^3. The threshold for initiating treatment remained 200 cells/mm^3 for the general population of treatment naïve HIV-infected adults. [51]

In September of 2011, initiation criteria were again adjusted, and currently all adult patients with CD4 counts of less than 350 cells/ mm^3 are eligible to start HAART. Due to exclusion criteria of the analyses for the individual aims of this dissertation, all included individuals began treatment under the 2004 or 2010 criteria for treatment initiation. [7]

At the time of treatment initiation, baseline labs included full blood count, hemoglobin assessment, and liver function tests carried out to determine most appropriate treatment regimens. In addition to the baseline visit (month 0), clinic appointments are typically scheduled at 1, 3, 6, and 12 months after beginning HAART. Again in accordance with national ARV treatment guidelines, viral load assessments are not performed prior to treatment initiation. The first viral load assessment is done 4 months after treatment initiation, along with a CD4 count, in order to assess virological and immunological response to HAART. [51]

Prior to April 2010, follow-up visits with full laboratory assessments were scheduled every six months after the first 6 months on treatment. With the updated guidelines, visits for routine care are now scheduled for every 12 months after the first year of treatment (with visits at 1,3,6, and 12 months the first year). Actual clinic visits may vary from these

guidelines, as clinicians can schedule more frequent visits if indicated. Patients can attend the clinic between standard follow-up visits for acute issues, including adverse events related to ARVs or suspected opportunistic infections. Non-standard laboratory tests can also be scheduled as needed. All clinical labs are processed by the National Health Laboratory Service (NHLS), which has a branch located in Helen Joseph Hospital.

ARVs are refilled and picked up from the onsite pharmacy at TLC. In most cases, appointments to pick up refills are scheduled monthly for the first six to twelve months, and every two months thereafter. If the patient is not stable on treatment, including being non-adherent, visits may remain on a one month schedule.

Antenatal care is not included in routine clinical services for HIV-infected pregnant women at TLC. Women who initiate HAART while pregnant, as well as those who become pregnant while on treatment, attend primary care clinics or specialized antenatal care clinics within the community for prenatal services. Maternal needs related to HIV, including refills of ARV prescriptions and routine laboratory assessments, are still handled by TLC during pregnancy and the postpartum period. Most women give birth in hospitals, where additional treatment with ARVs should be provided to mother and infants in order to prevent mother-to-child transmission. Follow-up for infants born to HIV-infected women, including HIV testing, is handled by yet another provider in a pediatric HIV clinic. During the period of our analysis, there was no routine communication between TLC and antenatal clinics, hospitals where women deliver, or pediatric HIV clinics. Any information in the patient record related to pregnancy is, therefore, informally assessed and highly dependent on patient self-report.

Data Management

TLC utilizes an electronic patient management system, Therapy Edge-HIV® (TE), to record and track all patient level data. Individual electronic records are continuously updated at each patient encounter with the clinic. (Table 3.2) Before 2007 all patient information was first recorded in paper records, and then transferred into TE by data capturers. Beginning in mid-2007 TLC transitioned away from using paper-based medical records, implementing live updates to TE as the patient moves through the clinic. The details of each clinic and pharmacy visit, as well as laboratory results, are updated in real time. In 2010, the NHLS electronic data management system became integrated with TE, allowing for immediate and accurate transfer or laboratory data into patient records. Data capturers are responsible for cleaning and verifying the data in TE, as well as dealing with missing information.

B. General Definitions and Inclusion Criteria

For each of our three aims, we selected individuals from the sub-cohort of all TLC patients who had ever initiated ART at the clinic, and who were ART-naïve prior to beginning treatment at the clinic. Patient enrollment ran between April 1, 2004 and September 30, 2011, although the enrollment period included in each of the specific aims varied in order to allow all participants adequate follow-up time for the question being asked. All analyses were restricted to those over 18 at treatment initiation.

Incident vs. Prevalent Pregnancy

We excluded women pregnant at the time of HAART initiation from our analyses. We will refer to existing pregnancies at the time of HAART initiation as prevalent

pregnancies. In contrast, incident pregnancies will be defined as those occurring after a woman has started on HAART. Women initiating HAART with a prevalent pregnancy tend to have less advanced HIV disease than those initiating treatment for their own health, as well as being younger and generally healthier.[123] Fundamental differences between women with prevalent and incident pregnancies that cannot be accounted for in the analysis could make causal interpretation of the results difficult if these women were analyzed together.

In relation to our specific aims, overall better health and different motivations for starting treatment among women pregnant and not pregnant at baseline may influence different patterns of adherent behavior. If women with prevalent pregnancies do have less advanced disease progression, it may also alter the severity and incidence of adverse events related to specific drugs.

C. Definitions and Methods Specific to Aim 1

Aim 1: To identify an optimal indicator for adherence derived from routinely collected pharmacy refill data.

This analysis included all adults who were ART-naïve and not-pregnant at baseline, and who initiated treatment between April 1, 2004 and July 31, 2011. This enrollment window allowed all patients at least two months on treatment before the end of follow-up (September 30, 2011), as the adherence indicators were based on adherence in the two months immediately prior to each pharmacy refill visit.

Definitions

Adherence (Exposure)

At TLC patients attend scheduled appointments at the clinic pharmacy to refill their prescriptions. Records of each scheduled refill date, as well as the actual date of pharmacy attendance, are maintained in TE®. As previously detailed, individuals typically refill their prescriptions approximately every 28 days early in the first several months after initiating HAART, and if there are known treatment concerns later in follow-up. Once patients are stable on treatment, they are usually scheduled to refill prescriptions approximately every 56 days.

In order to avoid missed doses due to unforeseen short delays in attending scheduled visits, two and four extra pills are dispensed respectively for each 28 and 56-day refill cycle. Because not all appointments could be scheduled exactly 28 or 56 days apart, we allowed some flexibility when categorizing pickup schedules as monthly or bimonthly. Visits scheduled within one week of either the standard 28 or 56 days apart were classified as one of these standard schedules, with the assumption that enough pills were dispensed to allow for complete pill coverage between visits.

For visits with a non-standard scheduled length between appointments, attendance measures were not calculated due to uncertainty about the number of pills dispensed and reasons for the non-standard scheduling. Using the scheduled and actual dates of pharmacy visits as the basis, we constructed and evaluated eight adherence measures. (Table 3.3)

In addition to pharmacy data, beginning in 2009, nurses began asking some patients about their adherence as a percentage of doses taken on time in the week prior to the current visit using the following scale: “All” (≥ 90%), “Most” (60-90%), “About half” (30-60%), “A

few" (10-30%) or "None" (<10%). We dichotomized self-reported response into patients taking 90% or more of doses on time and those taking less than 90%, i.e. "All" vs. other categories, and compared self-report to pharmacy indicators.

Virological Failure (Outcome)

Virological failure was defined as either failure to achieve suppression of plasma HIV to ≤400 copies/mL within six months of initiating HAART or as a viral load above 400 copies/mL after documented viral load suppression ≤400 copies/mL. [44, 124]

In order to maintain consistency between both adherence and viral load assessments, we used only viral load measurements with pharmacy data corresponding to either 2, 28-day cycles or 1, 56-day cycle in the two months immediately prior to the lab results. Adherence and viral load measurements that did not meet these criteria were excluded. Viral load was assessed on a routine basis, and pharmacy attendance was monitored at every pharmacy visit, meaning there were multiple assessments of both adherence and viral loads for most patients, and individuals could "fail" multiple times if multiple viral loads were greater than 400 copies/mL.

Analysis

We used logistic regression with generalized estimating equations to examine each 2-month adherence indicator as a predictor of virological failure. The odds ratios (ORs) and 95% confidence intervals (CIs) calculated with robust standard errors were examined to identify which measures of adherence most strongly associated with virological failure. We

also stratified logistic regression models by pharmacy refill schedule (monthly vs. bimonthly).

We used c- (or concordance) statistics, defined as the area under the under the receiver operating characteristic curve, to identify the measures of adherence best able to properly classify a case of virological failure or success.[125] Sensitivity, specificity, and predictive values, all with corresponding 95% confidence intervals, were also calculated. We examined the association between viral load and adherence for all eligible visits, as well as a stratified analysis by refill schedule.

D. Definitions and Methods Specific to Aim 2

Aim 2: To evaluate the effect of pregnancy and the postpartum period on adherence to HAART in HIV-infected women initiated on treatment prior to pregnancy.

Women with no prior ART exposure (including prior PMTCT regimens) before starting treatment at TLC were eligible for inclusion. As previously addressed, women with prevalent pregnancies were excluded. Pregnancy after the age of 45 was rare in this cohort, and women who become pregnant at older ages may be exceptional in other ways that cannot be accounted for in the analysis. Furthermore, in order to maintain positivity, [126] person-time in women 45 years of age and older will be excluded due the very small number of women exposed (experiencing pregnancy) in this age group.

Definitions

Not Pregnant, Pregnant, and Postpartum (Exposure)

The primary exposures of interest for Aim 2 were pregnancy and the subsequent postpartum period. Women who experienced an incident pregnancy during follow-up contributed both unexposed and exposed person-time. Women who never become pregnant during follow-up contributed only unexposed person-time.

As previously discussed, assessment of pregnancy is not part of routine follow-up care at TLC, but when a woman self-reports that she is pregnant, or when the condition is recognized by a clinician, information related to her pregnancy is entered into her TE® record. The recorded start and end dates of each gestational period are primarily estimates based on maternal self-report. . There will be substantial variance in both the gestational age at which a woman learns she is pregnant and when she reports it to her provider.

Exposure status was assigned for each month of follow-up based on self-reported dates of incident pregnancies, if applicable: not pregnant, pregnant, or postpartum. Clinically, the postpartum period is defined as the time from one hour after delivery to six weeks post-delivery. [10] Maternal changes (biological, physical and emotional) associated with recent delivery, however, likely extend beyond six weeks. For this reason, studies of adherence to ARVs postpartum have followed women for several months to two years after delivery. [117,122, 127] For our primary analysis, a fixed period of six months defined the postpartum period. At the end of the fixed postpartum period, women experiencing incident pregnancies were censored.

Adherence (outcome)

The outcome of interest was non-adherence, measured with a binary indicator of 100% pill coverage between pharmacy visits or less than 100% coverage. Details about the construction and selection of this adherence indicator are provided in the methods section for Aim 1.

Analysis

The longitudinal nature of the data collection makes it possible to assess effects of time-varying as well as time-fixed covariates, as well as changes in exposure status, with women able to contribute both exposed and unexposed person-time. In our analyses we accounted for both baseline and time-updated covariates as potential confounders. Baseline covariates were patient demographic and clinical characteristics assessed at or immediately prior to HAART initiation. Time-updated covariates were clinical indicators updated at different time points over patient follow-up. See Table 3.4 for a summary of baseline and time-updated characteristics.

Baseline characteristics at the time of HAART initiation for women meeting our inclusion criteria were described. Categorical covariates were compared using chi-square tests, while continuous covariates were compared using t-tests (means) or Wilcoxon rank-sum tests (medians). We used modified inverse probability of treatment weights to fit marginal structural log-binomial regression models in order to estimate relative risks of non-adherence during periods of pregnancy, postpartum, and non-pregnancy.

Marginal Structural Models

Longitudinal data from observational studies present unique challenges that cannot be addressed using more traditional epidemiologic methods for estimating causal effects. In particular, when a time-updated covariate confounds the relationship between the exposure and the outcome at one time point, yet also acts as a causal intermediate between the exposure and the outcome at a later time point, standard methods for controlling for confounding will produce biased effect estimates. [128]

Figure 3.1 demonstrates this concept. For Aim 2, we examined the relationship between incident pregnancy and adherence to ARVs. By design no one in our cohort was pregnant at HAART initiation. There are three time points (times 2,3, and 4) in Figure 3.1 representing 6 month intervals. At each follow-up time, the most recent CD4 count is associated with the likelihood of becoming pregnant. CD4 is also potentially associated with adherence if the degree of physical illness impacts motivation to take medication as prescribed. CD4 count, therefore, confounds the relationship between pregnancy and adherence. Pregnancy also affects subsequent CD4 count. An incident pregnancy (or not) at time 2 affects CD4 counts assessed at time 3, placing CD4 count on the causal pathway between incident pregnancy and adherence at time 4.

Using standard stratification methods to fix CD4 count at each follow-up time would control for confounding by CD4, but would also bias the estimate of the total effect as a result of controlling for a causal intermediate. Marginal structural models (MSM) offer an alternative approach to controlling for both time-fixed and time-varying confounding based on the concept of standardization rather than stratification.

MSM use inverse probability of treatment weights (IPTW) to control for confounding of a treatment (exposure) - outcome relationship due to a set of covariates by reweighting the data to account for selective observation. IPTW are calculated as the inverse of the probability that an individual experienced the exposure that she did given covariates unique to that person (represented as $1/ (P(E=e \mid Z=z))$. Application of this weighting structure to the data, in which the original observations are multiplied by their unique weights, creates a "pseudopopulation" where the association between the confounder and subsequent exposure is removed. [128,129] After controlling for baseline confounders (and assuming no unmeasured confounders), the potential outcome of non-adherence is modeled as if the exposure, pregnancy, were randomized. [130]

Typical IPTW calculations assume that there is a single transition in exposure state, from untreated(unexposed) to treated (exposed); once the transition occurs it is assumed that treatment continues until the end of follow-up, with the probability of receiving the treatment from that point forward fixed. [129- 133] This assumption does not hold for the exposures of pregnancy and postpartum.

Here, we were interested in three levels of exposure, and so the typical method of weight construction did not apply. Further, we could not treat non-pregnancy, pregnancy and postpartum as a simple polytomous exposure. In this special situation, all women who become pregnant transitioned from pregnancy (unless censored in the middle of pregnancy) into a third exposure category, the fixed postpartum stage. Further, the postpartum period could only be experienced by those women that first experienced pregnancy. The postpartum exposure is conditional on, but separate from, the pregnancy, and weights had to be calculated accordingly.

There is some variability to when a woman will transition from pregnancy to postpartum, and this second change in exposure was incorporated into the calculation of our IPT weights. First, weights were calculated for the inverse conditional probability of pregnancy for all women in the cohort. Then weights for the inverse conditional probability of becoming postpartum were calculated only among pregnant women, and only after pregnancy began. Weights for the two periods were multiplied across time, yielding a predicted probability of remaining non-pregnant for each month of follow-up where a woman was not pregnant or in the first month of pregnancy, and the predicted probability of remaining pregnant once a woman became pregnant through the first month of the postpartum. The probability at each month, t, is multiplied by the probabilities for all previous months of follow-up to create weights representing total history of exposure.

In the process of fitting IPTW, individuals with rare covariate patterns given their exposure status will be heavily weighted, and overrepresented in the "pseudopopulation." In order to reduce variance created by these up-weighted individuals, we used stabilized weights, constructed by multiplying the IPTW (conditional probability of treatment) by the probability of treatment conditional on baseline covariates only. The stabilized inverse probability of treatment weights (with weight A representing the pregnancy exposure and weight B for the postpartum exposure, calculated only for women experiencing pregnancy) are given as:

A.

$$SW_{it} = \prod_{k=0}^{t} \frac{\Pr[X_{ik} \mid \bar{X}_{ik-1}, Z_{i0}, \bar{C}_{ik-1} = 0]}{\Pr[X_{ik} \mid \bar{X}_{ik-1}, \bar{Z}_{ik-1}, C_{ik-1} = 0]}$$

B.

$$SW_{it} = \prod_{k=0}^{t} \frac{\Pr[Y_{ik} \mid \bar{Y}_{ik-1}, Z_{i0}, \bar{C}_{ik-1} = 0]}{\Pr[Y_{ik} \mid \bar{Y}_{ik-1}, \bar{Z}_{ik-1}, C_{ik-1} = 0]}$$

For weight A, the numerator is the probability of patient *i* having pregnancy exposure given her past exposure history up to the current month ($\bar{X}_{ik-1}$), her baseline covariates (Z_{i0}) and not being censored in the previous month of follow-up. The denominator is the probability of patient *i* having pregnancy exposure given her past exposure history up to the current month ($\bar{X}_{ik-1}$), her time-varying covariates ($\bar{Z}_{ik-1}$) including her baseline covariates, and not being censored in the previous month of follow-up. Inclusion of baseline covariates in the numerator stabilizes the model, but also means that the MSM no longer controls for the baseline measures. The baseline covariates are, therefore, included in the model with the exposure parameter estimate representing the marginal effect of exposure conditional on baseline covariates.

For weight B, the numerator is the probability of patient *i* entering the postpartum exposure given her past exposure history up to the current month ($\bar{Y}_{ik-1}$), her baseline covariates (Z_{i0}) and not being censored in the previous month of follow-up. Due to biological limitations on the gestational period, pregnancy was defined to end with a

probability of 1 10 months and later after the first month of pregnancy. The denominator is the probability of patient *i* being postpartum given her past exposure history up to the current month ($\bar{Y}_{ik-1}$), her time-varying covariates ($\bar{Z}_{ik-1}$) including her baseline covariates, and not being censored in the previous month of follow-up. The model is then fit using robust variance methods (GEE) to account for the repeated outcome assessments of the study design as well as the induced clustering from the pooled dataset.

The above equations are for estimates of the effect of pregnancy or postpartum in the absence of censoring ($\bar{C}_{ik-1} = 0$). Inverse probability of censoring weights (IPCW) were constructed in the same way as IPTW, with censoring replacing the exposure. IPTW and IPCW were multiplied together at each observation. We also considered that loss due to death was potentially more informative than censoring, and constructed inverse probability of death weights (IPDW) for inclusion in a separate model. Ultimately, neither the inclusion of death nor censoring weights had any effect on the estimates and were excluded from the main analysis and sensitivity analyses.

Sensitivity analyses

Several sensitivity analyses were conducted to assess the robustness of our risk ratio estimates when adjusting our exposure definitions to account for potential misclassification and other uncertainties.

1) Exclude first six months of follow-up, due to the fact that the occurrence of both non-adherence and drug-related adverse events are typically highest in the first several months after initiating HAART. [134,135]

2) Restricting the analysis only to women that experience pregnancy during follow-up: while it is problematic to condition inclusion at baseline based on future exposure status, we performed this sensitivity analysis to account for the fact that there may be fundamental differences between women who pregnant during follow-up and women who do not, and that it may bias our results to lump the unexposed person-time (non-pregnant) from both groups of women together.

3) Extended postpartum length: The fixed duration of the postpartum period was extended from 6 months to 12 months to account for social and psychological changes related to having a child that may impact adherence and may also extend beyond six months duration.

Analyses to account for errors in recorded pregnancy dates, including missing values:

4) Fixed duration of pregnancy: All women experiencing incident pregnancy were assigned an end date for the pregnancy that was 9 months from the indicated start date. A fixed postpartum period of six months was then assigned based on this new end date. Based on prior studies among HIV-positive pregnant women, it can be hypothesized that the median duration of the pregnancy will be between close to full-term (all four cited studies reported median gestation lengths of 39 weeks). [136- 139] Imputing a pregnancy end date nine months from the reported first month of pregnancy deals both with missing data, and potentially errors in the recorded last month of pregnancy.

5) Pregnancy start date moved 3 months earlier than recorded date: In order to account for the pregnancy start dates potentially being reported late, we moved the start date back by the equivalent of one trimester, and reassigned exposure statuses based on this new start date.

6) Pregnancy start date reassigned as 9 months before recorded end date: under the assumption that end dates of pregnancy are more memorable, and therefore more reliable, pregnancy start dates were reassigned as a fixed duration of 9 months from recorded end dates.

7) Multiple imputation for missing end dates: In order to account for the large number of missing pregnancy end dates (N=365), we used multiple imputation techniques to impute a duration of pregnancy, which was then used to assign a pregnancy end date. The postpartum period was then fixed at 6 months after the newly assigned end date.

E. Definitions and Methods Specific to Aim 3

Aim 3: To assess the impact of pregnancy and the postpartum period on frequencies of ARV-related drug toxicities, specifically tenofovir-associated renal toxicity.

Definitions

Not pregnant, Pregnant, and Postpartum (Exposure)

Incident pregnancy and the postpartum period were defined as in Aim 2. Women who were pregnant at baseline (HAART initiation) were again excluded. Using the discrete time model constructed from the data in Aim 2, we assigned exposure status (not pregnant, pregnant, postpartum) to each unit of person-time (month of follow-up). Women experiencing incident pregnancy were censored at the end of the fixed postpartum period.

Reduced Creatinine Clearance (Outcome)

Tenofovir, especially at high concentrations, has been shown to accumulate in the proximal tubules of the nephron of the kidney, potentially leading to renal failure, Fanconi

syndrome, proteinuria and tubular necrosis. Severe nephrotoxicity occurs infrequently, and in most cases, renal function can be restored with adjustments to drug regimens or dosing. [102] For this reason, regular monitoring of renal function in recommended for those on tenofovir. Baseline tests for creatinine clearance are also needed to determine appropriateness of treatment with tenofovir. A minimal baseline CrCl rate of 50 mL/min is recommended for those starting the drug. [140] With tenofovir now included in most first line regimens at the clinic, TLC has added creatinine clearance assessment to standard follow-up labs.

The creatinine clearance test is used to estimate the glomerularfiltration rate (GFR), which describes the flow rate of filtered material through the kidney. The creatinine clearance rate is the volume of blood plasma cleared of creatinine per unit of time. This test assesses how well the kidneys are functioning in terms of excreting substances. Creatinine is produced naturally by the body, and is filtered out of the blood stream by the glomerulus. [141] True creatinine clearance involves collecting both serum and urine samples, determining the creatinine removal rate in the urine and dividing it by the plasma creatinine concentration. [140] Due to small amounts of creatinine filtration through capillaries surrounding the kidneys, creatinine clearance tests tend to overestimate true GFR by 10-20%. This method is involves collection of multiple urine specimen and calculations, making routine clinical use impractical. [140]

A surrogate indicator derived using the Cockcroft-Gault formula and requiring only serum creatinine concentrations is a practical alternative for estimating the GFR. Creatinine clearance reported in TE® is estimated using this formula, and reported as ml/min cleared. [142] CrCl is highly dependent on age and weight, with the acceptable range for “normal” clearance being 90-139 mL/min for an adult male, and 80-125 mL/min for an adult female.

Pregnant women experience a 40-50% increase in serum creatinine, and calculations estimating CrCl in pregnant women should be adjusted accordingly. [142] See Tables 3.5 and 3.6 for definitions of renal function impairment for pregnant and non-pregnant women.

Analysis

Women with no prior ART exposure (including prior PMTCT regimens) before starting treatment at TLC and who were less than 45 years old at initiation were eligible for inclusion in this analysis. Baseline characteristics were stratified by tenofovir in the initial HAART regimen, and were assessed using standard descriptive statistics.

We reported the number of creatinine clearance assessments performed by pregnancy exposure status for women both on and not on tenofovir-containing regimens. Renal function was categorized according to a standardized scale (see Table 3.5). We also reported the outcomes a proportion of total creatinine clearance assessments in non-pregnant, pregnant, or postpartum women. CrCl results were compared between exposure categories by degree of severity using Fisher's exact tests. Exact methods were required due to a small number of moderate and severe outcomes occurring during pregnancy and the postpartum. Use of traditional approximate methods may produce invalid results when sample sizes are small.

A sensitivity analysis was performed among pregnant women, regardless of current tenofovir use, to adjust the CrCl grading scale to account for changes to kidney filtration that occur during pregnancy. The scale for non-pregnant and postpartum women remained the same as in the primary analysis.

The small number of outcomes in certain exposure categories precluded multivariate analysis. Rates were also calculated among only tenofovir users at the time of testing. Non-pregnant person-time was the referent group in all comparisons.

F. Tables and Figures

Table 3.1 Themba Lethu Clinic standard clinical practices

	2004 ARV Treatment Guidelines (April 1, 2004- April 2010)	**2010 ARV Treatment Guidelines** (April 1, 2010- September 30,2011)
Eligible for ART	CD4 count < 200 cells/mm^3 Irrespective of clinical stage (including pregnant women) **OR** WHO stage IV HIV disease Irrespective of CD4 count	CD4 count < 200 cells/mm^3 Irrespective of clinical stage **OR** CD4 count < 350 cells/mm^3 AND pregnant or active TB disease **OR** WHO stage IV HIV disease Irrespective of CD4 count
First Line ART Regimens For all eligible adults, including pregnant women* * NVP is preferred over EFV for women of childbearing age not on reliable contraception	d4T + 3TC +EFV OR d4T+ 3TC +NVP **OR** *If d4T is contraindicated:* AZT + ddI + LPV/r	TDF + 3TC + EFV OR TDF + 3TC + NVP **OR** *If TDF is contraindicated:* AZT + 3TC + EFV/NVP Those already on d4T based regimens with no side effects at the time of the guideline updated should remain on current regimen
Follow-up Schedule	Clinic visits at **1**,**3**,**6** and **12months** after treatment initiation Appointment at **month 4** for viral load and CD4 assessment After first year, follow-up appointments **every 6 months**	Clinic visits at **1**,**3**,**6** and **12** months after treatment initiation Appointment at **month 4** for viral load and CD4 assessment After first year, follow-up appointments **every 12 months**
Routine laboratory tests	Full blood count, hemoglobin, liver function tests: baseline, months 1,3,6 and 12 month visits, every 6 months after the first year Viral load: 4 months after treatment initiation and every 6 months after	Full blood count, hemoglobin, liver function tests, creatinine clearance: baseline, months 1,3,6 and 12 month visits, every 12 months after the first year Viral load: 4 months after treatment initiation and every 12 months after

Table 3.2. Data fields for routinely collected data among those in care at Themba Lethu Clinic *(Fox, et al., 2013) [7]*

Data fields	Variable list
Demographics	Name, national ID number, contact details, gender, date of birth, employment status, alcohol use, smoking history, ethnicity and education level
Clinical visit data	Date of visit (scheduled and actual), TB screening, urine analysis, vital signs, height, weight, description and duration of new symptoms and systems-based clinical examination (e.g. cardiology, neurology, and respiratory)
Laboratory results	ART initiation and monitoring bloods, including CD4 count, HIV viral load, full blood counts, liver function tests, renal function tests, TB microscopy and culture results, lactate levels and glucose and lipid profiles
Medication history	Date of start and stop of ART and non-ART medications, reasons for treatment discontinuation and self-reported treatment adherence
Clinical diagnoses	Pregnancy, opportunistic infections including TB, hepatitis, PCP, AIDS-related malignancies including Kaposi sarcoma, ART toxicities including peripheral neuropathy, anaemia, hyperlactataemia/lactic acidosis and lipoatrophy

Table 3.3 Indicators constructed from pharmacy refill and self-reported adherence data (Aim 1)

Indicator	Type	Definition
On time	Clinic attendance	Binary indicator of whether actual visit to pharmacy occurred on or before scheduled appointment
Less than 5 days late	Clinic attendance	Binary indicator of whether pharmacy refill occurred fewer than 5 days before the median time late among those not attending on time
Less than 30 days late	Clinic attendance	Binary indicator of whether pharmacy refill occurred before 30 days late or after
100% pill coverage	Pill possession	Binary indicator taking into account extra pills dispensed with last refill with on time attendance occurring before pills ran out (100% coverage)
> 90% pill coverage	Pill possession	Binary indicator taking into account extra pills dispensed with last refill; in the time between visits, were there pills for at least 90% of days
> 80% pill coverage	Pill possession	Binary indicator taking into account extra pills dispensed with last refill; in the time between visits, were there pills for at least 80% of days
Categorical	Clinic attendance/ pill possession	On time with enough pills for complete coverage between visits vs. late with enough pills between visits vs. late and not enough pills for coverage between visits
>90% doses on time in last week	Self-report	Based on nurse interview. Individuals reporting taking 90% of doses in the week before are adherent, compared to those with less than 90%

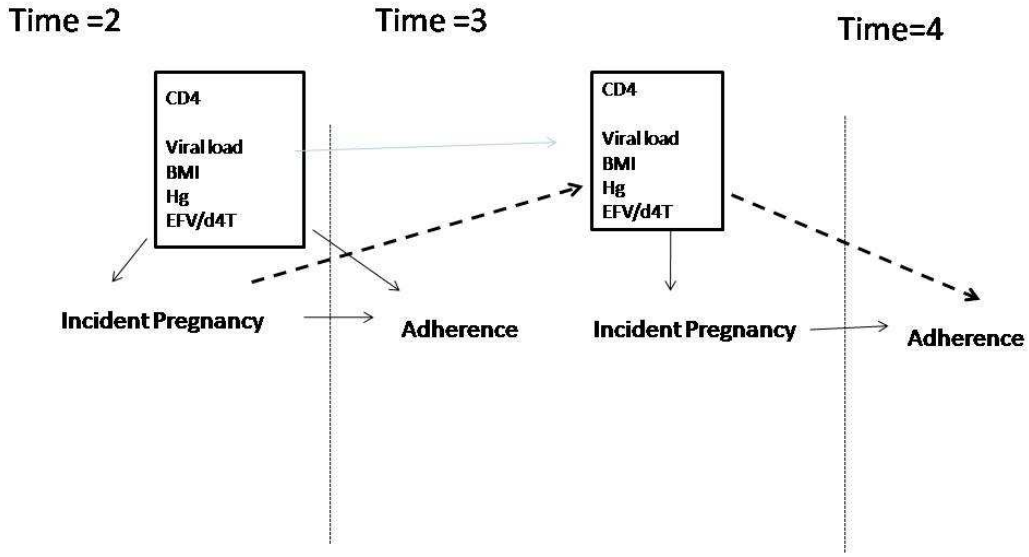
Table 3.4 Summary of baseline and time-updated covariates included in the analysis

Variable	Specification	Baseline, Time-updated	Description
Age	Continuous, cubic splines	Baseline	Age at enrollment
WHO Disease stage	Binary	Baseline	Patients were assessed at the time of treatment initiation as having stage 1,2,3,or 4 HIV disease according to WHO criteria; coded categorically as either disease state 1or 2 OR disease stage 3 or 4
Employment status	Binary	Baseline	Coded as employed or unemployed at enrollment
BMI (kg/m^2)	Continuous, cubic splines	Baseline, time-updated	Modeled as a continuous variable, described as both continuous (mean, SD) and categorical (baseline) according to the following categories: underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25.0 kg/m^2), obese ($\geq$ 30 kg/m^2)
CD4 Count ($cells/mm^{3)}$	Continuous, cubic splines	Baseline, time-updated	Modeled as a continuous variable, described as both a continuous (mean, SD) and categorically (at baseline) according to the following categories: $\leq$ 50 $cells/mm^3$, 51-100 $cells/mm^3$, 101-200 $cells/mm^3$, 201-350 $cells/mm^3$
Hemoglobin (g/dL)	Categorical	Baseline, time-updated	Coded as normal (>11.35 g/dL), moderately anemic (7.35-11.35 g/dL), and severely anemic (<7.35) for non-pregnant women (adjusted for altitude); categories for pregnant women: (>10.35 g/dL, 6.35-10.35 g/dL, <7.35 g/dL)
Viral load (copies/ mL)	Binary	Time-updated	Measured as a continuous value of number of viral copies, coded as virological failure (>400 copies/ ml) or not; not modeled as baseline covariate because viral load is not assessed regularly at baseline (missing for 80% of women)
Tuberculosis	Binary	Baseline	Indicates if diagnosed and on treatment for active TB at the time of treatment initiation; coded as yes/no
Efavirenz (EFV)	Binary	Baseline, time-updated	Binary indicatory of whether baseline (or current) regimen contains EFV
Stavudine (d4T)	Binary	Baseline, time-updated	Binary indicatory of whether baseline (or current) regimen contains EFV

Table 3.5 Classification of renal dysfunction according to creatinine clearance for adult, non-pregnant women+ *[32]*

Stage	CrCl
Normal	≥ 90 mL/min
Mild	60-89 ml/ min
Moderate	30-59 ml/min
Severe	< 30 ml/min

Figure 3.1. Simplified directed acyclic graph (DAG) of causal model for the effect of incident pregnancy on the risk of non-adherence. Only time-varying confounders are included in this diagram.



Chapter 4

Pharmacy-Based Measures of Adherence to Antiretroviral Therapy as a Predictor of Virological Failure

A. Introduction

Adherence to highly active antiretroviral therapy (HAART) is a major determinant of clinical outcomes in HIV infection. [143,144] A sustained high degree of adherence has been shown to be the strongest predictor of viral suppression among HIV-positive patients on HAART.[145, 146] Standardized, routine and cost-effective monitoring of adherence is thus necessary to identify patients who would benefit from targeted adherence support to prevent poor treatment outcomes. [147]

Several methods are commonly employed to assess individual degree of adherence in clinical settings. However, there is currently no consensus on a standard measure for routine use. [148] While direct assessments of adherence, including electronic monitoring (MEMS) and laboratory serum drug assays, are typically more accurate than indirect measures, they are costly and impractical in resource-limited settings with high HIV disease burden. [149] Pharmacy-based adherence measures, such as prescription refill data, are simple and objective methods for assessing compliance and use information that is often routinely collected for medical or pharmacy records.[150]

Adherence indicators calculated from pharmacy refill data can take a variety of forms. Measures commonly used assess timing of medication acquisition or enumerate doses available/taken, both with the intent of identifying gaps in treatment. [151,152] In most cases, dichotomous or categorical measures are created from continuous indices, using a variety of cutoffs which may or may not have clinical significance. [152,153] Assessment and reporting of adherence to HAART using pharmacy refill data have not been standardized, making comparison across time, between clinics, and between study populations difficult.

The purpose of this study was to optimize pharmacy-record-derived indicators of adherence to HAART by comparing measures of association between various pharmacy-refill adherence indicators and viral load suppression, while also considering the simplicity of each measure for routine use. These findings could contribute to the standardized use of routine pharmacy data to assess adherence among people on HAART in resource limited settings.

B. **Methods**

Study Population

We analyzed data from the Themba Lethu Clinic (TLC),an observational cohort of adult patients initiating treatment on HAART in Johannesburg South Africa. [7] The clinic is one of the largest providers of HAART in South Africa, and over 20,000 individuals have been started on HAART since the beginning of government treatment provision in April of 2004. At TLC, treatment and HIV-related care are provided free of charge.

Included in our analysis were treatment-naïve men and women initiating HAART between April 1, 2004 and July 31, 2011. Individuals were followed until they died, transferred care to another facility or were lost to follow-up. Patients still in care at the end of follow up (September 30, 2011) were administratively censored. Women starting HAART while pregnant were excluded due to potential fundamental differences in overall health and motivation for treatment initiation. [125]

Definitions

TLC patients attend scheduled appointments at the clinic pharmacy to refill their prescriptions for antiretroviral drugs and other medications. An electronic data management system maintains records of what drugs are dispensed, as well as the scheduled and actual dates of pharmacy attendance. Pharmacy visits are scheduled based on standard 28 or 56 day refill cycles. To avoid missed doses due to unforeseen short delays in attending scheduled visits, two and four extra pills are dispensed respectively for each 28 and 56-day refill cycle. Visits scheduled within one week of either the standard 28 or 56 days apart were still classified as one of these standard schedules, with the assumption that enough pills were dispensed to allow for complete pill coverage between visits. Visits scheduled outside of these standard refill periods were excluded from the analysis.

Based on the difference between the scheduled and actual dates of each visit,eight different adherence measures were calculated, assessing timing of clinic attendance, the proportion of visits covered by the medication dispensed at the prior visit, and a combination of timing and pill coverage. Adherence measures based on clinic attendance included(1) a simple binary indicator of presenting on or before the scheduled appointment date, (2)

coming late more or less than the median number of days late among all late visits at TLC and (3) coming more or less than 30 days late. Calculations of pill coverage included (4) a binary indicator of having complete or incomplete coverage, and based on a continuous measure of coverage, if the medication dispensed at the previous visit covered (5) 100%, (6) more than 90%, or (7) more than 80% of the time between visits. Finally, a categorical indicator combining attendance and pill coverage, categorized visits as (8) either on time, late with sufficient pill coverage, or late with missed doses.

For a subset of TLC visits, self-reported adherence was assessed during routine clinical visits. Adherence questions were administered at the discretion of the clinic nurse and participants were selected without a specific algorithm. Patients were asked to evaluate the number of prescribed doses taken on time in the week prior to the current visit using the following scale: "All" (≥ 90%), "Most" (60-90%), "About half" (30-60%), "A few" (10-30%) or "None" (<10%). We dichotomized self-reported response into patients taking 90% or more of doses on time and those taking less than 90%, i.e. "All" vs. other categories.

Virological failure was defined as a hybrid measure of failure to achieve suppression of plasma HIV to ≤400 copies/mL within six months of initiating HAART or a viral load above 400 copies/mL after documented viral load suppression ≤400 copies/mL.[124] To increase temporal association and predictive value of the adherence measure, only those pharmacy refill data visits corresponding to the two months prior to the viral load assessment were eligible for inclusion in the analysis. Viral load measurements without pharmacy data for the two months immediately prior to the assessment were also excluded. Because all eligible visits for individual patients were included, a single patient could contribute multiple visits and could "fail" multiple times if multiple viral loads were greater than 400 copies/mL.

For refills on a 28-day schedule, attending both visits by the scheduled date, one for each month before the viral load lab result, was required in order to be classified as "on time" for the entire two month period. Continuous measures were cumulative over both visits. For refills on a 56-day cycle, two- month adherence measures could be calculated from a single visit.

Statistical Methods

Baseline characteristics of individual patients at HAART initiation were described using standard descriptive statistics. We used generalized estimating equations (GEE) with a binomial distribution, logit link function, and independent correlation matrix to measure the association between each of the adherence indicators and virological failure while accounting for within-individual correlation. The odds ratios (ORs) and 95% confidence intervals (CIs) calculated with robust standard errors were examined to identify which measures of adherence most strongly associated with virological failure. We used c- (or concordance) statistics, defined as the area under the under the receiver operating characteristic curve, to identify the measures of adherence best able to classify a case of virological failure or success.[154] Sensitivity, specificity, and predictive values, all with corresponding 95% confidence intervals, were also calculated. We examined the association between viral load and adherence for all eligible visits, as well as a stratified analysis by refill schedule.

C. **Results**

A total of 8,695 adults contributed a total of 29,937eligible visits. The median age at HAART initiation was 37 years (IQR 31,43) and 63% (N=5505) of those starting treatment

were women. The mean baseline CD4 count was 103 cells/mm^3 (standard deviation: 74 cells/mm^3) with 32% having 50 cells/mm^3 or fewer. (Table 4.1) Of the 29,937 viral load assessments, 7% (N=4,095) indicated virological failure, either due to failure to suppress 400 copies/mL or less by six months (N=1259) of treatment or rebounding to over 400 copies after successful suppression (N=2836).

Adherence was high regardless of measure, with 84% of eligible visits occurring on or before the scheduled pharmacy visit date, and 88% occurring before pills from the last refill ran out. Among visits occurring late, the median time of actual attendance was five days after the scheduled visit. Most late visits occurred within several days of the scheduled visit, but 15% (N=737) occurred more than 30 days late. Accounting for extra doses dispensed with each refill, only 20% of those showing up late did not have enough pills to cover at least 80% of days between the two visits.

Independent of pharmacy refill schedule, all measures demonstrated increased probability of virological failure with lower adherence (Table 4.2).More extreme classifications of non-adherence showed stronger associations with virological failure, including a gap in treatment of 30 days or more (OR 2.56; 95% CI:2.16, 3.03)and having less than 80% pill coverage in the two months prior to viral load assessment(OR 1.89; 95% CI: 1.62, 2.20).However, simple binary measures of coming on time (OR 1.27, 95% CI: 1.16, 1.38) or having enough pills between visits (OR 1.26; 95% CI: 1.15, 1.39) were also associated with virological failure. The c-statistics, with a potential range of 0.5 to 1.0, were low for all of the assessed measures, ranging from 0.506 to 0.521 (data not shown).

All of the indicators had low sensitivity (Se) (Table 4.4), particularly those for the most non-adherent behaviors, coming 30 days or more late (Se: 5%) and having less than

80% pill coverage between visits (Se: 6%).The binary indicators for being on time (Se: 19%) and having complete pill coverage (Se: 14%) were associated with increased sensitivity while maintaining moderate specificity (Sp: 84% and Sp: 89%, respectively). Self-reported adherence assessment also performed comparably with these two indicators (Se: 13%; Sp: 88%).

When the relationship between adherence and virological failure was stratified by the refill schedule (28 days vs. 56 days), non-adherence between visits was more strongly associated with virological failure if refills were required every two months; that is, if only one visit to the pharmacy was required in the two months prior to the viral load assessment, rather than two. (Table 4.3) This held true for all pharmacy-based measures of adherence, although refill schedule seemed to have less of an impact on the indicators for coming on time and having complete pill coverage between visits.

Self-reported adherence assessment was performed at 64% of eligible visits. Those experiencing virological failure were more likely to be asked for self-assessment than those achieving virological suppression (17.2% vs. 12.8%, p=0.01).Among those assessed, 83% (n= 15,434) indicated taking >90% of their prescribed doses in the week prior to the appointment. Rate of virological failure was similar in those reporting suboptimal adherence (19%) and those reporting taking 90% of more of the prescribed doses (17%). In the subgroup of people with data on self-reported adherence, pharmacy refill indicators showed comparable associations with virological failure. Self-reported adherence showed a slightly weaker association with virological failure compared to pharmacy-based measures (OR 1.14; 95% CI: 1.02, 1.28).

D. Discussion

The study cohort had high adherence with nearly 90% of pharmacy appointments occurring before medication ran out, consistent with observations in similar settings. [155-157] This high level of adherence corresponds with low prevalence (7%) of virological failure seen in our cohort.

Independent of the type of adherence indicator calculated from pharmacy visit data, we were able to distinguish between true adherent and non-adherent behavior patterns. Notably, the two simplest indicators, binary assessments of whether an individual showed up to an appointment by the scheduled date and whether enough pills were available between visits, were shown to be adequate predictors of virological failure in comparison with more complex indicators. While the strongest association was found between being more than 30 days without antiretroviral (ARV) drugs and less than 80% coverage of ARVs between visits, these measures are not sensitive enough to predict most virological failures.

While non-adherence was more likely to occur when an individual had to refill drugs monthly, there was a stronger association between being non-adherent and experiencing virological failure if the prescription was refilled every two months instead of monthly. It is important to note that, in this setting, individuals with known issues or barriers to adherence are asked to pick up their medication more frequently than those with a record of high compliance. Therefore, among individuals who pick up drugs monthly, there may be other barriers to being fully adherent which cannot be assessed with pharmacy data, and on time pick-ups may make them appear more adherent than they actually are. [158] Similarly, being placed on a bimonthly pickup schedule may correspond more directly to overall pill-taking behavior, making on time pick-up a stronger indicator of true adherence in these patients.

The association between self-reported adherence assessment and virological failure was weaker than that of pharmacy-based measures of adherence, in agreement with other studies which suggest self-reported adherence is more biased and less accurate than pharmacy refill-based measures. [150,159] Overall, both self-reported and pharmacy measures had modest associations with virological failure. Conclusions about the value of self-report in monitoring adherence in our data are limited by the fact that self-reported adherence data was collected in a limited subset of individuals in our cohort.

A limitation of our study is the exclusion of refill data that did not fit the definition for either the standard 28- or 56-day cycles or did not have a corresponding viral load assessment. In addition to a decrease in power from the loss of these records, these exclusions also created gaps in the pharmacy refill data which made assessing adherence cumulatively or over longer periods of time not possible. Some studies have found that adherence over a longer period of time, for example four or six months prior to viral load assessment, may be stronger predictors of virological outcomes.[152]

In our study setting, there are few other resources for obtaining ARVs outside of the clinic pharmacy. This limits misclassification of non-adherent behavior among those attending pharmacy visits late, as it is unlikely they actually procured their medication from another source. The observed behavior, however, also represents the upper limit of potential adherence, in that if a patient does not possess drugs, they cannot take them; while mere possession of drugs does not guarantee drug intake, but only enables it. Thus, these measures may be generally regarded as less sensitive but more specific. [150]

While regression results indicated associations between virological failure and each of the adherence indicators, our odds ratios were modest and none of the c-statistics were

strong in their predictive value (a c-statistic of 0.5 is the equivalent of random classification, and none of the c-statistics associated with our indicators exceeded 0.521). In our analysis, 81% of virological failures occurred in those with complete pill coverage in the prior two months. This may reflect the crudeness of our adherence measurement, in which pharmacy refill does not directly correspond to taking doses as prescribed. The predictive ability of the indicators may also be in part due to the prevention paradox, in which most cases of virological failure occur among those who appeared fully adherent by our measures. [160,161] Virological failure in those with high degrees of adherence may be attributed to other factors, including drug resistant HIV strains and dosing issues. [162]

In assessing the value of different adherence indicators, a direct assessment of true adherent behavior would be a more specific and ideal referent standard. As this type of adherence data is rarely available, however, we selected a stricter standard of virological failure. In our analysis we were able to demonstrate the value and limitations of routinely collected pharmacy refill data for assessing adherent behavior. When pharmacy refill data is the best available source for monitoring adherence, on-time attendance and complete pill coverage, two simple binary indicators, perform as well as more complicated indicators of adherence in resource limited, high volume clinical settings, where rapid screening for non-adherence is critical. [163]

E. Tables

Table 4.1.Characteristics of 8,695 HIV-positive patients at time of HAART initiation in Johannesburg, South Africa.

Demographics	All patients
Age, years	37 (9)
Female	63.3
Unemployed	49.9
Clinical	
Body mass indexkg/m^2	22.6 (5.3)
WHO stage III or IV	42.0
Prevalent tuberculosis	17.9
Laboratory	
CD4 count $cells/mm^3$	103 (74)
CD4 category$cells/mm^3$	
≤ 50	32.0
51-100	20.9
101-200	36.3
201-350	10.9
Viral load+ $log_{10}copies/ml$	5.6 (6.2)
Viral load category* $copies/ml$	
401-10,000	17.4
> 10,000	82.6

Categorical variables are expressed as % total; continuous variables are expressed as mean (standard deviation).

+ Viral load at baseline was missing in 6761 (78%) patients.

*Those with viral loads <400 copies/ml at baseline were presumed to not be treatment naïve and were excluded from the analysis

Table 4.2.Associations between pharmacy-based indicators of adherence and virological failure

	All visits (n=29,937)	Visits with self reported adherence data (n=18,082)
	OR (95% CI)	**OR (95% CI)**
Pharmacy attendance		
Picked up prescription refill on or before scheduled date	1.	1.
Picked up prescription refill late	1.27 (1.16, 1.38)	1.42 (1.27, 1.60)
Picked up refill fewer than 5 days after scheduled date	1.	1.
Picked up prescription refill more than 5 days late[+]	1.38 (1.24, 1.53)	1.32 (1.15, 1.52)
Picked up refill fewer than 30 days after scheduled date	1.	1.
Picked up more than 30 days late	2.56 (2.16, 3.03)	1.70 (1.32, 2.18)
Percentage of Days Covered		
Had 100% coverage between pharmacy visits	1.	1.
Had < 100% coverage between pharmacy visits	1.26 (1.15, 1.39)	1.17 (1.03, 1.34)
Had ≥ 90% coverage between pharmacy visits	1.	1.
Had < 90% coverage between pharmacy visits	1.71 (1.50, 1.96)	1.34 (1.08, 1.67)
Had ≥ 80% coverage between pharmacy visits	1.	1.
Had < 80% coverage between pharmacy visits	1.89 (1.62, 2.20)	1.34 (1.11, 1.61)
Combination attendance and pill coverage		
Came on time, had enough pills	1.	1.
Came late, had enough pills	1.23 (1.62, 2.20)	0.88 (0.72, 1.07)
Came late, did not have enough pills	1.39 (1.24, 1.55)	1.34 (1.16,1.56)
Self-reported adherence		
Took ≥ 90% of prescribed doses in the week before assessment		1.
Took < 90% of prescribed visit		1.14 (1.02,1.28)

Table 4.3.Associations between pharmacy-based indicators of adherence and virological failure stratified by refill schedule (28 vs. 56 days).

	Adherence based on 2 visits	Adherence based on 1 visit
	OR (95% CI)	**OR (95% CI)**
Pharmacy attendance		
Picked up prescription refill on or before scheduled date	1.	1.
Picked up prescription refill late	1.17 (1.04, 1.33)	1.22 (1.09, 1.38)
Picked up refill fewer than 5 days after scheduled date	1.	1.
Picked up prescription refill more than 5 days late[+]	1.22 (1.04, 1.43)	1.46 (1.26, 1.68)
Picked up refill fewer than 30 days after scheduled date	1.	1.
Picked up more than 30 days late	1.85 (1.46, 2.34)	3.16 (2.52, 3.97)
Percentage of Days Covered		
Had 100% coverage between pharmacy visits	1.	1.
Had < 100% coverage between pharmacy visits	1.13 (0.98, 1.30)	1.32 (1.16, 1.51)
Had ≥ 90% coverage between pharmacy visits	1.	1.
Had < 90% coverage between pharmacy visits	1.34 (1.10, 1.62)	2.00 (1.66, 2.39)
Had ≥ 80% coverage between pharmacy visits	1.	1.
Had < 80% coverage between pharmacy visits	1.49 (1.20, 1.86)	2.17 (1.77, 2.67)
Combination attendance and pill coverage		
Came on time, had enough pills	1.	1.
Came late, had enough pills	1.15 (0.94, 1.42)	1.45 (1.24, 1.68)
Came late, did not have enough pills	1.26 (1.07, 1.48)	1.57 (1.25,1.98)

Table 4.4. Test characteristics for adherence measures identifying patients with virological failure.

Indicator	Sensitivity (95% CI)	Specificity (95% CI)	PPV^{+} (95% CI)	NPV$^{\pm}$ (95% CI)
Came after scheduled refill date	0.19 (0.18,0.20)	0.84 (0.83, 0.85)	0.18 (0.17, 0.19)	0.86 (0.85, 0.86)
Came 5 or more days late	0.12 (0.11, 0.13)	0.91 (0.91, 0.92)	0.19 (0.18, 0.21)	0.85 (0.85, 0.86)
Came more than 30 days late	0.05 (0.04, 0.06)	0.98 (0.98, 0.98)	0.30 (0.27, 0.34)	0.85 (0.85, 0.86)
Less than 100% pill coverage (pill count)	0.14 (0.13, 0.15)	0.89 (0.88, 0.89)	0.18 (0.17, 0.19)	0.85 (0.84, 0.86)
Less than 90% coverage (pill count)	0.07 (0.06, 0.08)	0.96 (0.95, 0.96)	0.23 (0.20, 0.25)	0.85 (0.85, 0.86)
Less than 80% coverage (pill count)	0.06 (0.05, 0.06)	0.97 (0.97, 0.97)	0.24 (0.22, 0.27)	0.85 (0.85, 0.86)
Fewer than 90% of doses taken (self-report)	0.13 (0.12, 0.14)	0.88 (0.88, 0.89)	0.19 (0.17, 0.21)	0.83 (0.82, 0.84)

+ Positive predictive value; ± Negative predictive value; the NPV and PPV only apply to this population or one with an identical prevalence

Chapter 5

The Effect of Pregnancy on Adherence to Highly Active Antiretroviral Therapy Among HIV-Infected Women Established on Treatment

A. Introduction

Increased access to highly active antiretroviral therapy (HAART) in human immunodeficiency virus (HIV)-infected pregnant women has dramatically decreased rates of mother-to-child transmission of HIV, in addition to improving maternal survival and clinical outcomes.[164] The benefits of HAART have been particularly evident in high HIV prevalence settings, such as South Africa, where the burden of the disease is concentrated in women of reproductive age.[2,10]

The effectiveness of HAART, however, depends on a person's ability to take medications as prescribed. A high degree of adherence to antiretroviral drugs is required for viral load suppression, is associated with prolonged survival and delayed HIV disease progression, and in the case of pregnant and breastfeeding women, reduced risk of mother to child transmission. [110,111,143,146] Among pregnant and postpartum women the consequences of failing to maintain adequate adherence (and therefore maximal viral suppression) are particularly significant due to increased risk to both mother and child.

A recent systematic review and meta-analysis examined published studies of adherence to ART both during and after pregnancy, finding suboptimal adherence in both periods, with a greater reduction in adherence in the postpartum phase. [6] Nearly all existing knowledge of adherence to ART in pregnancy, however, is derived from studies of women who initiated PMTCT regimens during pregnancy, rather than women receiving lifelong treatment with HAART and experiencing pregnancy subsequent to HAART initiation. Additionally, differences in patterns of adherence among pregnant and non-pregnant women drawn from the same population have not been assessed, limiting inferences about whether or not the degree of adherence observed during pregnancy is attributable to pregnancy alone or to another characteristic of the study population. [117,122]

There is a growing need to understand the effect of pregnancy on maternal responses to antiretroviral therapy among women established on treatment. Increased access to HAART means that more women are experiencing pregnancy after initiating combined antiretroviral treatment, particularly in resource limited settings. [15,53] Furthermore, with Option B+ gaining momentum in sub-Saharan Africa, more women initiating HAART during one pregnancy will remain on treatment following delivery, and potentially through subsequent pregnancies. [52] Using longitudinal data from a large cohort of HIV-infected women treated with HAART in South Africa, we examined the risk of non-adherence during pregnancy and 6 months postpartum in women established on HAART compared to adherence during periods of non-pregnancy.

B. **Methods**

Study population

We studied women who initiated HAART at the Themba Lethu Clinic in Johannesburg, South Africa. The Themba Lethu Clinic is one of the largest ART sites in South Africa, and has initiated over 20,000 adults on HAART since 2004; more than 12,000 patients remain in care. [7] We included previously antiretroviral therapy-naïve women initiating HAART at the Themba Lethu Clinic between 1 April 2004 (when treatment first became available at the clinic), and 31 March 2011. Women ages 18 and older were included; we excluded women over age 45 (censoring at age 46) because pregnancy is rare in women over age 45. Women were followed until they died, transferred care to another facility or were lost to follow-up. Women who experienced none of these outcomes and who remained in treatment at the end of data collection were administratively censored at the end of follow-up (30 September 2011).

Women initiating HAART during a pregnancy (*prevalent pregnancy*) were excluded from this analysis. Women initiating HAART during pregnancy are typically healthier than the general population of men and women beginning HAART for their own health, which could alter patterns of response to antiretroviral therapy and retention in care as well as adherence to HAART. [123,125]

Definitions

The primary factors of interest were new pregnancy after HAART initiation (*incident pregnancy*) and the subsequent postpartum period. The start and end dates of incident pregnancies were extracted from electronic patient medical records. These dates are primarily

noted in the record once a clinician recognizes the pregnancy or self-reported by the mother. Regardless of pregnancy outcome (which is not recorded in the present database), we defined the six months following the last recorded month of pregnancy as the postpartum period.

For each month of follow-up, a woman's exposure status was defined as 1) not-pregnant, 2) pregnant, or 3) postpartum. Person-time contributed after the end of the fixed postpartum period was excluded. For women with multiple pregnancies during follow-up, analysis was restricted to the first incident pregnancy after HAART initiation. For our primary analysis, women who experienced incident pregnancy but were missing either a start or end date for the pregnancy were excluded once they became pregnant. We performed a sensitivity analysis using multiple imputation for missing pregnancy dates (see below).

The outcome of interest was non-adherence. At each prescription refill appointment, adherence was assessed as the proportion of days with medication available in the 60 days prior to that pharmacy visit, creating repeated assessments within individual patients. Scheduled and actual dates of pharmacy attendance were compared, and a binary indicator of adherence, 100% pill coverage between pharmacy visits vs. less than 100% coverage, was calculated. This indicator was selected from among several candidate measures due to its ability to predict virological failure and relative ease of calculation for use in routine adherence assessment.[see CH 4]

Statistical Analyses

Baseline characteristics for all eligible women initiating HAART were reported with basic descriptive statistics. Selection of confounding variables for inclusion in multivariate analyses was based on substantive knowledge from existing studies and included the

following variables at baseline: age, employment status, WHO stage, treatment for tuberculosis, inclusion of efavirenz (EFV) or stavudine (d4T) in the initial HAART regimen, and initial CD4, hemoglobin and body mass index measurements. Baseline viral load was not included in the models as this information is not routinely collected at the initial clinic visit in this setting.

In this analysis we were concerned about the possibility of time-varying confounders affected by prior exposure. [128] Potential confounders of concern included time-updated measurements for CD4, viral load, hemoglobin, body mass index, EFV and d4T in the most recent HAART regimen. Time-varying confounding affected by prior exposure cannot be dealt with using traditional regression methods, as these may produce biased effect estimates; methods such as marginal structural models are needed to obtain unbiased estimates. [129, 130] Inverse probability weights accounting for multiple exposure transitions were calculated to control for bias due to confounding. [131,132]

Common practice for construction of inverse probability weights model a single transition between two exposure states; typically, a single transition from unexposed to exposed (for example, to HAART). [130] In our analyses, we wished to estimate the effect of pregnancy and the postpartum period (six months following pregnancy) on adherence to HAART. A novel weighting structure incorporating the cumulative probability of exposure transitions between three states was required (Figure 1): not-pregnant to pregnant (a transition for which all women were eligible, but which was experienced by only some women) and pregnant to postpartum (for which only pregnant women were eligible, and all which all pregnant women experienced).

We used these weights to fit marginal structural log-binomial regression models to estimate relative risks of non-adherence for assessments taken during periods of pregnancy, postpartum, and non-pregnancy. Generalized estimating equations were used to account for repeated adherence assessments within individuals.

In all models, restricted cubic splines were used to flexibly and efficiently control for the continuous and time-updated variables of age, CD4, viral load, and time-on-study. [165] Weights for censoring due to loss to follow-up and death were fit but not included in final models as they had minimal effect on model estimates. In order to reduce variance, all weights were truncated at the 0.1^{st} and 99.9^{th} percentiles. After truncation, mean of weights in primary analysis was 1.00.

Sensitivity Analyses

Several sensitivity analyses were performed to test analytic assumptions made in our primary analysis. Because pregnancy dates are based on self-report or clinician observation, we were concerned about the accuracy of the recorded pregnancy start and end dates, and potential exposure misclassification. We performed several adjustments to pregnancy dates including 1) shifting the pregnancy start date earlier by the equivalent of approximately one trimester, 3 months, to account for the possibility that many pregnancies may be recorded in the electronic medical record later in pregnancy (e.g., in second trimester), and 2) fixing pregnancy start dates 9 months prior to reported end dates. We also used 3) a fixed length of pregnancy nine months from the reported start date for individuals with missing end dates for pregnancy. A fixed duration of pregnancy allowed us to examine the effect of reporting error for end of pregnancy dates, as well as missing pregnancy end dates, which were common

(n=365) among incident pregnancies. We also 4) addressed missing pregnancy end dates using multiple imputation to fill in end dates.

The initial period after HAART initiation is associated with higher frequencies of drug-related adverse events and regimen changes, as well as greater risk of suboptimal adherence. [166] In order to account for this more unstable period of treatment we 5) performed a sensitivity analysis restricted to person-time contributed only after the first six months of treatment.

Clinical definitions of postpartum range from six weeks to a year and studies have examined postpartum periods as long as two years. Because we are accounting for both biological and lifestyle changes associated with giving birth, we were interested in a postpartum period of several months. In addition to a 6 month definition used in our primary analysis, we also 6) examined a 1 year postpartum period. For our final sensitivity analysis we 7) restricted inclusion in the dataset to those women experiencing pregnancy during follow-up, examining the effect of pregnancy and postpartum adherence only among those women experiencing all three exposures.

C. **Results**

A total of 7,510 previously treatment-naïve women initiating HAART at the clinic between April 1, 2004 and March 31, 2011 were eligible for inclusion in this analysis. Incident pregnancies on HAART were experienced by 896 women (Figure 5.2). Median follow-up time for all women was 27 months (IQR 11, 52), while median time from HAART initiation to first incident pregnancy was 19 months (IQR 9,33). The median recorded

duration of pregnancy among women with both reported start and end dates was 8 months (IQR 5, 9).

The median age at HAART initiation was 34 years (IQR: 30, 39 years). At baseline, clinical and laboratory indicators were similar regardless of age. (Table 5.1) Younger women (18-29 years old) were most likely to be unemployed (65%), compared to women 30-39 years old (54%) and 40-45 years old (47%). Women who were younger at the time of HAART initiation were also more likely to be underweight compared to women who were older (18-29 years old: 23% vs. 40-45 years old: 12%).

Overall, women had complete pill coverage between pharmacy visits two months prior 89% of the time. Adherence based on our binary indicator was nearly identical in both the pre-pregnant or never pregnant visits and during pregnancy, with 89.2% and 89.5% of pharmacy pickups during these periods, respectively, occurring before an individual ran out of pills. During the postpartum period, percentage of on-time pickup was slightly lower, with 84.8% of visits occurring before pills ran out.

Our primary analysis compared adherence during person-time contributed during pregnancy and the postpartum period with adherence in the non-pregnant person-time. In primary analysis, both crude and weighted models (Table 5.2) show no change in risk between non-pregnant and pregnant women (weighted incidence risk ratio [RR]: 0.95, 95% confidence interval [CI]: 0.78, 1.17), but an increased risk of non-adherence during the six month postpartum period following the end of a pregnancy (weighted RR: 1.46, 95% CI: 1.17, 1.82).

Results of our sensitivity analyses (Table 5.2) suggest that despite definitions used to classify pregnancy and postpartum exposed follow-up time, the estimated RRs for non-

adherence are relatively durable. Extension of the pregnancy period 3 months prior to the reported start date (postpartum vs. not-pregnant, RR=1.47, 95% CI: 1.20, 1.80), as well as adjustments to create a fixed 9 month pregnancy from reported end date (postpartum vs. not-pregnant, RR =1.39: 95% CI 1.17, 1.65), showed a qualitatively similar relationship between pregnancy exposure and adherence as the crude and primary weighted models.

A similar but less marked (as well as less precise) association was observed when the analysis was restricted to only women experiencing pregnancy, with the pre-pregnancy period as the referent exposure (pregnancy RR: 1.06, 95% CI: 0.63, 1.77; postpartum RR: 1.32, 95% CI: 0.88, 1.98). The exclusion of early adherence assessments from restricting to follow-up six months or later after HAART initiation also had little effect on the point estimates.

D. Discussion

In this analysis of HIV-infected women on HAART in South Africa, we found that the postpartum period following an incident pregnancy is associated with an increased risk of non-adherence, while the period of pregnancy itself was not associated with increased risks of non-adherence to HAART. The postpartum period appears to be time of greatest risk of non-adherence among those women experiencing pregnancy.

The finding of increased risk of non-adherence in the postpartum period is in agreement with previous adherence assessments after pregnancy. [6,167] Prior reports of postpartum assessment, however, have either compared the postpartum period only to pregnancy, or simply reported the degree of adherence without a comparative exposure period.

Decreased adherence in the postpartum period may be attributable to differing motivational factors. A woman may be more inclined to maintain a high level of adherence when she is pregnant and the ultimate goal is protecting her child from acquiring HIV. If her own health is not as high a priority, and in the absence of the desire to keep the child safe, particularly if a woman is not breastfeeding, adherence may fall off after the baby is born. [120] These specific motivations may be more of an issue among women initiating HAART during pregnancy, where treatment is primarily thought of as a means of PMTCT. In contrast, we analyzed women who were initiated on treatment for their own health prior to becoming pregnant; such women may be more invested in their own health regardless of motivation to protect their child from HIV. Women in our cohort demonstrated similar adherence behavior in both pregnant and non-pregnant periods during follow-up.

The challenges of recovering from giving birth and having a new baby in the home may also lead to an increased risk of non-adherence observed postpartum. [120, 168] Caring for an infant may create barriers to traveling and attending pharmacy appointments on time (which served as the indicator for adherent behavior in our analysis). The tendency among pregnant women to travel and stay with family around the time of delivery may also interrupt timely attendance at the clinic for prescription refills, in addition to increasing loss to follow-up. [169] Depression is common among HIV-infected individuals and is associated with suboptimal adherence to antiretroviral therapy, including postpartum women on long-term treatment, although we do not have data to evaluate this potential contributing factor in our cohort. [170-172]

Suboptimal compliance in pregnant women has been observed in other settings. [6,173] Decreased adherence during pregnancy has been attributed to pregnancy symptoms,

particularly those that interact with side effects of ARVs like nausea and fatigue, and maternal fears about taking potent drugs during pregnancy. [174] Sustained adherence during pregnancy observed in our study may be because women were on acceptable HAART prior to becoming pregnant, where studies observing decreased adherence associated with pregnancy primarily looked at women initiating HAART during pregnancy. Incidence of drug toxicities and adverse events is highest in the period immediately following treatment initiation, so women experiencing pregnancy symptoms as well as the challenge of a new treatment regimen may be at higher risk for poor adherence. The high degree of adherence maintained during pregnancy may also be attributed to antenatal care and increased exposure to health services during pregnancy, with women potentially receiving additional counseling and encouragement on the importance of taking their medication to prevent vertical transmission of HIV.

Reporting errors for pregnancy dates, and therefore misclassifying exposure, were the primary limitation of this study. If a clinician recognizing and recording a pregnancy is not associated with patient characteristics, including pharmacy attendance, errors in pregnancy dates would results in non-differential misclassification. Non-differential misclassification generally results in effect estimates being biased toward the null, meaning that any effect of pregnancy or postpartum on adherence may be attenuated in the observed effect. It is also plausible however, that women that present at the clinic later in pregnancy may also attend pharmacy refill appointments late.

Sensitivity analyses to account for exposure misclassification had little effect on the relationship between adherence and period of pregnancy. The overall durability of our estimates suggests that exposure misclassification has less of an impact than suspected.

Furthermore, results of our multiple imputation sensitivity analysis for missing pregnancy end dates indicated the same general relationship between pregnancy and adherence, with increased risk of non-adherence in the postpartum period but not during pregnancy.

When the effect of pregnancy and postpartum was examined only among women that eventually experienced pregnancy at some point during follow-up, the postpartum was not as strongly associated with non-adherence as when comparing the postpartum with all non-pregnant person time. One possible explanation of this differing result is that our primary analysis may be overestimating the true effect of pregnancy and postpartum among women that become pregnant, and that there may be differences in adherence among women who eventually become pregnant during follow-up and those that do not. When comparing women who experienced pregnancy during follow-up and those that did not by both baseline characteristics and clinical attributes at the median time of first incident pregnancy (14 months on treatment), the two groups of women were comparable. Differences could be attributed to unmeasured characteristics, but baseline comparisons between the two groups of women based on future exposure status should be limited.

In comparative assessments, pharmacy-based measures have generally produced less biased estimates compared to other indirect adherence measures, including self-reported adherence. [150] The use of pharmacy refill data, however, is not without limitations. The observed behavior of refilling prescriptions on time represents the upper limit of potential adherence, in that if a patient does not possess drugs, they cannot take them. [152] Furthermore, mere possession of drugs does not guarantee adherent behavior, but only enables it. Thus, these measures may be generally regarded as less sensitive but more specific.

Despite restrictions to our analysis, our study has the benefit of a large cohort and high quality data. Our findings are likely generalizable to similar resource limited settings where the incidence of women becoming pregnant after initiating HAART for their own health is increasingly common, yet still largely unstudied.

We found that among women established on HAART prior to becoming pregnant, adherence overall was high and equivalent in pregnant and non-pregnant women. The postpartum period of the six months following the end of pregnancy was associated with an increased risk of non-adherence, although most refills still occurred on time. Our findings suggest that in contrast to women starting ART while pregnant, women who demonstrate adherent behavior prior to pregnancy sustain a high degree of adherence during pregnancy. This finding emphasizes that early support and interventions to establish optimal adherence are important for long-term outcomes, and may protect against potential barriers to adherence that arise later in treatment, such as pregnancy. The drop in on-time prescription refills during the postpartum period may indicate an additional critical window for increased vigilance or interventions in clinical settings with large patient populations and limited resources.

E. Tables and Figures

Table 5.1. Characteristics of treatment-naïve women at time of HAART initiation at the Themba Lethu Clinic, by age at baseline

Characteristic	Age at initiation		
	18-30 years old *n=2190*	30-40 years old *n=3986*	40-45 years old *N=1334*
Clinical/demographic			
Unemployed	1392 (65.1)	2102 (53.8)	607 (46.5)
Body mass index *kg/m²*			
< 18.5 *kg/m²*	478 (23.5)	596 (15.9)	153 (12.2)
18.5 – 24.9	1142 (56.0)	1964 (52.3)	646 (51.5)
25.0 – 29.9	304 (14.9)	789 (21.0)	288 (23.0)
≥ 30.0	113 (5.6)	408 (10.9)	167 (13.3)
WHO stage III or IV	837 (44.0)	1503 (42.8)	470 (41.1)
Tuberculosis (treated)	387 (17.7)	670 (16.8)	195 (14.6)
Laboratory			
CD4 count *cells/mm³*	107 (79)	103 (75)	108 (76)
CD4 category *cells/mm³*			
≤ 50	665 (32.3)	1221 (32.5)	369 (29.6)
51-100	367 (17.8)	804 (21.4)	250 (20.1)
101-200	747 (36.3)	1315 (35.0)	464 (37.2)
201-350	278 (13.5)	420 (11.2)	164 (13.2)
Viral load category[+]			
401-10,000*copies/ml*	86 (19.8)	138 (18.1)	54 (22.4)
> 10,000	349 (80.2)	626 (81.9)	187 (77.6)
Hemoglobin category[*]			
Normal	909 (41.5)	1635 (41.0)	592 (44.4)
Moderately anemic	1059 (48.4)	1953 (49.0)	611 (45.8)
Severely anemic	222 (10.1)	398 (9.9)	131 (9.8)

Categorical variables are expressed as number (% total); continuous variables are expressed as mean (standard deviation)

[+]:Viral load at baseline was missing in 6761 (78%) patients.*Those with viral loads <400 copies/ml at baseline were presumed to not be treatment naïve and were excluded from the analysis

*: adjusted for altitude, hemoglobin categories for non-pregnant women: normal: > 11.35 g/dL, moderately anemic: 7.35-11.35 g/dL, severely anemic: < 7.35 g/dL

Table 5.2. Association of incident pregnancy with adherence from main and sensitivity analyses.

Model	RR	95% CI
Crude		
Pregnancy	0.94	0.80, 1.10
Postpartum	1.40	1.19, 1.65
Weighted		
Pregnancy	0.95	0.78, 1.17
Postpartum	1.46	1.17, 1.82
Sensitivity Analyses (weighted models)		
Only among women experiencing pregnancy ±		
Pregnancy	1.06	0.63, 1.77
Postpartum	1.32	0.88, 1.98
Established on HAART+ *		
Pregnancy	0.96	0.78,1.19
Postpartum	1.49	1.20, 1.85
Start of pregnancy 9 months from end date		
Pregnancy	0.97	0.86, 1.12
Postpartum	1.39	1.17, 1.65
Fixed pregnancy length (9 months)		
Pregnancy	1.00	0.87, 1.17
Postpartum	1.23	1.01, 1.49
Start of pregnancy 3 months earlier		
Pregnancy	0.90	0.75,1.09
Postpartum	1.47	1.20, 1.80
Postpartum period of 12 months		
Pregnancy	1.08	0.80, 1.48
Postpartum	1.49	1.18, 2.09
Multiple imputation analysis		
Pregnancy	0.97	0.81, 1.13
Postpartum	1.34	1.11, 1.57

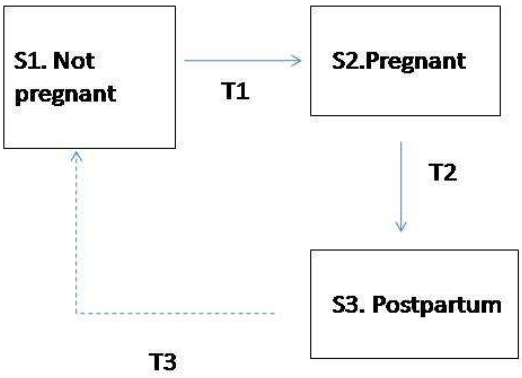
RR: risk ratio; 95% CI: 95% confidence interval; Models use "Pre-pregnancy/never pregnant" as the reference group.

±: referent group is pre-pregnancy person-time among those that became pregnant during follow-up

+: HAART: highly active antiretroviral therapy

*: analysis among alive and in care at 6 months post-HAART initiation

Figure 5.1. Transitions between states of pregnancy exposure



S1. **Not pregnant** All women initiating HAART start out not pregnant. The majority remain in this exposure category through follow-up

S2. **Pregnant** Some women become pregnant during follow-up after treatment initiation., transitioning from not-pregnant to pregnant (T1).

S3. **Postpartum** All women experiencing incident pregnancy transition to the postpartum period at the end of pregnancy (T2)

S1. **Not pregnant** After a fixed period of 6 months women transitioned back to not-pregnant (T3). In our analysis , person-time after the postpartum period was censored and this transition was not modeled.

Chapter 6

Reduced Creatinine Clearance Related to Tenofovir Use Among Non-Pregnant, Pregnant, and Postpartum Women

A. **Introduction**

Women of reproductive age have been disproportionately affected by HIV in South Africa, with an overall prevalence as much as four times that of men the same age, and up to 30% of pregnant women infected.[2] Highly active antiretroviral therapy [HAART] has been shown to be protective against maternal morbidity and mortality, and effectively reduces the risk of mother-to-child transmission of HIV. [4,175]

Tenofovir disoproxil fumarate (TDF) is currently recommended as part of the nucleoside reverse transcriptase inhibitor (NRTI) backbone in first-line HAART regimens for non-pregnant adults, due to its efficacy and low toxicity.[176,177] It is also recommended that women effectively treated with TDF prior to becoming pregnant remain on treatment with TDF throughout their pregnancies. [51] In general, TDF remains a category B drug for administration to pregnant women, due to concerns about potential effects on renal function, as well as limited current data on use of the compound during pregnancy, particularly among ART-experienced women. [178]

South African national guidelines recommend routine assessment of renal function for all individuals taking TDF. Monitoring of adverse events related to the drug, however, is

complicated by the challenges of clinically detecting renal impairment in resource-limited settings.

The purpose of this investigation was to evaluate the implementation of clinical monitoring for renal impairment, as well as the incidence of nephrotoxicity among those assessed, in non-pregnant, pregnant, and postpartum women on TDF from a clinical cohort in Johannesburg South Africa.

B. Methods

We analyzed data from the Themba Lethu Clinic (TLC) in Johannesburg, the largest public-sector antiretroviral treatment site in South Africa. [7] Women initiating HAART at TLC between April 1, 2004 and March 30, 2011, and who were previously treatment-naive and between the ages of 18 and 45 were included in this analysis. Women who were pregnant at the time of HAART initiation were excluded due to differences in immune function and overall health at baseline compared to women starting treatment solely for protection of their own health.

All data were extracted from electronic medical records collected and maintained for routine clinical use. Pregnancy exposure was assessed using self-reported or clinician indicated start and end months for each pregnancy, with month assigned from time of HAART initiation. The postpartum period was defined as the six months immediately following the last reported month of pregnancy. Women experiencing a first incident pregnancy on HAART were censored after the postpartum period. Otherwise, women were followed until they died, transferred care, were lost to follow-up, or until the end of data collection (September 30, 2011). The first six months on HAART were excluded from the

analysis for all women given that the early period of treatment is not comparable to long-term treatment in terms of both drug-induced adverse events and incidence or pregnancy. [105]

In order to assess renal impairment, we estimated creatinine clearance (CrCl) using serum creatinine and the Cockroft-Gault equation [179]:

$$\text{Creatinine clearance} = \frac{[(140-\text{age})*(\text{weight (kg)})*(0.85 \text{ for females})]}{(72*\text{Creatinine mg/dL})}$$

The results were categorized according to the US National Foundation's Kidney Disease Outcome Quality Initiative: Normal (≥ 90 ml/min), mild (60-89 ml/min), moderate (30-59 ml/min) and severe (< 30 ml/min). [180] Creatinine clearance increases 30-50% during pregnancy.[181] We performed sensitivity analyses, increasing thresholds for degrees of impairment by both 30% and 50% for assessments occurring in pregnant women. For these analyses, moderately impaired renal function was defined as <78 ml/min or <90 ml/min in pregnant women.

Baseline characteristics were stratified by inclusion of TDF in the initial HAART regimen, as well as period of HAART initiation. Women were classified as starting treatment before or after implementation of new treatment guidelines beginning in April of 2010, which included TDF as a recommended first-line NRTI. Baseline characteristics were assessed using standard descriptive statistics.

We reported crude rates of CrCl assessments by pregnancy exposure and inclusion of TDF in the most current HAART regimen. Renal function was assessed as the proportion of

CrCl assessments indicating normal function, or mild, moderate or severe renal impairment. CrCl results were reported by pregnancy exposure and current TDF exposure.

C. **Results**

A total of 7,534 were eligible for inclusion in this analysis. Overall, the mean age at HAART initiation was 34 years old, and nearly 50% (N=3,686) of women had a baseline CD4 cell count less than 100 cells/mm^3 (median of 95 cells/mm^3 (interquartile range: 36-165)). Prior to the implementation of updated treatment guidelines in April of 2010, 82% (N=5,134) of women starting HAART were placed on a regimen of stavudine, lamivudine and efavirenz, and 9% (N=540) on stavudine and lamivudine with nevirapine. Only 2% (N=141) of patients were on initial regimens containing tenofovir. With the shift from stavudine to tenofovir-based regimens, 75% (N=989) of women starting HAART in April of 2010 or later were prescribed tenofovir, lamivudine, and efavirenz, with an additional 6% (N=75) started on the same regimen with nevirapine in place of efavirenz.

After initiating HAART, 918 women experienced at least one pregnancy on treatment. More than 70% of women had at least one laboratory result for creatinine clearance on record. Over half (N=11,256) of these results occurred after April of 2010, when guidelines changed to include CrCl assessment as part of routine follow-up among those on TDF. Renal function was also assessed in individuals not on TDF on a non-routine basis. Among those on HAART regimens not containing TDF, creatinine clearance was not assessed more frequently among pregnant (Incidence Rate Ratio (IRR): 1.09, 95% Confidence Interval (CI): 0.94, 1.26) and postpartum women (IRR: 1.03, 95% CI: 0.86, 1.28) compared to non-pregnant women. (Table 6.1) For women taking TDF, rates of CrCl testing

did not differ between non-pregnant and pregnant women (IRR: 0.92, 95% CI: 0.77, 1.13), but postpartum women did appear to be tested less frequently (IRR: 0.66, 95% CI: 0.52, 0.80).

Overall, moderate to severe renal dysfunction occurred infrequently, regardless of pregnancy exposure, with 2.8% (95% CI: 2.5%, 3.28%) of assessments in non-pregnant women, 1.7% (95% CI: 0.2%, 3.14%) in pregnant women, and 2.3% (95% CI: 0.3%, 4.3%) in postpartum women indicating creatinine clearance of less than 60 mL/min. Among those assessed for CrCl, a higher proportion of non-pregnant women not on TDF were found to have moderate (4.0% vs. 0.4%) and severe (1.7% vs. 0.3%) renal impairment compared to non-pregnant women on TDF-based HAART regimens. (Table 6.1) Moderate to severe renal impairment was indicated in very few CrCl assessments for pregnant and postpartum women, regardless of current TDF exposure.

Our sensitivity analyses, which adjusted the thresholds for moderate and severe renal impairment by 30% and 50% to account for the range of potential changes in filtrations rates associated with pregnancy, identified 12 to 32 CrCl results indicating moderate renal impairment, respectively. Increasing the cut-off for severe impairment by 50%, identified one additional case, while the 30% increase identified no additional cases of severe renal impairment.

D. Discussion

Among 7,534 ART naïve women initiating HAART and followed-up over more than 206,000 person-months on treatment, we found that moderate and severe reductions in creatinine clearance were rare among both those on TDF-based regimens and not. Among

those on TDF, the low occurrence of patients with moderate or severe renal impairment is comparable to those seen in similar settings. [91, 106, 182]

The dramatic increase in TDF use in first line regimens, as well as the increased frequency of CrCl assessment after April of 2010 indicates efficient uptake of revised treatment guidelines in this clinical setting. While women taking TDF were more likely to be assessed for renal impairment, pregnancy itself did not seem to be an indicator for more frequent testing. This held true for women both on TDF-based regimens and non-TDF based regimens. Given that pregnancy itself alters filtration rates of creatinine [183], increased vigilance of renal function among women receiving a drug known to induce nephrotoxicity may be indicated. It is a limitation of our study that pregnant women attending TLC do not receive any prenatal services from the facility. It may be that additional monitoring for nephrotoxicity is occurring for some women as a part of their follow-up outside of TLC.

Changes in glomerular filtration rates associated with pregnancy do not occur uniformly across the gestational period, with fluctuations throughout trimesters that potentially carry over into the early part of the postpartum period. [183] The self-reported nature of the pregnancy start and end dates used to assign exposure status in this analysis introduce concerns about exposure misclassification, and limit the ability to account for altered kidney function throughout individual pregnancies, rather than applying broad sensitivity analyses. Nonetheless, results of our sensitivity analyses accounting for higher rates of filtration among pregnant women identified several additional cases of moderate renal impairment. It is unclear from the available data whether the clinicians caring for pregnant women also adjusted the CrCl lab results to take pregnancy into account in clinical decision making regarding regimen selection.

While TDF is associated with nephrotoxicity, we observed more frequent CrCl results indicating moderate to severe renal impairment among non-pregnant women on non-TDF-based regimens, compared to non-pregnant women on the drug. This may be due to differences in indication for assessment between the two groups of women. Routine assessment of CrCl is suggested every six months by the South African national treatment guidelines for all adults on TDF [51]. Most individuals tested under this policy are likely stable on treatment with no signs of reduced renal function. CrCl assessment among those not on TDF, however, is performed when there are clinical indicators suggesting potential issues with kidney function. Moderate and severe CrCl results among pregnant and non-pregnant women were rare, limiting comparisons by drug regimens.

The primary contribution of our analysis is the inclusion of women on existing TDF-based regimens at the time of pregnancy. Studies indicating increased risk of renal adverse events have primarily focused on ART-naïve individuals initiating tenofovir-based regimens. The few studies that have looked at long-term use of tenofovir use in pregnancy have also focused on women starting ART during pregnancy.[106,182] In general, the initial phase of treatment is associated with increased drug-related adverse events, issues with adherence and more frequent drug substitutions as people are first encountering complex regimens with common side effects and potentially high pill burdens.[184] The exclusion of this more variable time on treatment may at least partially explain the low incidence of renal insufficiency experienced by the women in our cohort, regardless of pregnancy exposure.

With more women of reproductive age accessing treatment with the intention of lifelong treatment, incident pregnancies among HIV-positive women will continue to increase. Assessing maternal safety of ARVs during pregnancy and the postpartum will be

critical for managing care in these women. Our study suggests that tenofovir is not associated with increased risk of renal impairment, regardless of pregnancy exposure. Appropriate monitoring of CrCl during the course of treatment is critical, however, particularly in pregnancy and the postpartum, as the impact of naturally occurring changes in kidney function on the risk of TDF-induced nephrotoxicity is still unclear.

E. Tables

Table 6.1 Rates of creatinine clearance (CrCl) assessment, and proportion of tests indicating impaired renal function by pregnancy exposure status and current HAART regimen

	Pregnancy Exposure					
	Not pregnant (N=13,845[+])		Pregnant (N=297[+])		Postpartum (N=218[+])	
	N	%	N	%	N	%
Non-TDF-based regimen						
Rate Ratio of CrCl assessment[α]	1.		1.09 (0.94, 1.26)		1.03 (0.86, 1.23)	
Total assessments	7,003	50.6[*]	177	59.6[*]	122	56.0[*]
Normal (>90ml/min)	5,084	72.6	165	94.3	103	84.4
Mild (60-89 ml/min)	1,330	19.0	8	4.6	17	13.9
Moderate (30-59ml/min)	279	4.0	2	1.1	2	1.6
Severe (<30 ml/min)	117	1.7	2	1.1	0	0.0
TDF-based regimens						
Rate Ratio of CrCl assessment[α]	1.		0.92 (0.77, 1.13)		0.66 (0.52, 0.80)	
Total assessments	6,842	49.4[*]	120	41.1[*]	96	44.0[*]
Normal (>90ml/min)	5,677	83.0	110	91.7	80	83.3
Mild (60-89 ml/min)	1,109	16.2	9	7.5	13	13.5
Moderate (30-59ml/min)	33	0.4	1	0.8	2	2.1
Severe (<30 ml/min)	23	0.3	0	0.0	1	1.0

+: number of total serum creatinine clearance assessments performed in each exposure category
α: expressed as the number of laboratory assessments performed per person-month of follow-up in each exposure category, with non-pregnant assessments serving as the referent
* : expressed as proportion of total assessments per pregnancy exposure category

Chapter 7

Discussion

A. **Summary of Findings**

The results of this dissertation suggest that for HIV-infected women experiencing incident pregnancy after initiation of long-term HAART, pregnancy and the postpartum create few additional challenges to safe and optimal treatment. While continuation of HAART during and after pregnancy appeared well-tolerated overall, we were able to identify potential aspects of treatment for increased vigilance or additional study.

In Specific Aim 1 we constructed adherence indicators derived from pharmacy refill data and based on either timing of pharmacy attendance or pill possession. The eight indicators, in addition to a self-reported adherence assessment, were compared based on ability to predict subsequent virological failure in the cohort of adult men and women on HAART at the Themba Lethu Clinic. Regardless of indicator, adherence was high overall, with 84% or more of assessments classified as “adherent”, dependent on the definition. High levels of adherence corresponded with the low observed prevalence of virological failure. While the indicators each demonstrated a comparable and modest degree of association with virological response, sensitivity for each was very low, potentially indicating that pharmacy data overestimated true levels of adherence in our cohort. The binary indicator for 100%

pill coverage (yes or no) between pharmacy visits performed comparably to more complex measures calculated from the same data, and was selected as the adherence indicator to be applied in Specific Aim 2.

For Specific Aim 2 we examined the effect of incident pregnancy and the subsequent postpartum period on risk of non-adherence. The period of pregnancy was identified using start and end dates indicated in the medical record, and the postpartum period was fixed as the six months following the end of pregnancy. In order to control for confounding by time-varying covariates, we used marginal structural models fit with inverse probability of treatment weights adapted for the unique exposures transitions between not pregnant, pregnant, and postpartum. Compared to refills placed prior to pregnancy or by women who never experienced pregnancy during follow-up, being pregnant was not associated with any changes in risk of non-adherence. Women in the postpartum period, however, were more likely to attend their pharmacy appointments after running out of medication. Despite concerns about exposure misclassification and missing data, results of sensitivity analyses indicated that the relationship between non-adherence and pregnancy exposure was durable.

In Specific Aim 3, we used the same pregnancy exposure classifications created in Specific Aim 2 to examine the effect of pregnancy and the postpartum on the occurrence of tenofovir-associated nephrotoxicity. We observed that both the use of tenofovir and the frequency of creatinine clearance assessments increased over time, corresponding to changes in treatment guidelines implemented in 2010. Overall, moderate and severe renal impairment were relatively rare adverse events, regardless of ART regimen. Women on tenofovir were more likely to receive a CrCl assessment, but non-pregnant women on non-tenofovir-based HAART regimens were more likely to have a lab result indicating renal impairment than

non-pregnant women taking tenfovir. This is perhaps due to women on tenofovir being screened for routine purposes, as opposed to clinically indicated assessments performed more frequently for women not on tenofovir. For CrCl assessments performed in pregnant and postpartum women, limitations related to assigning pregnancy exposure made it challenging to assess the impact of altered kidney function attributed to pregnancy on tenofovir-induced nephrotoxicity.

B. **Public Health Significance**

HIV prevalence is extremely high among women of reproductive age in South Africa. As access to ART continues to expand, women between the ages of 18 and 49 will be one of the largest groups initiating and continuing on lifelong treatment for HIV. Pregnancy after the initiation of HAART is also common in this setting. In our cohort specifically, more than half (52%) of women between the ages of 18 and 25 who start treatment at the Themba Lethu Clinic will become pregnant within five years of initiating HAART (among those alive and in care at 5 years). [44] Understanding the effects of pregnancy on maternal responses to HAART, therefore, is critical for optimizing long-term HIV care for women, particularly in resource limited settings such as South Africa. Despite the growing incidence of pregnancy among women established on HAART, most of what is currently known about ART during pregnancy may not apply to these women.

The results of our analyses are particular insightful in context of changing recommendations for initiating lifelong ART, both globally and within South Africa. The South African National Department of Health has recently (April 2013) updated PMTCT guidelines to recommend initiating all pregnant women on triple regimen ART for the

duration of pregnancy and breastfeeding, regardless of CD4 at baseline. Women with CD4 counts below 350 cells/mm^3 prior to initiating treatment will be encouraged to remain on HAART after breastfeeding is discontinued. This treatment strategy is known as Option B. [13] Additionally there is growing momentum for the implementation of Option B+ in resource limited settings, which would initiate all HIV-infected pregnant women on lifelong HAART regardless of CD4 count. [184] While the feasibility of implementing these strategies remains questionable, and there are additional concerns about preferential access to treatment for women and increased drug resistance, whether or not these policies should be implemented is beyond the scope of this research. With any increase in the number of young women starting ART while pregnant and remaining on treatment for the remainder of their reproductive years, there will be a corresponding rise in incident pregnancies among women established on HAART.

C. **Strengths and Limitations**

To our knowledge, this is one of the first longitudinal studies to examine the effect of pregnancy on adherence to ART, as well the incidence of drug toxicities, in women not pregnant at the time of HAART initiation. Most of what is currently understood about maternal responses to treatment with antiretroviral drugs during pregnancy comes from analyses of women taking antiretrovirals explicitly for the purposes of PMTCT, or initiating lifelong maternal treatment during pregnancy. Women who initiate HAART during pregnancy are systematically different from women who start HAART due to advanced HIV disease and subsequently become pregnant. Women who initiate treatment during pregnancy tend to be younger and healthier (higher CD4 at initiation, less likely to be underweight,

etc.), and beginning HAART at an earlier stage of their disease progression, which may alter long-term clinical and virological responses to treatment. [185]

Given that adherence is a behavior that can be modified by psychological and social factors, it also important to consider that women experiencing prevalent and incident pregnancies may have different motivations for adhering to treatment. Although women starting lifelong ART during pregnancy are in need of treatment for their own health, the motivation to initiate promptly and take pills as prescribed may still be primarily stem from a desire to prevent mother-to-child transmission. In our cohort, the relatively high degree of adherence observed before, during, and even after pregnancy, compared to what has been seen in other studies, may indicate that establishing adherent behavior early in treatment makes women less vulnerable to challenges, including being pregnant and caring for an infant, that may interrupt taking medication as prescribed.

For Aims 2 and 3 we restricted our analyses of adherence (in a sensitivity analysis) and tenofovir-induced renal impairment to follow-up time after the first six months on treatment. The early phases of treatment are associated with more frequent side effects and adverse events, as well as drug substitutions. [105] In part related to more frequent adverse events, adherence is also more volatile in the first weeks and months of treatment with HAART. One of the problems with looking at drug-related adverse events or adherence in women initiating HAART during pregnancy is that it may not be possible to distinguish the effects of pregnancy and postpartum from the effects of starting new treatment.

In addition to the clear distinction between prevalent and incident pregnancy, our study featured several design and analytical advantages for assessing our specific aims. We analyzed high quality, prospectively collected data from the Themba Lethu Clinic. The

overall patient population and number of incident pregnancies were much larger than most prior studies of pregnant women on HAART, particularly in sub-Saharan Africa. We had the additional advantages of extensive patient-level data collected for routine medical purposes, and subject to both ongoing validation and cleaning. Longitudinal data collection enabled women experiencing incident pregnancy to contribute follow-up time to non-pregnant, pregnant, and postpartum exposure periods. The ability to compare both pregnant and non-pregnant women within the same cohort is another distinct advantage of this study.

The time-varying nature of our data required application of innovative epidemiologic methods, including marginal structural models with a weighting structure appropriate for the unique exposures of pregnancy and postpartum.

Despite the overall benefits of using extensive and rich data from the Themba Lethu Clinic, there are still important limitations to acknowledge. Exposure misclassification due to errors in reporting pregnancy dates is one of the greatest potential sources of bias in our study. As previously described, start and end dates for pregnancies occurring after treatment initiation are based primarily of patient self-report. Pregnancy is not routinely assessed as part of follow-up at TLC. Pregnancy start dates should be entered as the general time of conception, with end dates corresponding to the date of delivery, miscarriage or termination. While difficult to confirm, we estimate that pregnancies are commonly recorded as starting much later than the true dates of conception, shortening the exposure time, and misclassifying the first months of pregnancy as non-pregnant person-time. This is supported by the fact that the median duration of pregnancy seen in our cohort eight months.

Early pregnancy loss due to miscarriage and elective termination are common in this setting. Data from the DART trial indicated that 35% of pregnancies occurring among

women on treatment ended in either miscarriage or termination. [114] Given the suspected tendency to recognize pregnancy later in gestation, we may be missing many cases of pregnancies that end before they are recognized in this cohort. If the effect of pregnancy on adherence or drug-related adverse events is seen earlier in pregnancy but not later, our results could be biased towards the null due to this missing data.

Furthermore, there are currently no data on pregnancy outcomes in TE. In addition to helping distinguish whether a pregnancy listed as 4 months long is the result of a recording error or a pregnancy that ended before term, it would be valuable to know whether the effect of pregnancy and postpartum on our outcomes of interests extends to birth outcomes.

Missing data also poses a threat to the validity of our results. Forty percent of pregnancies had no end date corresponding to a given start date. We used imputation methods to assess the impact of this missing data. Given our concerns about the accuracy of recorded start dates, however, basing imputations on varying durations from the start of pregnancy may be problematic.

D. Future Research

This dissertation adds to our knowledge of the effects of pregnancy on maternal responses to HAART among women initiated on treatment prior to pregnancy. Our findings, along with limitations of the current work, also raise additional questions for future research.

While we restricted pregnancies included in our analysis to first incident pregnancies on HAART, we did not have data to assess the impact of parity on these outcomes. Pregnancies prior to initiating ART are inconsistently noted in individual medical records. Censoring women after the postpartum period of their first pregnancy on treatment also

limited the ability to assess the effect of subsequent incident pregnancies on our outcomes of interest. Biological changes related to pregnancy and potentially related to tolerability and effectiveness of treatment may be restricted to the actual period of pregnancy and immediately postpartum. It is also possible that pregnancy, either before or after treatment initiation, induces long-term physical changes that alter drug metabolism or other factors related to drug tolerance. The number of pregnancies a woman has experienced, as well as the number of children currently in her care, could impact motivation and behaviors related to treatment. If there is a cumulative physical effect for any of these factors, assessing parity prior to treatment, as well as multiple pregnancies on HAART would be critical. As long-term longitudinal studies of pregnancy among women on HAART are essentially non-existent, these questions are currently unexplored.

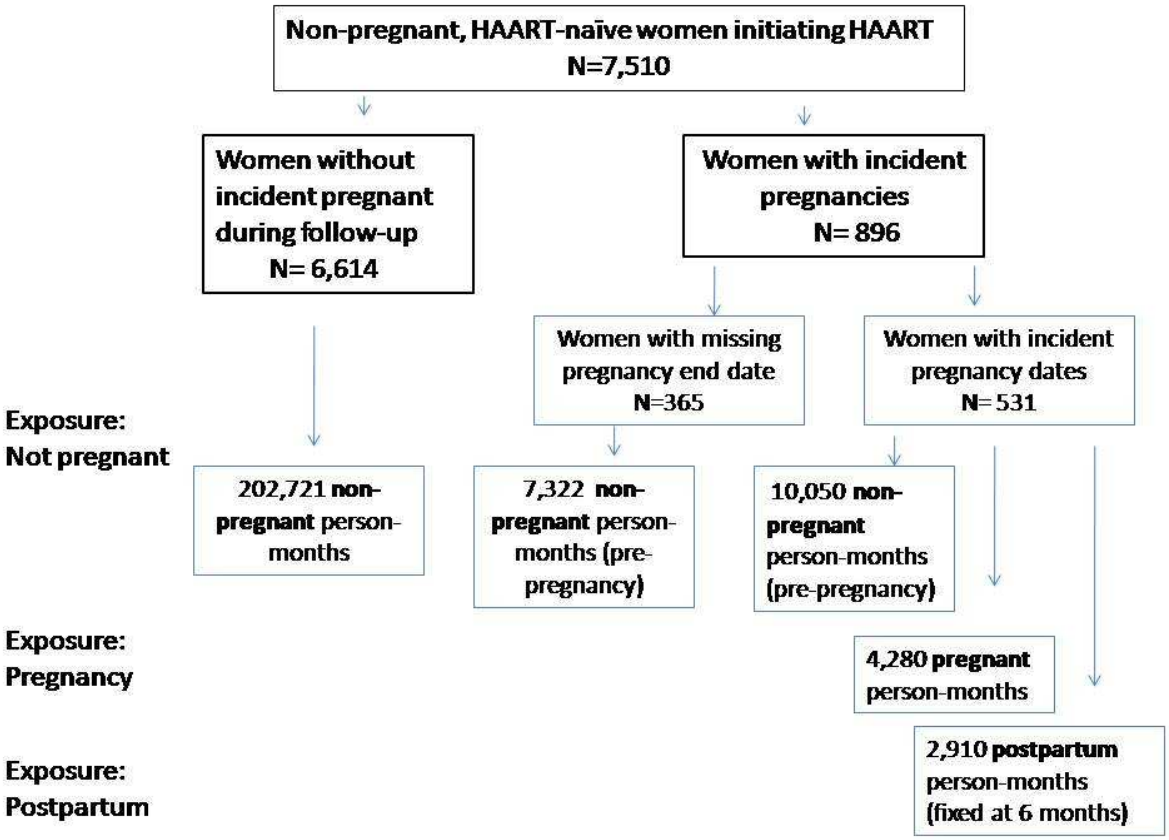
Given concerns about the accuracy of pharmacy-based refill measures based on the results of the analysis in Aim 1, the development and validation of an adherence indicator that could be incorporated into routine care, or calculated from routinely collected data, would have both clinical and research implications. An analysis of this nature could also potentially identify factors beyond adherence that result in poor virological responses, highlighting areas for increased clinical interventions. These factors may have been overlooked under current clinical practice, with cases of virological failure attributed primarily to poor adherence. Additionally, a highly valuable source of data for answering questions related to treatment with ART during pregnancy would be multi-generational data linking records of antenatal care, HIV-related services including treatment, birth outcomes, and infant follow-up. In our setting, these areas of care are currently handled by different providers at separate facilities. The ability to link data from these multiple sources would

allow for a more complete picture of the effect of pregnancy on maternal responses to treatment. From a clinical perspective, piecing together this information could highlight points of care that require strengthening. From a research perspective, this type of data could elucidate where there are true gaps in knowledge as opposed to simply missing information. The primary goal behind understanding HIV treatment during pregnancy is to protect the short- and long-term health of the mother, as well as protecting the health of the child; these outcomes can be difficult to assess when data from parts of clinical follow-up are missing.

Appendix A

Specific Aim 2: Allocation of Person-Time

Figure A.1. Allocation of person-time by pregnancy status contributed by HAART-naïve women initiating treatment



Appendix B

Specific Aim 3: Supplemental Tables

Table B.1. Characteristics of treatment-naïve women at treatment initiation, stratified by initial HAART regimen and period of initiation

Characteristic	Baseline HAART regimen			
	Before April 2010		After April 2010	
	aTDF	No TDF	TDF	No TDF
	N= 141	N=6,089	N=1096	N=208
Clinical/demographic				
Age *years*	33 (6)	34 (6)	34(6)	34 (6)
Unemployed	78 (55.3)	3452 (57.8)	479 (45.1)	104 (51.5)
Body mass index *kg/m^2*				
< 18.5	28 (25.0)	1045 (18.1)	128 (12.8)	29 (17.1)
18.5 – 24.9	58 (51.8)	3117 (53.9)	512 (51.1)	75 (44.1)
25.0 – 29.9	16 (14.3)	1092 (18.9)	241 (24.1)	39 (22.9)
≥ 30.0	10 (8.9)	633 (9.2)	121 (12.1)	27 (15.9)
WHO stage III or IV	51 (53.1)	2361 (43.2)	334 (38.8)	70 (48.6)
Tuberculosis (treated)	32 (22.7)	1061 (17.4)	132 (12.0)	29 (13.9)
Laboratory				
CD4 category *cells/mm^3*				
≤ 50	40 (31.5)	1959 (33.3)	213 (22.9)	48 (31.8)
51-100	30 (23.6)	1215 (20.7)	153 (16.4)	28 (18.5)
101-200	34 (26.8)	2094 (35.6)	364 (39.1)	43 (28.5)
201-350	23 (18.1)	610 (10.4)	201 (21.6)	32 (21.2)
Hemoglobin category*				
Normal	40 (28.4)	2580 (42.4)	471 (43.0)	58 (27.9)
Moderately anemic	73 (51.8)	3028 (49.7)	452 (41.2)	79 (38.0)
Severely anemic	28 (19.9)	481 (7.9)	173 (15.8)	71 (34.1)

Categorical variables are expressed as number (% total); continuous variables are expressed as mean (standard deviation

*: adjusted for altitude, hemoglobin categories for non-pregnant women: normal: > 11.35 g/dL, moderately anemic: 7.35-11.35 g/dL, severely anemic: < 7.35 g/dL

Table B.2. Creatinine clearance assessment scales and case estimates adjusted for altered glomerular filtration during pregnancy

	Standard	N [+]	30% [*]	N [+]	50%[#]	N [+]
Normal	≥ 90 mL/min	285	≥ 117 mL/min	184	≥ 135 mL/min	141
Mild	60-89 mL/min	17	78-117 mL/min	106	90-134 mL/min	128
Moderate	30-59 mL/min	3	39-77 ml/min	15	45-89 mL/min	35
Severe	<30 mL/min	2	<39 mL/min	2	< 45 mL/min	3

+: the number of CrCl assessments classified in each category of nephrotoxicity

*: thresholds increased by 30% for all categories of renal impairment; 30% is believed to be the low end of the range estimated change in glomerular filtration in pregnancy

#: thresholds increased by 50% for all categories of renal impairment; 50% is believed to be the high end of the potential change in glomerular filtration that occurs in person.

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